

UNIVERSITÀ CATTOLICA DEL SACRO CUORE

Sede di Piacenza

Dottorato di ricerca per il Sistema Agro-alimentare

Ph.D. in Agro-Food System

Cycle XXXVII

S.S.D. AGR02



UNIVERSITÀ
CATTOLICA
del Sacro Cuore

Developing Operational Carbon Farming Strategies to Unlock the Climate Benefits of Soil Carbon Sequestration: From Agronomic Practices to MRV Tools

Coordinator:

Ch.mo Prof. Paolo Ajmone Marsan

Candidate: Marta Bertola

Matriculation n: 5116124

Academic Year 2023/2024



UNIONE EUROPEA
Fondo Sociale Europeo



Ministero dell'Università
e della Ricerca



PON
RICERCA
E INNOVAZIONE
2014 - 2020

REACT EU

La borsa di dottorato è stata cofinanziata con risorse del Programma Operativo Nazionale Ricerca e Innovazione 2014-2020, risorse FSE REACT-EU Azione IV.4 “Dottorati e contratti di ricerca su tematiche dell’innovazione” e Azione IV.5 “Dottorati su tematiche Green”

UNIVERSITÀ CATTOLICA DEL SACRO CUORE

Sede di Piacenza

Dottorato di ricerca per il Sistema Agro-alimentare

Ph.D. in Agro-Food System

Cycle XXXVII

S.S.D. AGR02



UNIVERSITÀ
CATTOLICA
del Sacro Cuore

Developing Operational Carbon Farming Strategies to Unlock the Climate Benefits of Soil Carbon Sequestration: From Agronomic Practices to MRV Tools

Coordinator:

Ch.mo Prof. Paolo Ajmone Marsan

Tutor:

Dr. Andrea Ferrarini

Prof. Stefano Amaducci

Candidate: Marta Bertola

Matriculation n: 5116124

Academic Year 2023/2024



UNIONE EUROPEA
Fondo Sociale Europeo



Ministero dell'Università
e della Ricerca



PON
RICERCA
E INNOVAZIONE
2014 - 2020

REACT EU

Ricerca e Innovazione 2014-2020, risorse FSE REACT-EU Azione IV.4 "Dottorati e contratti di ricerca su tematiche dell'innovazione" e Azione IV.5 "Dottorati su tematiche Green". La borsa di dottorato è stata cofinanziata con risorse del Programma Operativo Nazionale

Marta Bertola

Developing Operational Carbon Farming Strategies to Unlock the Climate Benefits of Soil Carbon Sequestration: From Agronomic Practices to MRV Tools,
216 pages.

PhD thesis, Università Cattolica del Sacro Cuore, Piacenza, Italy (2024)

Tables of contents

CHAPTER 1	8
General introduction	9
1.1 Climate Change and the Role of Agriculture	10
1.2 Carbon Farming: Definition and European regulation	11
1.3 Carbon Farming Practices and their agronomic and environmental co-benefits	13
1.4 Challenges and Criticisms of Carbon Farming	16
1.5 Measurement, Reporting, and Verification (MRV) Methods in Carbon Farming	18
1.6 Research objectives.	20
1.7 Thesis outline and experimental approach	21
CHAPTER 2	24
Early impacts of marginal land-use transition to Miscanthus on soil quality and soil C storage across Europe	25
2.1 Introduction	26
2.2 Materials and Methods	28
2.3 Results	32
2.4 Discussions	39
2.5 Conclusion	47
CHAPTER 3	61
Carbon farming solutions for the industrial tomato supply chain: evaluating agronomic practices eligibility and MRV tools accuracy on lead commercial farms in northwestern Italy.	62
3.1 Introduction	63
3.2 Materials and Methods	68
3.3 Results	78
3.4 Discussions	86
3.5 Conclusion	97
CHAPTER 4	105
Targeted supplementation of mineral and organic nutrients enhances carbon incorporation from wheat residue into mineral-associated soil organic matter fraction	106
4.1 Introduction	107
4.2 Materials and Methods	110
4.3 Results	117
4.4 Discussions	125
4.5 Conclusion	132
CHAPTER 5	141
Integrating proximal soil sensing into SOC stock accounting for carbon farming applications: a case study in Northern Italy	142
5.1 Introduction	143
5.2 Materials and Methods	147
5.3 Results	161
5.4 Discussions	181
5.5 Conclusions	189
CHAPTER 6	194
General conclusions	195
Overarching Insights and Contributions	199
REFERENCES	201

....

*Questa tesi è dedicata alle mie nonne,
alla loro forza, al loro coraggio
e alla loro determinazione.*

Chapter 1

General introduction

Marta Bertola

1.1 Climate Change and the Role of Agriculture

The increasing concentration of greenhouse gases (GHGs) in the atmosphere, primarily due to human activities, has become one of the most pressing environmental challenges of our time. The latest IPCC Assessment Report (IPCC, 2023) estimates global net anthropogenic GHG emissions at 59 ± 6.6 GtCO₂-eq in 2019, reflecting a 12% (6.5 GtCO₂-eq) increase from 2010 and 54% (21 GtCO₂-eq) rise since 1990. Climate change, driven by this accumulation of GHGs, is altering global weather patterns, raising sea levels, and increasing the frequency of extreme weather events. These changes present significant risks to ecosystems, biodiversity, and human societies, particularly those dependent on stable agricultural systems for food production and livelihoods (Lal, 2004a).

Agriculture, a vital sector for food security and economic development, is both a contributor to and a potential solution for climate change. Globally, agriculture, forestry, and other land use (AFOLU) account for approximately 21% of total net anthropogenic GHG emissions (Nabuurs et al., 2023), driven by activities such as livestock production, soil management, rice cultivation, synthetic fertilizer use, and deforestation for cropland expansion. Agricultural emissions are primarily composed of methane (CH₄) and nitrous oxide (N₂O), gases with a much higher global warming potential than carbon dioxide (CO₂). Additionally, land misuse and soil mismanagement have contributed to the degradation of soil organic matter (SOM) and the release of stored soil organic carbon (SOC) back into the atmosphere (Lal, 2004b). This loss of SOC not only exacerbates climate change but also weakens the resilience and fertility of agricultural lands.

However, agriculture holds immense potential to mitigate climate change through carbon (C) sequestration and improved land management practices. Soils represent the largest terrestrial reservoir of organic C, storing more C than the atmosphere and vegetation combined (Scharlemann et al., 2014). Observations suggest that more than half of SOC is chemically or physically associated with soil minerals (Georgiou et al., 2022). Yet, current SOC levels in surface and deep soil layers utilize only 42% and 21% of the soil's mineral capacity, respectively (Georgiou et al., 2022). This gap between soils' C storage potential and current levels presents an opportunity: by restoring SOM, the capacity of soils to sequester atmospheric CO₂ can be enhanced, turning soils into powerful C sinks. (Bruni et al., 2021; Martin et al., 2021; Minasny et al., 2023). This dual function — reducing emissions while capturing C — positions agriculture as a key sector in the global effort to combat climate change.

Beyond its potential for SOC sequestration, sustainable agricultural practices can improve soil health, boost biodiversity, and enhance ecosystem services such as water retention and nutrient

cycling (Lal, 2004b). These benefits are especially important in the context of a changing climate, where increased variability in rainfall patterns and extreme weather events place additional stress on agricultural systems. By adopting practices that prioritize soil health and resilience, farmers can contribute to climate mitigation while simultaneously improving productivity and sustainability.

Despite these promising opportunities, the agricultural sector faces numerous challenges in integrating climate mitigation strategies. The complexity of agricultural systems, combined with regional variations in climate, soil types, and cropping patterns, makes it difficult to apply a one-size-fits-all approach to reducing emissions and increasing SOC sequestration. Furthermore, economic and social conditions, particularly for smallholder farmers, may hinder the adoption of climate-friendly practices (Lal, 2009). Addressing these challenges will require coordinated efforts across policy, research, and practice to ensure that agriculture plays a central role in climate change mitigation.

This introduction sets the stage for exploring innovative solutions within agriculture, particularly the emerging opportunities given by carbon farming in terms of decarbonization of agriculture sector and contemporary soil health improvement. Understanding agriculture's dual role in both contributing to and mitigating climate change underscores the potential of land-based strategies that can simultaneously address C reduction and support sustainable food production.

1.2 Carbon Farming: Definition and European regulation

Carbon farming (CF) is a business model designed to reward land managers for C sequestration while adhering to ecological principles. It involves a strategic approach to land management aimed at capturing and storing atmospheric CO₂ in soils, vegetation, and ecosystems through agricultural practices. At its core, CF seeks to enhance the natural ability of agricultural lands to function as C sinks, offsetting a portion of the emissions that contribute to climate change (IPCC, 2023).

The concept of CF gained global attention following the implementation of the Kyoto Protocol in 2004, as a potential solution to the dual challenge of mitigating climate change and promoting sustainable agriculture. By focusing on increasing SOC sequestration and improving the long-term soil health, CF aims to make agricultural landscapes not only more productive but also more resilient to the impacts of climate change. Its goals include capturing atmospheric CO₂, enhancing biodiversity, improving soil health, and boosting ecosystem services such as water retention and nutrient cycling.

In Europe, CF has become a key component of the European Union's climate strategy, particularly in light of the EU's goal to achieve C neutrality by 2050. Recognizing the potential of agricultural lands as a significant C sink, the European Commission has launched initiatives to promote CF as part of the broader European Green Deal. The Green Deal, introduced in 2019, sets ambitious climate and environmental goals, including a 55% reduction in GHG emissions by 2030 and the restoration of degraded ecosystems. CF is seen as an essential practice to help meet these objectives by capturing C in soils and reducing emissions from agricultural activities. In 2024, the European Parliament approved a new regulation on the certification of C removals - **Carbon Removals and Carbon Farming (CRCF) Regulation** - which aims to establish a transparent and robust framework for certifying C removals of different land-based practices, including CF (European Parliament and Council of the European Union, 2024). It is designed to provide a reliable mechanism for farmers to earn C credits, which can be traded in voluntary or regulated markets, creating an economic incentive for adopting climate-friendly practices. Under this certification framework, farmers engaging in CF would be able to demonstrate the amount of C sequestered in their soils and potentially receive financial compensation through the sale of C credits to companies or organizations seeking to offset their emissions.

The regulation places a strong emphasis on the need for high-quality C credits, ensuring that the C stored in soils is measurable, verifiable, and permanent (Paul et al., 2023; Smith, 2012). To support this, the European Commission has proposed an EU quality standard for the certification of C removals along four **QU.A.L.I.TY** criteria:

Quantification ensures that C removals are accurately measured using robust and transparent methods. This involves precise monitoring techniques, models, and verification processes to track the amount of C sequestered in soils, forests, or other ecosystems, providing reliable data on the effectiveness of CF and other sequestration activities.

Additionality requires that the C removal would not have occurred without the specific intervention. This ensures that the practices adopted lead to new and measurable reductions in atmospheric C, rather than rewarding activities that would have happened under business-as-usual scenarios. Additionality is key to ensuring that CF projects contribute meaningfully to climate mitigation.

Long-term storage addresses the long-term stability of C stored in soils or vegetation. C sequestered through farming or forestry must remain stored over extended periods to effectively mitigate climate change. The EU emphasizes practices that prevent C re-release due to land-use

changes, environmental degradation, or extreme weather events, ensuring the longevity of captured C.

Sustainability ensures that C removal projects align with broader environmental and social goals. Practices must not only sequester C but also protect biodiversity, support ecosystem health, and contribute to socio-economic development. This criterion ensures CF is a holistic approach to sustainable land management, avoiding negative trade-offs.

The regulation outlines best practices for meeting these criteria, drawing on relevant legislation, while acknowledging that solutions may vary across different C removal approaches.

Looking ahead, the EU is positioning itself as a leader in promoting CF not only as a tool for climate mitigation but also to support rural economies and improve food security. By 2028, the European Commission aims to develop a fully operational C removal certification system with stringent criteria for environmental integrity and socio-economic benefits. This system is expected to play a central role in the EU's broader efforts to establish a circular economy, reduce emissions, and support a sustainable food system.

1.3 Carbon Farming Practices and their agronomic and environmental co-benefits

The success of CF lies in the selection and implementation of agricultural practices designed to maximize C capture and storage in soils, minimize C losses, and reduce GHG emissions from farming activities (Lal, 2004b; Paustian et al., 2016). These practices, rooted in sustainable land management and regenerative agriculture, play a crucial role not only in mitigating climate change but also in improving soil health, enhancing soil biodiversity, reducing soil erosion, runoff and water pollution (Smith, 2012). For many CF practices applied to cereal crops it was reported increased crop resilience to extreme weather and an increase of net farm income thanks to the above-mentioned agronomic co-benefits that tighter with reduced gasoline consumption for tillage lead to an average cost reduction to grow crops per hectare.

CF practices can be broadly categorized based on their primary objectives: increasing C input to the soil, reducing carbon losses, and lowering GHG emissions (Lal, 2004b). While the greatest potential for SOC sequestration lies in the restoration of marginal and degraded lands to grassland, forest, or wetlands, the focus here is on strategies applicable to agricultural lands (Lal, 2004a; Paustian et al., 2016).

1.3.1 *Increasing Carbon Inputs to Soil*

Practices aimed at increasing C inputs to soil from both above- and belowground biomass include: (1) using improved varieties or species with larger root systems that deposit C in deeper layers, where C turnover is slower (Kell, 2011); (2) adopting crop rotations that enhance C inputs (Burney et al., 2010); (3) increasing residue retention (Bolinder et al., 2020); (4) planting cover crops during fallow periods for continuous C inputs (Poeplau and Don, 2015); and (5) applying organic amendments (Hua et al., 2014). Cultivating **deep-rooted crops**, such as perennial grasses and certain cover crops, enhances belowground C inputs and the incorporation of this biomass into the subsoil can improve the long-term stability of C (Agostini et al., 2015; Chimento et al., 2016). For instance, SOC accumulation significantly increase when degraded or marginal soils are converted to perennial vegetation (Don et al., 2012; Xu et al., 2022). Additionally, there is potential in breeding crops with larger and deeper root systems to enhance SOC sequestration (Glover et al., 2007). Although increasing root biomass has not traditionally been a focus for crop breeders, the genetic plasticity of most crops allows substantial changes in root traits (Bell et al., 2010; Cox et al., 2006). Selecting root systems that are better adapted to water and nutrient limitations could increase root C inputs while improving crop yields (Kell, 2011; Lilley and Kirkegaard, 2011). Improved **crop rotations** that provide greater C inputs is another effective strategy. High-biomass species like maize or sorghum, for example, increase belowground C inputs and residue returns to the soil (Morari et al., 2006). **Incorporating residues** into the soil, rather than removing or burning them after harvest, enriches the soil with organic matter while improving soil structure and nutrient cycling (Dămățircă et al., 2023). **Cover cropping** during fallow periods is another important practice to ensure year-round C inputs. By growing cover crops, such as legumes, grasses, or brassicas, during periods when the soil would otherwise be bare, C captured through photosynthesis is deposited into the soil through root biomass and decaying plant material (Jordon et al., 2022). This practice also prevents erosion, reduces nutrient leaching, and promotes nutrient cycling for subsequent crops (Blanco-Canqui and Ruis, 2020). Lastly, the application of **organic amendments**, such as compost, manure, or biochar, effectively increase C inputs. Organic amendments add significant amounts of organic matter to the soil, promoting microbial activity and improving soil aggregation (Cowie et al., 2013). These inputs not only enhance SOC sequestration but also improve soil fertility, water retention, and microbial diversity, which contribute to overall soil health and resilience (Diacono and Montemurro, 2011). However, the climate mitigation potential of external plant-derived C inputs like composts or biochar is controversial due to GHG emissions associated with offsite biomass removal,

transportation, and processing (Paustian et al., 2016). Moreover, adding C-rich plant biomass without considering other nutrients (N, P, S) can lead to the depletion of the SOC pool through accelerated mineralization, a phenomenon known as the "positive priming effect" (Bastida et al., 2019). Therefore, the nutrient requirements for biomass humification and conversion into stable humic substances and organo-mineral complexes must be considered to prevent SOC priming (Coonan et al., 2020b; Kirkegaard et al., 2023).

1.3.2 Reducing Carbon Losses from Soil

While increasing C inputs is essential, reducing the loss of stored C from agricultural soils is equally important. C losses occur through soil erosion, oxidation, and land management practices that disturb soil structure, such as tillage (De Rosa et al., 2023). CF practices that minimize soil disturbance and maintain soil cover are crucial for maintaining SOC stability (De Rosa et al., 2023). **Conservation tillage**, including no-till or reduced tillage, is one of the most effective practices for limiting C losses (Lal, 2004b). By minimizing the mechanical disruption of soil, conservation tillage preserves soil aggregates, protects SOC, and reduces its oxidation or microbial degradation (Bailey et al., 2019). Over time, this can result in gradual SOC accumulation, improved soil structure, and enhanced water infiltration (Bai, 2018). Maintaining a perennial soil cover by cultivating perennial herbaceous or woody crops, as well as integrating agroforestry systems, also reduces C losses (Lal, 2009). Permanent root systems help anchor soil, reducing erosion risk and protecting C stored in the topsoil. Additionally, retaining crop residues as mulches and incorporating cover crops into rotations conserve soil moisture, promote microbial activity, and support SOC stabilization (Lal, 2015).

1.3.3 Reducing Greenhouse Gases Emissions

Beyond enhancing SOC sequestration and reducing C losses, CF practices also focus on reducing other potent GHG emissions, such as CH₄ and N₂O, which are released from soils through agricultural activities. **Improved fertilizer management** is key to reduce N₂O emissions, which primarily arise from excessive use of synthetic N fertilizers (Paustian et al., 2016; Smith et al., 2007). Optimizing the timing, placement, and quantity of fertilizer applications helps reduce reactive nitrogen in the soil that is converted into N₂O (Paustian et al., 2016; Smith et al., 2007). The use of commercial additives that slow ammonium oxidation can further reduce N₂O emissions by promoting gradual nutrient release and minimizing N leaching and volatilization (Paustian et al., 2016; Smith et al., 2007). Methane emissions, particularly from rice paddies and wetlands, can also be mitigated by modifying

water management practices. In rice paddies, alternating wetting and drying reduces anaerobic conditions that promote CH₄ production, although this may increase N₂O production and reduce net mitigation effects (Paustian et al., 2016). Similarly, wetlands, which are natural CH₄ sources due to anaerobic decomposition, can be managed to balance CH₄ reduction while maintaining their C sequestration benefits (Smith et al., 2007). For example, maintaining water levels that reduce prolonged periods of complete saturation can help balance the trade-offs between C storage and CH₄ production (Smith et al., 2007).

1.3.4 Benefits to Soil Health and Quality

In addition to SOC sequestration and GHG reduction, CF practices provide numerous benefits to soil health and quality (Bai, 2018). Soils managed using CF techniques generally exhibit improved structure, increased organic matter content, and enhanced biological activity (Cowie et al., 2013). These improvements lead to better water retention, particularly important in drought-prone areas, and more efficient nutrient cycling, supporting sustainable crop production (Lal, 2015).

Soils rich in organic C are more resilient to erosion and compaction, as organic matter binds soil particles together, forming stable aggregates (Bongiorno et al., 2019a). This improved structure allows for better root penetration and water infiltration, reducing the risk of runoff and nutrient loss (Lal, 2015). Increased microbial biomass and diversity in C-rich soils also improve nutrient availability and disease suppression fostering healthier and more productive agricultural systems (Bongiorno et al., 2019b).

In conclusion, CF practices not only help mitigate climate change but also enhance soil quality and support sustainable agriculture. By building healthier, more resilient soils, CF contributes to long-term food security and the overall sustainability of farming systems.

1.4 Challenges and Criticisms of Carbon Farming

While CF holds considerable promise as a climate mitigation strategy, its widespread adoption and long-term effectiveness face several challenges and criticisms. These challenges stem from scientific, technical, social, and economic factors, as well as concerns over the scalability and sustainability of CF practices in various agricultural contexts (Smith and Torn, 2013; Smith, 2012). One major scientific issue is the uncertainty around the **permanence** of C sequestration in soils. C stored through improved management can be re-emitted to the atmosphere via processes such as soil erosion, decomposition of SOM, and disturbances caused by land-use changes or extreme weather events (Dynarski et al., 2020). Even when best practices are followed, natural events like

droughts, floods, or wildfires can disrupt the C balance, diminishing the overall climate benefits (Smith, 2005). In contrast to permanent reductions of other GHG, sequestered C is non-permanent, posing a risk of future release (Smith, 2012), which raises concerns about CF's long-term effectiveness. Another significant challenge is soil SOC **saturation**. Soils have a limited capacity to store C due to physical and chemical properties, and once saturation is reached, sequestration rates decrease, limiting the long-term potential of CF practices (Guillaume et al., 2022; Moinet et al., 2023). The timeline for reaching saturation depends on factors such as soil type, climate, and management practices, but many agricultural soils may reach a point where additional C inputs yield diminishing returns in terms of storage (Angers et al., 2011). This limitation questions the scalability of CF as a sustainable climate solution over the long term. **Biophysical constraints** also hinder to the widespread implementation of CF. In croplands with unsaturated topsoil, increased net primary productivity and additional nutrient inputs are necessary to sustain SOC increases (Martin et al., 2021; Richardson et al., 2014). Access to organic inputs like crop residues, compost, or manure is not always reliable, and logistical costs, such as transporting these materials, further complicate adoption (Poulton et al., 2018). Exporting organic matter from one area to another may also result in SOC losses elsewhere, negating the overall C sequestration benefits due to **leakage** or displacement.

Economic constraints also present major challenges. While CF could provide financial incentives through C credits, the profitability remains uncertain, especially for small-scale farmers (Barbato and Strong, 2023). Transition costs, such as acquiring new equipment, can be prohibitive, and the financial returns from C credits are often low and unstable due to market volatility. (Barbato and Strong, 2023). There is consistent social-scientific evidence that farmer motivations for adoption of CF on their land are driven by perceptions about the co-benefits of such activities, rather than by financial returns or the idea of sequestering C (Barbato and Strong, 2023). **Social barriers** factors, including lack of familiarity with CF practices and concerns over costs, further complicate adoption, despite potential financial incentives (Barbato and Strong, 2023).

Moreover, over-reliance on CF as a climate solution may divert attention from more urgent actions, such as reducing fossil fuel emissions (Baveye, 2020). CF should be viewed as part of a broader climate strategy rather than a standalone solution. Lastly, the success of CF depends on **robust and transparent Measurement, Reporting, and Verification (MRV) systems** (Smith, 2012). Without reliable methods for accurately measuring C sequestration and monitoring its permanence, the credibility of CF as a climate solution remains in question. Current technologies face challenges

in balancing accuracy with cost, and many farmers lack access to the necessary tools and expertise for implementation (Brummitt et al., 2024). This complexity adds another layer of difficulty to verifying C sequestration claims.

1.5 Measurement, Reporting, and Verification (MRV) Methods in Carbon Farming

Measurement, Reporting, and Verification (MRV) systems are integral to the success of CF initiatives, as they must ensure the **accuracy** and **reliability** of C sequestration outcomes and reductions in GHG emissions (Guan et al., 2023; Paul et al., 2023; Smith, 2012). **Robust** MRV frameworks should build trust among stakeholders—farmers, policymakers, and investors—by promoting **transparency** and **accountability** in carbon trading and offset markets. Beyond regulatory compliance, MRV systems are critical for assessing the real impact of CF on climate mitigation. However, the challenge lies in developing **reliable**, **scalable**, and **cost-effective** MRV methods particularly within diverse agricultural contexts where resources and capacities may differ (Guan et al., 2023). Practical MRV solutions are necessary, especially for farmers, to ensure accurate measurements without imposing excessive financial burdens (Paul et al., 2023).

Field-based measurement approaches remain one of the primary methods for assessing SOC levels and monitoring C fluxes (Guan et al., 2023; Smith et al., 2020). These approaches traditionally involve measuring both SOC concentration and soil bulk density to quantify SOC stocks. A key recommendation for SOC stock assessment is the use of an “equivalent mass basis” approach, particularly when comparing stocks across different land uses and management practices that may affect SOC concentration and bulk density (Wendt and Hauser, 2013). Direct measurement involves collecting soil from various locations to account for the spatial variability of SOC content across different fields and conditions. However, the need for a large number of soil samples to minimize potential errors in SOC stock estimation (Smith et al., 2020) makes this approach labor-intensive and costly. These constraints make large-scale measurement campaigns challenging, especially when trying to maintain precision.

Recent technological innovations have expanded the possibilities for enhancing MRV systems in CF. **Soil proximal sensing** is one such innovation, providing direct point measurements in both field and laboratory settings, as well as large-scale soil mapping (Guan et al., 2023; Smith et al., 2020). Proximal sensing technologies, such as ultraviolet (UV), visible and infrared spectroscopy, electromagnetic induction, electrical resistivity, and gamma radiometric sensor, can estimate various soil parameters, including SOC content, moisture levels, clay content, and sand fraction

(Rossel et al., 2010). These technologies, once equipped with a well-developed library or calibration set, allow for more frequent and detailed soil assessments without relying heavily on intensive sampling, thus reducing both time and labor costs (Rossel et al., 2010). By combining proximal sensing with traditional field-based measurements, a more comprehensive and efficient approach to monitoring SOC and other soil health indicators can be achieved. In addition to proximal sensing, **remote sensing** technologies provide promising opportunities for monitoring C fluxes over larger areas with greater efficiency. These technologies can involve linking data from unmanned aerial vehicles (UAVs), airplanes, or satellites directly to soil properties, inferring changes in SOC through shifts in vegetation, or incorporating remote imagery as a covariate in digital soil mapping (Smith et al., 2020). Remote sensing is particularly valuable for collecting data across vast regions, complementing field-based measurements to offer a more comprehensive understanding of C dynamics in agricultural systems. **Process-based modeling** tools such as Daycent and RothC are also instrumental in simulating C dynamics within agricultural ecosystems (Guan et al., 2023; Smith et al., 2020). These models rely on algorithms that incorporate various factors—including soil properties, vegetation, climate, and management practices—to predict C fluxes and sequestration potential over time. These models are particularly useful for assessing the long-term impacts of different agricultural practices on SOC levels, providing insights into how modifications to land management can enhance C sequestration. However, the accuracy of these models depends heavily on the quality and availability of input data, as well as the assumptions made in the model's design. **Carbon accounting tools** play a pivotal role in quantifying GHG emissions reductions associated with CF practices (Guan et al., 2023). Tools such as the Cool Farm Tool offer user-friendly platforms to estimate the C footprint of a farm and track improvements in management practices over time (Hillier et al., 2011). These tools often integrate data from field measurements, remote sensing, and modeling to provide a holistic view of C dynamics.

However, one of the key challenges in MRV systems is finding a **balance between accuracy and cost** . More precise measurement techniques often come with increased financial and logistical demands, which can be prohibitive for smallholder farmers or those operating under tight budgets. Therefore, while technological innovations have improved MRV systems' accuracy and scalability, their practical implementation must consider the economic feasibility for a wide range of agricultural stakeholders. One potential approach for the quantification of field-level C outcomes in agricultural soils is through an integration of sampling, sensing, and modeling, which was defined by Guan et al. (2023) as the “System-of-Systems” solution.

1.6 Research objectives.

The transition to sustainable agriculture and climate mitigation has placed CF at the forefront as a crucial solution for supporting C removals and overall soil quality improvements. Several EU projects considered in this research, including GRACE, ClieNFarms, Farms4Climate, and MARVIC, collectively address the urgent need for cost-effective climate neutral and climate resilient agronomic solutions aligned with CF principles and operational MRV systems to enable the rewarding of net climate benefits generated at farm level and across supply chains.

- **GRACE** (<https://www.grace-bbi.eu/>) focuses on cultivating non-competitive biomass crops like Miscanthus on marginal lands, transforming these areas into productive landscapes that enhance SOC sequestration while generating high-value bio-based products.
- **ClieNFarms** (<https://clienfarms.eu/>) aims to co-develop and scale systemic, locally relevant solutions for climate-neutral and climate-resilient farming systems by integrating organizational, technical, and financial innovations, creating economically viable CF models.
- **Farms4Climate** (<https://www.farms4climate.eu/>) adopts a farmer-centered living lab approach to develop and test agroecological practices, supported by digital tools for measuring, reporting, and verifying C removals, thereby enabling farmers to access C markets.
- **MARVIC** (<https://www.project-marvic.eu/>) works on developing context-specific MRV frameworks for public or private certification schemes and OPC (operational processing chain) that empower farmers to engage in CF program and benefit from climate finance mechanisms.

Together, these projects emphasize shared goals of enhancing SOC storage, promoting climate-smart farming practices, and providing practical pathways for farmers to contribute to and benefit from the emerging soil C economy.

In this context, this thesis explores operational CF strategies across various systems and scales to unlock the potential of the soil C economy. The primary aim is to enhance SOC sequestration and improve soil quality through science-based, practical CF practices, while enabling effective monitoring and verification to support the soil C economy.

Each chapter contributes to this overarching goal:

- Chapter 2: Investigates the effects of land-use transitions, specifically the conversion of marginal lands to Miscanthus cultivation, on enhancing SOC storage and improving soil quality. This chapter examines how such transitions can transform underutilized landscapes into productive systems that contribute to SOC sequestration and sustainable land management.

- Chapter 3: Develops and evaluates practical CF practices tailored to the industrial tomato supply chain in northwestern Italy. This chapter assesses the efficiency of short-term SOC sequestration while highlighting the delivery of environmental co-benefits such as improved soil quality and reduced GHG emissions. Additionally, it integrates accurate and cost-effective MRV tools to ensure the scalability and economic viability of these practices within CF frameworks.
- Chapter 4: Explores the microbial mechanisms underlying SOC stabilization, specifically the incorporation of straw residues into the soil with targeted nutrient supplementation, a practice explored in Chapter 2. This approach aims to enhance the efficiency of C incorporation into stable soil fractions, addressing a critical requirement for long-term C storage—a cornerstone of effective CF initiatives.
- Chapter 5: Advances the accuracy of SOC stock predictions by integrating on-the-go proximal soil sensing technologies with remote sensing and auxiliary data, ensuring reliable assessment and monitoring, which are crucial for CF systems.

Together, these studies aim to provide actionable insights into CF's role in supporting sustainable agriculture, enhancing soil health, and contributing to both climate mitigation (through certified removals) and adaptation (via improved soil resilience).

1.7 Thesis outline and experimental approach

To address the research objectives outlined in the previous section, a multifaceted approach was adopted. Field experiments were conducted to examine two distinct cropping systems—a perennial cropping system and an arable cropping system—to evaluate their contributions to SOC sequestration and soil quality improvement. An incubation experiment under controlled conditions was designed to investigate the microbial mechanisms underpinning SOC stabilization. This experiment aimed to assess the efficiency of targeted agronomic practices in promoting long-term C storage, a critical component of sustainable CF strategies. Finally, a comprehensive field campaign across northern Italy was undertaken to develop accurate and cost-effective SOC accounting approach to support MRV activities, ensuring reliable assessment and scalability of CF practices. This integrated approach provides actionable insights into enhancing SOC sequestration while supporting the operationalization of CF initiatives.

Chapter 2 focuses on evaluating the short-term potential of four promising *Miscanthus* hybrids, highly productive perennial grasses, to sequester SOC and enhance physical, chemical, and biological soil quality across six marginal sites in Europe. The study examines the impact of

transitioning land use to *Miscanthus* cultivation from former cropland and grassland, with a focus on SOC dynamics and the feasibility of sustaining these high-yielding genotypes on unfertilized marginal lands. Additionally, the role of plant C inputs from these hybrids as a key driver of SOC sequestration is explored.

Chapter 3 assesses the eligibility of CF practices specifically designed for the industrial tomato supply chain in northwestern Italy, with a focus on their potential for future C crediting. The study investigates the ability of these practices to generate C credits and deliver environmental co-benefits through trials conducted on five Lead Commercial Farms (LCFs) in the Piacenza province. A hybrid MRV methodology is proposed, integrating field measurements with remote sensing technologies and a biogeochemical model to ensure scalability, reliability, and adaptability within CF programs. The criteria for certifying C removals and emission reductions align with the European Commission's newly proposed Carbon Removal Certification Framework (CRCF) and the VERRA VM0042 methodology. Adhering to these frameworks ensures that the CF practices tested meet stringent standards of credibility and consistency, thereby building stakeholder confidence in C markets, and supporting the advancement of CF initiatives.

Chapter 4 investigates the microbial mechanisms that regulate the balance between SOC mineralization and stabilization in mineral soil fractions through a controlled incubation experiment. Building on the practices explored in Chapter 2, the study focuses on the balanced addition of nutrients to straw residues incorporated into the soil as a strategy to enhance the humification efficiency of C-rich organic matter and promote SOC stabilization. This stabilization is linked to the "microbial-C pump" mechanism introduced in the chapter, highlighting the role of microbial processes in driving long-term C storage. The stabilization of SOC into mineral fractions is crucial, not only as a cornerstone of effective CF initiatives but also as a source of sustained soil fertility. Stable SOC contributes to long-term C storage while gradually releasing nutrients that support crop productivity, reinforcing the dual benefits of CF for climate mitigation and agricultural sustainability.

Chapter 5 develops a proximal sensing-based regional machine learning (ML)-based prediction models to estimate key soil properties, such as clay content, SOC concentration, and BD for on-the-go SOC stock mapping. Additionally, a Smart Soil Sampling approach is introduced to optimize soil sampling at the field level, minimizing the number of samples required while enabling local calibration procedures. The ultimate goal is to produce high-resolution SOC and BD maps at the field level, which can serve as inputs for calculating SOC stock maps and generating uncertainty maps.

This approach provides valuable tools for precise, field-scale SOC stock spatial accounting and supports CF applications such as model initialization and climate smart fertilization strategies.

Finally, **Chapter 6** integrates the findings from the previous chapters, synthesizing key insights and outcomes. It also addresses the limitations of the methods employed and discusses both the opportunities and the critical needs for future research in the field of CF. This reflection aims to guide further advancements and refine strategies for enhancing SOC sequestration and improving agricultural sustainability.

Chapter 2

Early impacts of marginal land-use transition to *Miscanthus* on soil quality and soil C storage across Europe

Marta Bertola¹ | Elena Magenau² | Enrico Martani^{1,11} | Mislav Kontek³ | Chris Ashman⁴ | Vanja Jurišić³ | Isabelle Lamy⁵ | Jason Kam⁶ | Flavio Fornasier^{7a,b} | Jon McCalmont⁸ | Luisa M. Trindade⁹ | Stefano Amaducci¹ | John Clifton-Brown¹⁰ | Andreas Kiesel² | Andrea Ferrarini¹

¹ Department of Sustainable Crop Production – Università Cattolica del Sacro Cuore, Piacenza, Italy

² Department of Biobased Resources in the Bioeconomy, Institute of Plant Breeding, University of Hohenheim, Stuttgart, Germany

³ Department of Ag Technology, Faculty of Agriculture, University of Zagreb, Zagreb, Croatia

⁴ Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, UK

⁵ University Paris-Saclay, INRAE, AgroParisTech, UMR EcoSys, 91110 Palaiseau, France

⁶ Terravesta Ltd., Lincoln, Lincolnshire, UK

^{7a} CREA, Research Centre for Viticulture and Enology, Unit of Gorizia, Italy

^{7b} SOLIOMICS srl, Via del Cottonificio, 129/B 33100 Udine, Italy

⁸ King's College, University of Aberdeen, Aberdeen, UK

⁹ Wageningen University & Research, Plant Breeding, Wageningen, The Netherlands

¹⁰ Department of Agronomy and Plant Breeding, Research Centre for Biosystems, Land-Use and Nutrition (iFZ), Justus Liebig University, Gießen, Germany

¹¹ Davines Group – Rodale Institute European Regenerative Organic Center (EROC), Parma, Italy

This chapter has been published in the journal

Global Change Biology and Bioenergy (2024), Volume 16,

DOI: <https://doi.org/10.1111/gcbb.13145>

2.1 Introduction

Miscanthus, a perennial herbaceous crop with C₄ photosynthesis, has received considerable attention as a multi-purpose crop that can provide large amounts of biomass for the growing bioeconomy and simultaneously offering environmental benefits. Novel *Miscanthus* hybrids are being developed in Europe as promising biomass crops for the bioenergy and bio-based industry, rural diversification, to face climate changes and global energy security (Clifton-Brown et al., 2017). Its nearly year-round soil cover reduces soil erosion and run-off, while the deep and dense rooting system ensures a more efficient use of nutrients and water, making this crop a low-input species that minimizes nutrient losses (Anderson et al., 2011; Ferrarini et al., 2017b). Moreover, its strong tolerance to environmental stresses makes *Miscanthus* a suitable crop for marginal land and degraded soils affected by heavy metal contamination, erosion, flooding, drought, salinity, and heavy clay soils (Brami et al., 2020; Islam et al., 2023; Pidlisnyuk et al., 2014; Xu et al., 2021). On such lands, *Miscanthus* enhances soil quality by improving soil structure (Schrama et al., 2016; Winkler et al., 2020), increases soil organic carbon content (Clifton-Brown et al., 2007; Pidlisnyuk et al., 2014; Richter et al., 2015), microbial biomass (Emmerling et al., 2017) and the overall belowground functioning (Chen et al., 2020; Hargreaves and Hofmockel, 2014; Kane et al., 2023). However, several studies highlighted that the effects of *Miscanthus* on soil quality are site-dependent and related to soil properties (Brami et al., 2020; Emmerling et al., 2017; Rowe et al., 2016; Ruf et al., 2018), intensity of contamination (Brami et al., 2020) and previous land use (Brami et al., 2020; Rowe et al., 2016; Ruf et al., 2018). For instance, land use change from grasslands to commercial rhizome-based *Miscanthus x giganteus* negatively impact soil quality, while land use change from intensively managed arable lands is more likely to have a beneficial impact on soil quality (Emmerling et al., 2017; Ruf et al., 2018). This is mainly related to changes in soil organic carbon (SOC) content, as priming of existing soil organic matter (SOM) in response to the input of easily decomposable carbon from this crop occurred in grasslands soils characterized by high level of initial SOC (Richter et al., 2015; Rowe et al., 2016; Ruf et al., 2018; Zatta et al., 2014). However, on the long-term, these authors suggested that establishment of perennial bioenergy crops on degraded and marginal lands could provide long-term improvements to SOC sequestration and soil quality. Most of these studies addressed the potential contribution of *Miscanthus* cultivation to deliver climate and other ecosystem benefits using the standard *Miscanthus x giganteus* genotype (Don et al., 2012). However, there is a significant knowledge gap on the soil carbon storage potential of novel *Miscanthus* hybrids. In particular, the contribution of plant-based C inputs of high-yielding

hybrids to soil C sequestration and overall, to soil quality improvement is unknown. *Miscanthus* hybrids can act as a sink or even a source of atmospheric carbon according to their plant growth habit, rooting pattern, as well as the original SOC content and nutrient availability of the site before *Miscanthus* plantation (Agostini et al., 2015; Richter et al., 2015). Only few recent studies compared the carbon storage capacity of *Miscanthus sinensis* and *Miscanthus sacchariflorus* with that of the commercially grown *Miscanthus × giganteus* (Ouattara et al., 2021; Richter et al., 2015).

Advances in *Miscanthus* domestication are needed to breed novel genotypes to suit a wider range of ecological conditions and increase the efficiency of carbon capture and thus soil C sequestration (Clifton-Brown et al., 2007). Within the BBI demonstration GRACE project (grant agreement ID 745012) a number of novel rhizome- and seed-based *Miscanthus* hybrids were cultivated across European marginal lands to assess their potential to secure feedstock for the bio-based economy (Awty-Carroll et al., 2022; Clifton-Brown et al., 2023; Impollonia et al., 2022a; Impollonia et al., 2022b; Magenau et al., 2022, 2023; Shepherd et al., 2023). In this study, four promising *Miscanthus* hybrids (two novel seed-based hybrids *M. sinensis* × *sinensis* [*M. sin×sin*] and *M. sacchariflorus* × *sinensis* [*M. sac×sin*]; a novel *M. sacchariflorus* × *sinensis* [*M. sac×sin*] rhizome-based clone; and a standard *M. × giganteus* clone [*M×g*]) were chosen to evaluate their short-term potential (4 years after land use change from marginal land) to 1) sequester soil C in topsoil (0-30 cm) and 2) improve chemical, biological and physical soil quality. A set of representative European marginal sites were chosen to identify the effects of land use change both from grasslands and arable lands to *Miscanthus* cultivation. In southerly sites, some hybrids reached mature yield levels at the end of the second growing seasons, while in more northerly sites mature yield levels required 3 to 4 years depending on soil and climatic factors (Awty-Carroll et al., 2022; Magenau et al., 2022). Once successfully established, standard *Miscanthus × giganteus* performed well in all locations, while *M. sin×sin* hybrids were found to be better adapted to Northern Europe, and two novel *M. sac×sin* hybrids were more productive in Southern Europe. In this work it was hypothesized that after 4 years from establishment higher yielding hybrids across diverse pedoclimatic conditions would have the highest contribution to soil C sequestration thanks to their higher belowground plant C input to soil. To evaluate the impact of these novel *Miscanthus* genotypes on soil quality, multiple parameters were assessed including soil physical (bulk density and porosity), chemical (content and stock of SOC, total N, P, and K, plant available P and K, Ca, and Mg), and biological (microbial biomass and enzymatic activity) indicators.

2.2 Materials and Methods

2.2.1 Experimental design and sampling strategies

The field experiment was established in plot scale trials in 2018, at six locations across Europe, arranged along a North-East to South-West transect (Fig. 2.1). The six trial sites are located:

- at Trawsgoed (TWS) near Aberystwyth, UK (52°24'59.8"N, 4°04'02.6"W);
- at Oberer Lindenhof (OLI) near Stuttgart, Germany (48°28'42.1"N, 9°18'41.0"E);
- at Chanteloup (CHV) near Paris, France (48°58'34.9"N, 2°01'57.9"E);
- near Zagreb (ZAG), Croatia (45°85'05.55"N, 16°17'77.7"E);
- near Piacenza at PAC1 (45°00'11.70"N, 9°42'35.4"E) and PAC2 (44°50'40.32"N, 9°35'04.9"E).

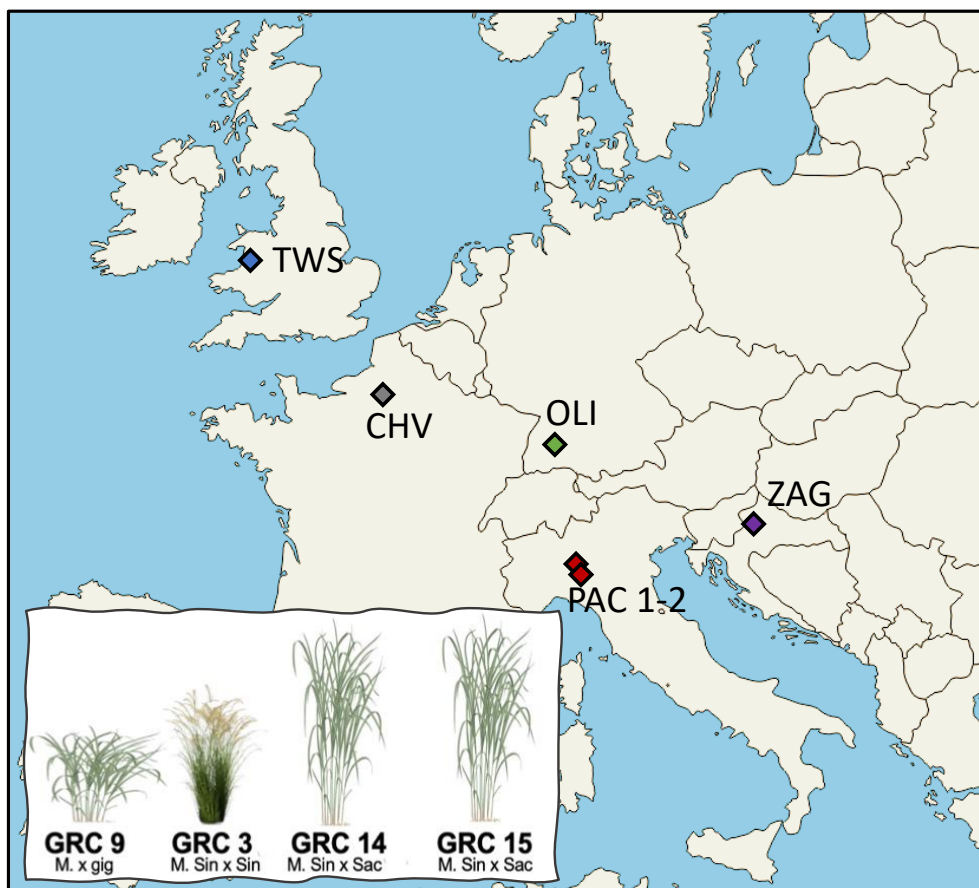


Figure 2.1 Map of the sites over a range of European marginal lands. Sites in order of decreasing latitude: TWS (Aberystwyth, Wales, UK) [Aberystwyth University, IBERS], CHV (near Paris, France), OLI (near Stuttgart, Germany) [University of Hohenheim], ZAG (Zagreb, Croatia) [University of Zagreb], PAC 1 and 2 (Po Valley, Italy) [Università Cattolica del Sacro Cuore]. The panel at the bottom left of the figure show the four high-yielding *Miscanthus* hybrids selected for this study: GRC9, a standard rhizome-based clone of *M. × giganteus*; GRC 3 (*M. sin × sin*) and GRC 14 (*M. sac × sin*), two novel seed-based hybrids; GRC15 (*M. sac × sin*), a novel rhizome-based clone.

Experimental sites had different land uses before *Miscanthus* transplanting: PAC1, OLI, and ZAG were marginal arable lands, while TWS and PAC2 were semi-improved permanent marginal grasslands. CHV was a polluted site by hundred years of irrigation with raw wastewater coming from

Paris and its suburbs on sandy soils occupied by market gardens and orchard. Information on climatic conditions, previous land uses, soil type and marginality factors are thoroughly described in [Awty-Carroll et al. \(2022\)](#) and [Magenau et al. \(2022\)](#). Field trials were planted with 14 *Miscanthus* seed- and rhizome-based hybrids in a randomized complete block design with four replicates. This study analyses the impact of four of these 14 hybrids on soil properties: GRC 3 (*M. sin×sin*) and GRC 14 (*M. sac×sin*), two novel seed-based hybrids; GRC15, a novel rhizome-based clone (*M. sac×sin*); and GRC9, a standard rhizome-based clone of *M.xgiganteus*. Detailed hybrid information is presented in (Awty-Carroll et al., 2022; Magenau et al., 2022), including planting density, biomass yield, growth rate and nutrients offtake. An initial soil sampling was conducted in autumn 2017 (T₀ sampling) before the establishment of field trials, collecting six soil cores at 30 cm depth in each field trial which were then divided into 0-10 and 10-30 cm sections. At each location bulk density (BD) samples were collected and measured for the same depth intervals. In spring 2022, 4 years after establishment (T₄ sampling), soil and bulk density sampling was performed by extracting four replicates of 0-30 cm soil cores for each *Miscanthus* hybrid. Two soil cores were collected in three different plant positions (inter-row, edge of the plant and center of the plant) according to the sampling strategy described by Martani et al. (2020). Soil cores collected were then split into 0-10 and 10-30 cm sections and combined to have one sample per each soil depth layer per plot. Soil samples were taken to the labs, where one subsample was air-dried and sieved at 2 mm for physicochemical analysis and another subsample was stored at -18 °C for microbiological analysis.

2.2.2 Soil quality indicators

2.2.2.1 Soil quality physicochemical indicators

Air-dried soil samples were weighted and analyzed by Dumas's combustion method with elemental analyzer (SoliTOC, Elementar) to determine soil organic carbon (SOC) and total N (TN) concentration (g kg⁻¹) respectively. Total phosphorous (P), potassium (K), calcium (Ca), and magnesium (Mg) were analyzed through microwave digestion method and read with an ICP-OES, while plant available phosphorous (P₂O₅) and plant available potassium (K₂O) were analyzed with the ammonium lactate method (Egnér et al., 1960). Soil BD (g cm⁻³) was calculated dividing dry soil weight by the volume of the known cylinder used, while soil porosity (Por) was calculated from BD (1- BD/2.65). Variations in SOC and soil nutrients concentration (g kg⁻¹) were calculated as the difference between the values measured after 4 years from plantation and the values measured before the beginning of the experiment.

2.2.2.2 Soil quality biological indicators

Soil biological quality was evaluated by measuring soil enzymes activities and microbial biomass. The effect of *Miscanthus* spp. cultivation on soil microbial community activity was determined via measurements of enzyme activity (EA). The assay based on the procedure of Ferrarini et al. (2021) tested 16 hydrolytic enzymes involved in the principal nutrient cycles, namely: β -glucosidase (betaG), β -1,4-glucanase (cell), β -1,4-xylanase (xilo), involved in C cycling; N-acetyl- β -D-glucosaminidase (chit), leucine amino-peptidase (leu), arginin amino-peptidase (argi) and aspecific protease (aprot) involved in N cycling; acid- (acP) and alkaline- (alkP) phosphomonoesterase, phosphodiesterase (bisP), pyrophosphate-phosphodiesterase (piroP), inositol-P phosphatase (inositP) involved in P cycling; arylsulfatase (aryS); butyrate esterase (butir) and fluorescein diacetate hydrolysis (fda) involved in the hydrolysis of ester bonds; and peroxidase (perox) involved in redox reaction of soil organic matter cycle. All measurements were taken in triplicate and the activities were expressed as nanomoles of MUF (or AMC) g^{-1} dry soil h^{-1} . Microbial biomass (MB) was determined using the double-stranded DNA (dsDNA) content as a proxy (Bragato et al., 2016). DNA was extracted as described by (Fornasier et al., 2014). The soil dsDNA content was corrected for soil moisture content and expressed as nanograms dsDNA g^{-1} dry soil.

2.2.3 Soil organic carbon and nutrients stock changes calculation

SOC and soil nutrients stocks (Mg ha^{-1}) were calculated across sites using the equivalent soil mass (ESM) approach proposed by Wendt & Hauser (2013). ESM's (Mg ha^{-1}) were calculated using a cubic spline function to provide estimates that correspond to 0-10 cm and 10-30 cm soil depth across all sites to ensure comparable SOC and nutrients stock estimates responding to changes in bulk density between sites and the two sampling times T_0 (time-zero) and T_4 (year 4). Stocks values were calculated by multiplying the ESM of the soil layer by its C or nutrient concentration. Stocks were calculated also as cumulative soil mass of SOC and nutrients in the 0-30 cm soil layer. In this study, SOC sequestration is expressed both as 1) SOC stock variation ($\Delta\text{SOC stock in Mg ha}^{-1}$), calculated as the difference between SOC stock measured after 4 years from plantation and the SOC stock measured before the beginning of the experiment and 2) yearly carbon sequestration rate (CSR in $\text{Mg ha}^{-1} \text{ y}^{-1}$), calculated dividing the $\Delta\text{SOC stock Mg ha}^{-1}$ by the years of *Miscanthus* cultivation. We used here the term "SOC sequestration" as SOC stock measured change from time zero as discussed in Don et al. (2023), by assuming that SOC stock is under equilibrium for the business-as-usual scenario with no land use change to *Miscanthus*.

2.2.4 Plant C input to soil calculation

Plant C input to soil from above- and belowground biomass components were estimated as described in [Martani et al. \(2023\)](#) for perennial biomass crops with the addition of the formula to estimate C input from rhizomes (Tab. S2.3). Total C input to soil was calculated on a yearly basis as the sum of the C input from the following plant organs: C from stubble (C_s), C from roots (C_r), C from root exudates (C_e), and C from rhizomes (C_{rhiz}). C input from weeds (C_w) was considered negligible and was excluded from the calculation. C_s , C_r and C_e were calculated as the sum of the 4 growing seasons while C_{rhiz} was calculated only for three growing seasons since in the first year of establishment the rhizome biomass (RB) is negligible. In order to calculate belowground C input from C_r and C_e in the 0-10 and 10-30 cm layers, the “beta” root distribution model was applied using a β coefficient value of 0.967 (see Chimento and Amaducci, 2015). In order to calculate C_{rhiz} , RB was estimated using an allometric function derived from literature where RB and cumulative aboveground biomass over the growing seasons (CumAGB) have been measured. A detailed description of RB estimation is reported in the Supplementary Materials. To apply the formula of table S3 the data on annual yields were obtained from Awty-Carroll et al. (2022) and Magenau et al. (2022) for the same plot/hybrid/year/site combination, while information about harvest index (HI) was obtained from Martani et al. (2023) and root to shoot (R:S) ratio values from dedicated roots sampling at PAC1 and OLI sites.

2.2.5 Statistical analysis

Soil variables (BD, Por, SOC, TN, TP, TK, P_2O_5 , K_2O , Ca and Mg concentration and stock) as well as single EA and MB were analyzed using a three-way mixed-model ANOVA for complete randomized block design. Analyses were performed using R: *nmle* (Pinheiro et al., 2012) and *emmeans* (Lenth et al., 2018) packages. *Miscanthus* hybrids (Hybrid), site (Site) and block were considered as random effects to represent the experimental designs of the different trials while Site x Hybrid x sampling time interactions were used as fixed factors. Mixed model was run independently for each soil layer (0-10, 10-30 and 0-30 cm). Annual rate of SOC and nutrients stock changes were analyzed with a two-way mixed-model ANOVA for complete randomized block design. *Miscanthus* hybrids, site and block were considered as random effects while their interaction as fixed effect. Mixed model was run independently for each soil layer (0-10, 10-30 and 0-30 cm). Log transformations or square root transformations were performed to satisfy assumptions of normality and heteroskedasticity when needed. If the transformation did not meet the criteria, ‘function weights’ were used in the linear

mixed model effect formula to consider the non-homogeneous variance structure introduced by the factors studied. The function *emmeans* was used to estimate the marginal means and Tukey HSD post-hoc tests were used to assess significant differences between treatments when the F-tests indicated statistically significant effects. All test results were considered statistically significant at $p \leq 0.05$.

A distance-based Redundancy Analysis (dbRDA) based on Bray-Curtis's distance was used to assess the effects of depth and time on soil enzyme activities and how they are affected by soil properties (SOC, TN, TP and MB). dbRDA was performed separately on each site and run on a three step basis as described in Ferrarini et al. (2021). A similarity percentage (SIMPER), coupled with Wilcoxon signed-rank tests with $n=9999$ permutations, was used to determine the enzyme activities that contributed most to the Bray-Curtis dissimilarity between T_0 and T_4 sampling times.

A backward stepwise multiple linear regression analysis was applied according to Ferrarini et al. (2021) to discriminate and rank the most important soil variables (initial SOC, TN, TP and TK concentration, C:N, C:P, N:P and C input) in explaining the SOC sequestration (Δ SOC stock in Mg ha^{-1}) at two soil depths, 0-10 cm and 10-30 cm, and for two different environments: the Northern environment (NE) that includes TWS, OLI and CHV sites and the Southern environment (SE) that includes ZAG, PAC1 and PAC2 sites. Moreover, a correlation analysis was conducted to identify significant relationships between changes in SOC concentration (Δ SOC g kg^{-1}) and enzymatic activities (Δ EA)

2.3 Results

2.3.1 Effects of *Miscanthus* cultivation on soil quality indicators

Four years after planting *Miscanthus* on the six widely distributed European sites, most of the soil quality indicators were significantly affected ($p < 0.05$) by the land use transition (Tables S2.4 a-b). "Site" effect was significant for all soil physical, chemical, and biological indicators, while the effect of "Time" was significant for 7 out of 10 soil physicochemical indicators and for 3 out of 10 biological indicators. Moreover, no significant "Hybrid" effect was detected for any soil quality indicators, therefore they will not be considered hereafter.

2.3.1.1 Effects of *Miscanthus* cultivation on soil physical and chemical indicators

The BD increased significantly at ZAG and PAC2 sites both in 0-10 cm (+11.7% and +83.8% respectively) and 10-30 cm (+14.4% and +10.7% respectively) layers (Fig. S2.2 a,c) and OLI in the top

layer (+16.4%), while in all other sites its variation was not significant. Estimates of soil porosity changes have an opposite behavior to BD (Fig. S2.2 b,d). Overall, across sites, SOC concentration ($\text{g}\cdot\text{kg}^{-1}$) decreased by -2.9% (-1.4 g kg^{-1}) in the 0-10 cm layer and increased by +6.4% ($+1.5 \text{ g kg}^{-1}$) in the 10-30 cm layer (Fig. S2.3 a,e). In the 0-10 cm layer, SOC content significantly decreased in TWS, by -5 % (-2.6 g kg^{-1}) and PAC2 by -37% (-8.6 g kg^{-1}), while significantly increase in CHV by +13% ($+3.8 \text{ g kg}^{-1}$) (Fig. S2.3 a). In addition, SOC content increased by +33% at TWS in the 10-30 cm layer ($+10.1 \text{ g kg}^{-1}$) (Fig. S2.3 e). Similarly to SOC, concentration of TN (g kg^{-1}) was reduced in the upper layer by -4.9% (-0.3 g kg^{-1}) and enhanced in the lower layer by +19% ($+0.2 \text{ g kg}^{-1}$). In TWS and PAC2, TN content was reduced by -24.3% (-1.2 g kg^{-1}) and -32.5% (-0.7 g kg^{-1}) in the first 0-10 cm respectively, while in the 10-30 cm layer TN increased by +39.2% ($+0.9 \text{ g kg}^{-1}$) and +49% ($+0.4 \text{ g kg}^{-1}$), respectively (Fig. S2.3 b,f). In contrast, at CHV TN content (g kg^{-1}) significantly decreased in both layers by -16% (-0.3 g kg^{-1}) on average (Fig. S2.3 b,f). PAC1 was the only site where TN content significantly increased in both layers, on average by +13.8% ($+0.2 \text{ g kg}^{-1}$). Generally, across sites, total P content increased in the soil profile by +11.6% ($+0.06 \text{ g kg}^{-1}$) with significant increases at CHV (+30.1%; $+0.2 \text{ g kg}^{-1}$), OLI (+8.7%; $+0.09 \text{ g kg}^{-1}$) and PAC1 site (+34.8%; $+0.1 \text{ g kg}^{-1}$). However, TP significantly decreased in ZAG -15.6% (-0.1 g kg^{-1}) and in TWS in 10-30 cm layer by -5% (-0.08 g kg^{-1}) (Fig. S2.4 a,e). Available P significantly increased in all sites by +129% ($+0.2 \text{ g kg}^{-1}$), with the highest increment in PAC1 +418.9% ($+0.5 \text{ g kg}^{-1}$) (Fig. S2.3 c,g). Moreover, a significant depletion of total K concentration was observed with *Miscanthus* cultivation across sites corresponding to -21% (-0.3 g kg^{-1}), which was significant in TWS (-49.3%; -0.7 g kg^{-1}), ZAG (-45.7%; -0.3 g kg^{-1}), PAC1 (-25.3%; -0.3 g kg^{-1}) and PAC2 (-39.9%; -0.3 g kg^{-1}) (Fig. S2.4 b,f). Available K generally increased in the first layer, on average by +31.8% ($+0.1 \text{ g kg}^{-1}$), while it decreased in the 10-30 cm layer by -11.5% (-0.06 g kg^{-1}), except at PAC1 where it had the highest increment in both layers (+71.3%; $+0.2 \text{ g kg}^{-1}$) (Fig. S2.3 d,h). Ca content (g kg^{-1}) was not significantly affected by *Miscanthus* cultivation (Fig. S2.4 c,g), while Mg concentration significantly decreased in the 10-30 cm layer in TWS, PAC1 and PAC2 sites with an average decrease of -5.1% (-0.4 g kg^{-1}) (Fig. S2.4 d,h).

2.3.1.2 Effects of *Miscanthus* cultivation on soil biological indicators

Cultivation of *Miscanthus* positively affected biological soil quality measured as microbial biomass (MB, measured as dsDNA content) and enzymatic activity (EA) (Tab. S2.4b). In TWS, CHV, ZAG and PAC1 sites, the MB significantly increased along the soil profile, showing the highest increment in CHV (+10.9%) followed by TWS (+6.3%), PAC1 (+3.8%) and ZAG (+3.5%) (Fig. S2.5 a,b).

However, MB decreased significantly in OLI (-2.9%) and PAC2 (-15.5%) mainly in the 0-10 cm, while no significant differences were detected in the 10-30 cm layer in PAC2. Considering the previous land uses of these sites, in managed grassland such as TWS and PAC2, the differentiation of EA was more evident in the top layer with separation along axis 1 accounting for 59.7% in TWS ($F:69.3$; $P = 0.001$) and 32.3% in PAC2 ($F:107.9$; $P = 0.001$) (Fig. 2.2 a,b). Differently, land use change from former arable lands such as OLI, ZAG and PAC1 showed a clearer differentiation in the entire soil profile considered, with the separation along axes 1 (F range: 91.2 to 149.8; $P = 0.001$) and accounting for 37.5% to 48.7% of the total variance. dbRDA results from multivariate analysis on C-, N-, P- and S-cycling enzymes indicated that the soil parameters that had the highest influence on EA distribution across sites were mostly MB and SOC (Fig. 2.2). MB and SOC were in turn positively correlated in all sites as also highlighted by the correlation analysis between SOC and EA changes (Tab. S2.5). A south-north environmental gradient controlling EA activity was observed (Fig. 2.2). EA was found to be mainly governed by MB and SOC in southern environments, while availability of total nutrients (TN and TP) significantly controlled EA in northern environment such as OLI and TWS. On average, the activities of C-acquiring, esterase, and P-acquiring enzymes under *Miscanthus* cultivation were significantly increased in most of the sites (Fig. 2.3). However, PAC2 showed a reduction for all EA in the 0-10 cm and for N-acquiring enzymes in the 10-30 cm layer. In addition, in TWS most of the EA decreased in the 10-30 cm layer, while in OLI N-acquiring enzymes were reduced in the top layer (Tab. S2.6). SIMPER analysis showed that esterase activity (measured as *butir*) was the enzyme activity that increased significantly in both soil layers and contributed the most to overall EA dissimilarities between T_0 and T_4 sampling time (52.5% in 0-10 and 55% in 10-30 cm layer). *AlkP* was significantly increased and contributed to the overall dissimilarities in TWS, CHV, OLI and PAC1 sites in the top layer as well as in the lower layer in CHV, OLI, ZAG and PAC1 sites (Fig. 2.3). At PAC2, EA were significantly decreased after 4 years of cultivation, in particular in the 0-10 cm layer were *alkP* and 3 N-cycling enzymes (*aprot*, *leu* and *argi*) contributed to 80 % of the overall dissimilarity. Furthermore, a significant decrease of N-acquiring enzymes activity like *aprot* in the top layer was observed in CHV, OLI, PAC1, PAC2 and in the lower layer of ZAG and PAC2 sites (Fig. 2.3).

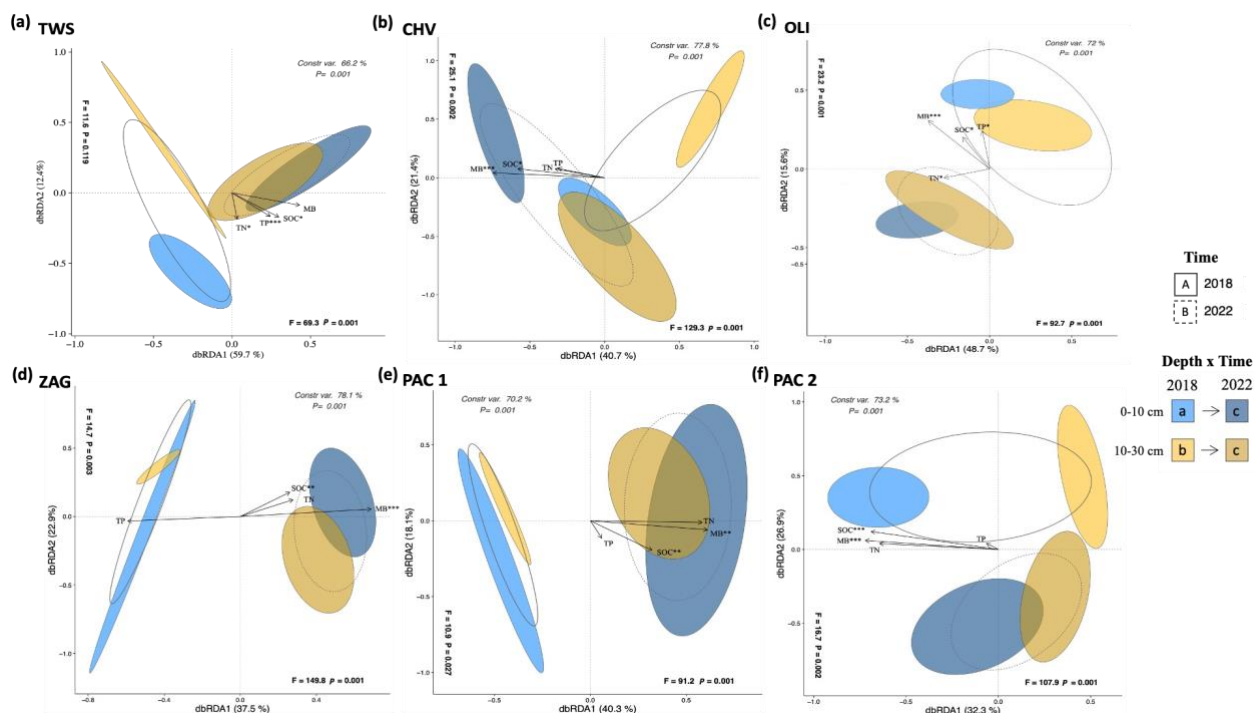


Figure 2.2 dbRDA plots showing shifts in C-, N-, P- and S-cycling enzyme activities (MUF or AMC gdrysoil⁻¹ h⁻¹) among marginal sites ([a] TWS, [b] CHV, [c] OLI, [d] ZAG, [e] PAC1, [f] PAC2) in topsoil layers (0–10 cm and 10–30 cm) from T0 to T4. The interaction of the factors “Depth” and “Time” is positioned as multivariate centroids surrounded by 95% confidence interval ellipsoids. Soil variables (SOC, TN, TP and MB), represented as arrows that significantly contributed to EA differentiation along axis are indicated with: *, **, *** for $p < 0.05$, $p < 0.01$, $p < 0.001$ respectively. dbRDA, distance-based Redundancy Analysis; EA, enzyme activity; MB, microbial biomass; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus

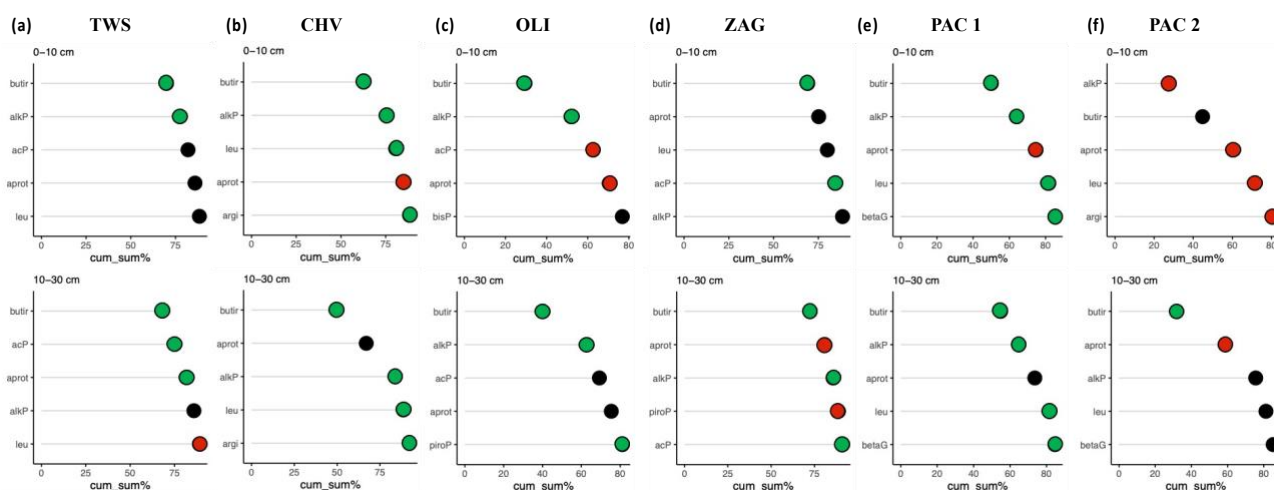


Figure 2.3 SIMPER analysis showing enzyme activities that contributed most (>80%) to the Bray–Curtis dissimilarity between T0 and T4 sampling times, in 0–10 cm (figures above) and 10–30 cm (figures below) soil layers, among marginal sites ([a] TWS, [b] CHV, [c] OLI, [d] ZAG, [e] PAC1, [f] PAC2). Different colors indicate a significant positive (green) or negative (red) contribution to the Bray–Curtis's dissimilarity.

2.3.1.3 Soil nutrients stock variation after 4 years of *Miscanthus* cultivation

After 4 years of *Miscanthus* cultivation, total K, Ca and Mg stocks were significantly reduced compared to time zero sampling in the 0–30 cm layer (Fig. 2.4f–h, Tab S2.7b). Total K stock showed the highest cumulative reduction ($-0.2 \text{ Mg ha}^{-1} \text{ y}^{-1}$) across sites, which was significant in TWS (-0.4

Mg ha⁻¹y⁻¹), ZAG, PAC1 and PAC2 (-0.3 Mg ha⁻¹y⁻¹ in all 3 sites). Ca stock overall decreased on average by -0.06 Mg ha⁻¹y⁻¹ among sites, but significantly only at ZAG (-0.6 Mg ha⁻¹y⁻¹) and PAC1 (-1.8 Mg ha⁻¹y⁻¹). Moreover, Mg stock was lowered by -0.1 Mg ha⁻¹y⁻¹, but significantly only in the 10-30 cm layers (Fig. S2.7, Tab. S2.7a). The variation of Mg stock was significant in the lower layer at TWS (-0.2 Mg ha⁻¹y⁻¹), PAC1 (-0.2 Mg ha⁻¹y⁻¹) and PAC2 (-0.4 Mg ha⁻¹y⁻¹). On the contrary, available P, available K, TN, and TP stocks cumulative increased over time in the 0-30 cm layer, respectively by +0.16 Mg P₂O₅ ha⁻¹y⁻¹, +0.01 Mg K₂O ha⁻¹y⁻¹, +0.03 Mg TN ha⁻¹y⁻¹ and +0.04 Mg TP ha⁻¹y⁻¹ (Fig. 2.4b-e, Tab. S2.7b). The highest increment was found for available P, which resulted significantly enhanced in all sites and substantially at CHV and PAC1 (both +0.4 Mg ha⁻¹y⁻¹). Increased stocks of available K were found mainly in the upper layer (+0.03 Mg ha⁻¹y⁻¹) and decreased in the lower layer (-0.02 Mg ha⁻¹y⁻¹) (Fig. S2.6, Tab. S2.7a). Moreover, significant variations in TN and TP stocks were different across sites. TN was enhanced only in the 0-30 cm at PAC1 (+ 0.2 Mg ha⁻¹y⁻¹), while decreased at CHV (-0.2 Mg ha⁻¹y⁻¹). In addition, at TWS and PAC2, TN stock was reduced in the upper layer (-0.2 Mg ha⁻¹y⁻¹), while it was increased in the lower layer (+0.3 Mg ha⁻¹y⁻¹) (Fig. S2.6, Tab. S2.7a). Similarly, for TP stock a significant increase in 0-30 cm was found at CHV (+0.2 Mg ha⁻¹y⁻¹), OLI (+0.06 Mg ha⁻¹y⁻¹) and PAC1(+0.2 Mg ha⁻¹y⁻¹), while it was reduced at ZAG (-0.1 Mg ha⁻¹y⁻¹) (Fig. S2.7, Tab. S2.7a).

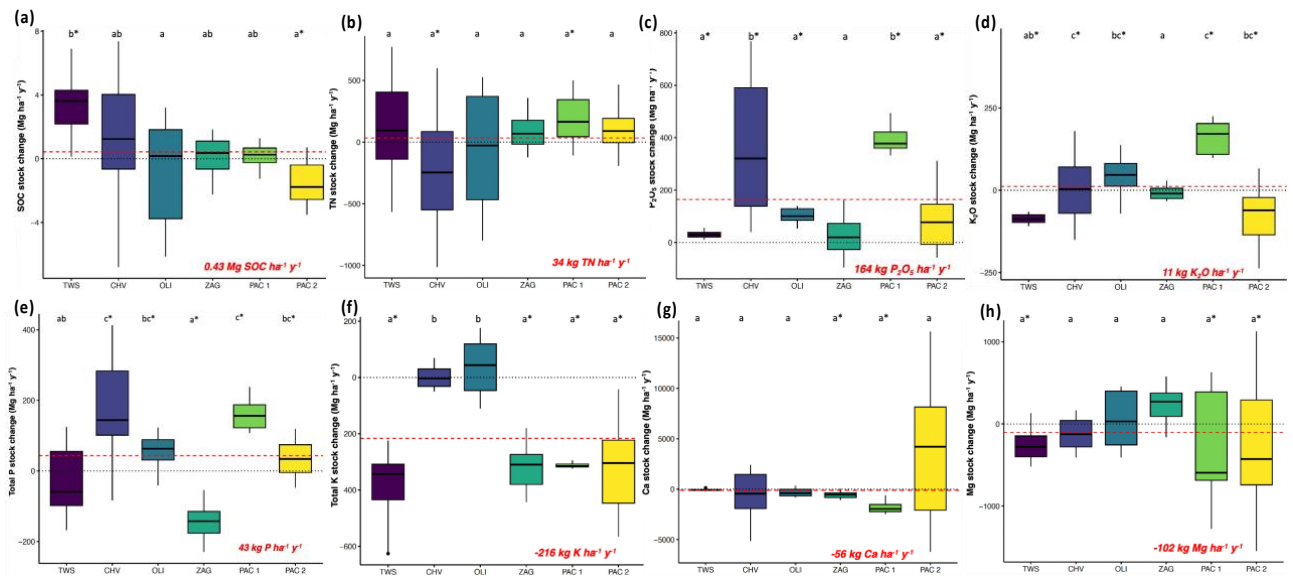


Figure 2.4 Rate of ([a] SOC stock [Δ stock Mg ha⁻¹ year⁻¹] and soil nutrients, [b] total N, [c] available P, [d] available K, [e] total P, [f] total K, [g] Ca, [h] Mg) stock variation (Δ stock Mg ha⁻¹ year⁻¹) in the topsoil layer [0–30 cm], in 4 years of *Miscanthus* cultivation among marginal sites. Different letters denote statistically different means among marginal sites (Tukey's test, p: 0.05), while (*) show statistically different variations from T0 to T4 (Tukey's test, p: 0.05) within the same site. SOC, soil organic carbon.

2.3.2 Effects of *Miscanthus* cultivation on SOC stock and drivers of soil C sequestration

After 4 years of *Miscanthus* cultivation soil organic carbon stocks (Mg SOC ha⁻¹) showed significant variations on former grasslands (TWS, CHV and PAC2) with opposite behaviors in the upper and lower layers (Fig. 2.4a-S2.6, Tab. S2.7a-b). In the upper layer of TWS and PAC2, SOC stock was significantly reduced by -0.5 Mg ha⁻¹y⁻¹ and -2.1 Mg ha⁻¹y⁻¹ respectively, while in CHV SOC increased by +1.0 Mg ha⁻¹y⁻¹ (Fig. S2.6). On the contrary, SOC stock significantly increased in the 10-30 cm layer by +3.7 Mg ha⁻¹y⁻¹ and +0.6 Mg ha⁻¹y⁻¹ respectively at TWS and PAC2, while no significant differences were detected at CHV (Fig. S2.6). In other marginal sites, that were former arable lands, SOC stock after *Miscanthus* establishment did not show significant changes (Fig. 2.4a-S2.6). Nevertheless, it was estimated that *Miscanthus* cultivation had an average plant C input to soil of 3.9 Mg C ha⁻¹ in four years across sites (Tab. 2.1). Estimated plant C input values (as a sum of above and belowground inputs) varied greatly across sites and within genotypes, showing the greatest values at PAC1 (5.3 Mg C ha⁻¹). Generally, the standard clone *M*×*g* (GRC9) showed the lowest average plant C input over four growing seasons (2.6 Mg C ha⁻¹), with the lowest value at CHV (0.3 Mg C ha⁻¹) and the highest at OLI (4.0 Mg C ha⁻¹). *M. sac*×*sin* hybrids, such as GRC14 and GRC15 showed the highest plant C input in southern environment (SE) like ZAG (5.1 Mg C ha⁻¹ GRC 15), PAC1 (7.5 Mg C ha⁻¹ GRC 14) and PAC2 (6.1 Mg C ha⁻¹ GRC 14). In contrast, the *M. sin*×*sin* hybrid (GRC3) showed higher C input values in northern environment (NE) such as CHV (4.5 Mg C ha⁻¹y⁻¹), OLI (6.5 Mg C ha⁻¹y⁻¹) and TWS (5.3 Mg C ha⁻¹y⁻¹). Overall, the contribution to the estimated plant C input to soil from *Miscanthus* derives mainly from roots (47%), followed by roots exudates (21%) and rhizomes (20%) (Tab. S2.8). Despite considerable estimated values of plant C added to the soil by *Miscanthus* hybrids across sites in four years, other factors were identified as drivers of soil C sequestration (measured as ΔSOC stock changes in Mg SOC ha⁻¹ after 4 years of *Miscanthus* cultivation compared to initial SOC storage). The results of the multiple linear stepwise regression showed overall good R² values of the models for the northern and southern European environments, except in 10-30 cm in south Europe (Tab. 2.2). In northern environment R² were 35.4% in 0-10 cm and 64.1% in 10-30 cm layer, while in southern environment R² were 92.8% in 0-10 cm and 13.9% in 10-30 cm depth (Tab. 2.2). In both environments, in the top layer the initial concentration of soil carbon was identified as the main driving factor of soil C sequestration, showing a relative importance (% to R²) of 28% in northern Europe and 31% in southern Europe and a significant ($p < 0.001$) negative correlation. Moreover, initial values of TN and C:P ratio (SOC:TP stoichiometric ratio of concentration values) had a positive correlation with ΔSOC stock in both

environments. In addition, initial TP values had a positive correlation in northern environment (27% to R^2) and N:P a negative correlation in southern environment (19% to R^2). In the lower layer of northern Europe, TK had the highest relative contribution to the model (38%) followed by C:N (19%), TP (18%), C (15%) and N:P (10%). Moreover, considering the correlation between variations of SOC concentration ($\Delta\text{SOC g kg}^{-1}$) and changes in EA (ΔEA), it is worth noting that a significant positive correlation ($p < 0.0001$) was found with a redox enzyme (Δperox) (Tab. S2.5).

Table 2.1 Estimated total C input to soil (Mg ha^{-1}) as the sum of C inputs from the following *Miscanthus* organs: C from stubble (Cs), C in roots (Cr), C in root exudates (Ce), and C in rhizomes (Crhiz).

Note: Lowercase letters denote significant differences ($p > 0.05$, Tukey test) among hybrids in a particular site, while uppercase letters denote significant differences ($p > 0.05$, Tukey test) among sites and hybrids.

	GRC 14	GRC 15	GRC 3	GRC 9	
TWS	2.67 ^a	3.58 ^a	5.33 ^b	2.92 ^a	3.62 ^{AB}
CHV	3.25 ^{bc}	2.49 ^b	4.46 ^c	0.30 ^a	2.63 ^A
OLI	3.10 ^a	4.59 ^b	6.55 ^c	4.07 ^{ab}	4.58 ^{BC}
ZAG	3.89 ^{ab}	5.07 ^b	3.41 ^a	3.10 ^a	3.86 ^B
PAC 1	7.48 ^c	5.31 ^b	4.90 ^{ab}	3.47 ^a	5.29 ^C
PAC 2	6.05 ^c	3.78 ^b	2.59 ^{ab}	1.93 ^a	3.59 ^{AB}
	4.41 ^A	4.14 ^A	4.54 ^A	2.63 ^B	3.93

Table 2.2 Summary statistics of the multiple regression models obtained for SOC sequestration (measured as Δ SOC stock changes in Mg C ha after 4 years of cultivation TABLE 2 compared to initial SOC storage) for Northern (TWS, OLI, CHV) and Southern (ZAG, PAC1, PAC2) environments.

Note: Initial values (as g kg) of carbon and nutrients at sampling were considered as predictors.

Abbreviations: SE, standard error; SOC, soil organic carbon.

*Metrics are normalized to sum to 100% of adjusted R^2 . Superscript lowercase letter denotes significant differences among predictors (^a $p < 0.001$, ^b $p < 0.01$ Bonferroni test) as assessed by bootstrap ($n=1000$) measures of relative importance.

Type of Environment	Depth (cm)	Variable	Prop. of variance %	Residual SE	Adjusted R^2	F-Statistic	p-Value	Significant Predictors (% relative importance) *
Northern	0-10	Δ SOC stock (Mg C ha ⁻¹)	35.42	6.075	0.2773	4.607	0.0019	C content (28%) ^a Total P content (27%) ^b Total N content (18%) ^b C:P ratio (14%) ^b C input (13%)
	10-30		64.10	3.627	0.5937	13.57	1.294e-07	K content (38%) ^a C:N ratio (19%) ^b Total P content (18%) ^b C content (15%) ^a N:P ratio (10%) ^a
Southern	0-10		92.84	1.517	0.9199	109	2.2e-16	C content (32%) ^a C:P ratio (24%) ^a N:P ratio (19%) ^a Total N content (19%) ^a K content (6%) ^b
	10-30		13.94	1.58	0.0369	1.361	0.2585	C:N ratio (30%) ^b C content (26%) ^b Total N content (22%) ^b Total P content (11%) N:P ratio (11%)

2.4 Discussions

2.4.1 Effects of *Miscanthus* on soil quality indicators

The effect of land-use transition to *Miscanthus* on soil physical parameters was site-specific. Overall, BD increased in the upper layer mainly in those sites characterized by heavy textured soils such as OLI, PAC2 (clay loam texture) and ZAG (silty loam texture) (Awty-Carroll et al., 2022). Likewise, in these sites soil porosity was reduced, and this might negatively impact soil structure, gas exchange and water movement (Winkler et al., 2020). The increase in BD during *Miscanthus* cultivation is a consequence of the no tillage regime of this crop (Guzman et al., 2019) and also of

the use of heavy machinery during harvesting operations under wet soil conditions (Hargreaves et al., 2019; Zatta et al., 2014). However, TWS, CHV (sandy-loam soils) and PAC1 (clay loam soil) did not show any significant variation of soil physical properties. These results confirm that the impact of the no tillage perennial crop regime on soil physical quality is site-specific. Indeed, other studies revealed either a reduction (Das et al., 2016; Kahle et al., 1999; Lemus and Lal, 2005; Martani et al., 2020), or an increase (Brami et al., 2020; Guzman et al., 2019) of BD after planting *Miscanthus*, making difficult to establish which is the key factor determining the impact on physical soil quality.

In this study, after 4 years of cultivation, variations of SOC content in marginal lands were significantly influenced by previous land uses as confirmed by several studies (Brami et al., 2020; Dondini et al., 2009; Emmerling et al., 2017; Rowe et al., 2016; Ruf et al., 2018; Zatta et al., 2014; Zimmermann et al., 2013). SOC content significantly decreased in former grasslands, which were characterized by higher contents of SOC, while in former arable lands SOC concentration did not vary significantly. Several studies have reported that variations in SOC content can be site-dependent and related to mean annual temperature (Emmerling et al., 2017; Poeplau and Don, 2014), or soil texture (Brami et al., 2020; Emmerling et al., 2017; Kahle et al., 2001; Ruf et al., 2018). In this study, under marginal land conditions, the trend of SOC sequestration during *Miscanthus* cultivation was affected by initial SOC content as identified in other non-marginal lands converted to *Miscanthus* (Emmerling et al., 2017; Rowe et al., 2016; Ruf et al., 2018; Zatta et al., 2014; Zimmermann et al., 2013). Generally, ploughing operations conducted before planting *Miscanthus* on grassland, along with the subsequent disruption of soil aggregates, is considered a contributing factor to the significant SOC loss in C-rich soils (Poeplau and Don, 2014; Zimmermann et al., 2013) which can be accelerated by the input of easily decomposable organic substrate, a phenomenon ascribed to the priming effect (Dondini et al., 2009; Kuzyakov, 2002; Richter et al., 2015; Zatta et al., 2014). Variation of SOC content after land use changes to *Miscanthus* was also identified as the main driver of changes of physical and biological soil quality indicators. For instance, aggregate stability (Brami et al., 2020; Emmerling et al., 2017; Ruf et al., 2018; Winkler et al., 2020), microbial biomass and microbial activity (Brami et al., 2020; Emmerling et al., 2017; Ruf et al., 2018), were higher after conversion from arable lands to perennial crops, but lower when converted from grasslands. This is confirmed also in this study, where SOC concentration was significantly correlated to MB ($p < 0.001$) in most of the sites as well as to EA variations in all sites (Fig. 2.2). The high quantity of estimated plant C input to soil, especially the more easily decomposable roots and root exudates (on average $0.67 \text{ Mg C ha}^{-1} \text{ y}^{-1}$), may have stimulated an increase of the microbial turnover, which resulted in a

significant increment of the microbial biomass in most of the sites in four years of *Miscanthus* cultivation. However, at PAC2 site, land use change from a 15-year-old meadow to *Miscanthus* significantly depleted SOC and MB agreeing with other reports (Brami et al., 2020; Ruf et al., 2018). This study also highlighted the importance of plant C input in activating the overall belowground functioning. The physiological shift of the microbial community was mainly governed by the stimulation of the activity of esterase (*butir*), involved in the hydrolysis of ester bonds, and alkaline phosphomonoesterase (*alkP*), involved in organic P mineralization to inorganic P available for plant uptake. The increase in esterase (*butir*) activity could reflect an increment of the organic matter decomposition rate, which was probably related to the large amounts of unprotected fresh root litter from fine roots turnover and production of root exudates (Wittmann et al., 2004). The availability of easily utilizable C led also to a reduction of extracellular protease (“*aprot*”) production due to catabolic repression (Vranova et al., 2013). Indeed, fresh root turnover enriches soil with free amino acids, inhibiting microbial proteolytic activity (Vranova et al., 2013). Production of P acquiring enzymes was instead indicative of a microbial community with high P demands. Indeed, to gain access to P, microbes synthesize extracellular enzymes that decompose organic matter and release plant available P. Moreover, the high correlation between MB and the enzymatic activity ($p < 0.001$ in dbRDA) was reflected in the reduction of the microbial community physiological capacity in PAC2, where the activity of all enzymes analyzed decreased in the top layers as well as the enzymes associated with N-cycling in the 10-30 cm layer.

In this short-to medium field experiment, where no fertilization was applied at transplanting nor during the growing seasons, *Miscanthus* cultivation led to a significant depletion of some soil macronutrients, while others were significantly enhanced. Among all sites, total K was the plant macronutrient that showed the greatest depletion ($-860 \text{ kg K ha}^{-1}$), followed by Mg (-410 kg ha^{-1}) and Ca ($-240 \text{ kg Ca ha}^{-1}$) (Fig. 2.4f-h). On the contrary, topsoil under *Miscanthus* were on average enriched of available P, TP, TN, and available K respectively by $+640 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$, $+160 \text{ kg P ha}^{-1}$, $+120 \text{ kg N ha}^{-1}$, and $+40 \text{ kg K}_2\text{O ha}^{-1}$ (Fig. 2.4b-e). However, soil nutrient stocks varied greatly among sites depending on initial soil nutrient content and crop yield. Indeed, the extent of the nutrient recycling under *Miscanthus* cultivation depended on soil nutrient content available before conversion to *Miscanthus* plantation and by nutrient offtake during the growing seasons (Magenau et al., 2022). If *Miscanthus* is harvested in the spring time when the plants are most senescence only a small proportion of nutrients is removed by harvesting because 60 to 80% is remobilized in rhizomes and recycled in the system through fallen leaves and the turnover of rhizomes and roots

(Amougou et al., 2011; Beuch et al., 2000; Christian et al., 2006; Magenau et al., 2022; Ruf et al., 2017; Strullu et al., 2011). For nutrient budget calculations to determine fertilizer requirements, the seasonal dynamics of nutrient contents in the above ground biomass and the harvest time must be taken into account (Cadoux et al., 2012). In this study total K stock is highly depleted after 4 growing seasons at all sites, as K is the primary nutrient absorbed by *Miscanthus* (Cadoux et al., 2012; Magenau et al., 2022; Pidlisnyuk et al., 2014). Using plant nutrient concentration measured by Magenau et al. (2022) and plant yield measured by Awty-Carroll et al. (2022) in the same sites and for the same genotypes, on average K offtake was 96 kg ha⁻¹, 89 kg ha⁻¹, and 226 kg ha⁻¹ respectively for *Mxg*, *M. sinxsin* and *M. sacxsin* hybrids after 4 years of *Miscanthus* cultivation. Overall, the highest nutrient offtakes were found for the higher-yielding *Miscanthus* hybrids, GRC14 (304.8 kg ha⁻¹) followed by GRC15 (146.3 kg ha⁻¹) (Magenau et al., 2022). During four growing seasons, the highest nutrients requirement of the *M. sacxsin* hybrids resulted also in an offtake of N (120 kg N ha⁻¹) and P (29 kg P ha⁻¹) compared to *Mxg* (respectively 56 kg N ha⁻¹ and 13 kg P ha⁻¹) and *M. sinxsin* (respectively 80 kg N ha⁻¹ and 17 kg P ha⁻¹). We also observed that soils with high initial total K content, such as those previously occupied with grasslands (TWS and PAC2), led to luxury uptake compared to poorer soils (CHV and OLI). On these sites, it was indeed observed the most significant depletion of TK stock compared to other sites with an average depletion of -1.4 Mg K ha⁻¹, while in CHV and OLI no significant variations were detected. According to Cadoux et al. (2012), nutrients removed at winter harvest are greatest, by proportion, for K (38-57%) and approximately equal for N and P (36-40%). These values confirm the findings on soil nutrient depletions and on nutrients offtake and clearly show that most of the nutrients are translocated during senescence in belowground biomass. In addition, nutrient remobilization from rhizome to aboveground plant biomass were lowest for K (7-14%), N (9-21%) and P (18-36%), while the proportion of nutrients that were recycled either by translocation or leaf fall were lower for K (50%) and higher for N and P (60%). This indicates that, in the long term, *Miscanthus* hybrids have a higher nutrient requirement for K followed by N and P, and that K might become a limiting factor for growth on marginal site where no fertilization at planting is applied. In this study, plant available K was yearly accumulated in the topsoil (+30 kg ha⁻¹y⁻¹) as a result of the nutrient mineralization from plant litter deposited on soil surface after harvesting (Fig. S2.6) (Cadoux et al., 2012; Kahle et al., 2001). However, the simultaneous reduction in the 10-30 cm layer (-20 kg ha⁻¹y⁻¹) indicates that this mechanism of nutrient recycling did not occur in this layer, where the amount of nutrients absorbed by the plant is greater than that derived from the decay of litter and roots (Fig. S2.6). The increment of available

P (+160 kg P₂O₅ ha⁻¹y⁻¹) among all sites indicate that *Miscanthus* has the potential to sustain P recycling by increasing the amount of plant available phosphorous in upper soils layers. This was suggested also by [Ferrarini et al. \(2021\)](#) who found an increase of available P (+ 240 kg P ha⁻¹y⁻¹) under *Miscanthus* cultivation of which averaged 30 % from organic sources. Ferrarini et al. (2021) and Stutter et al. (2015) proposed that under unfertilized perennial crops, with high C-input, the increase of available P in soil is dominated by the increase of organic P forms deriving from the biological turnover of plant litter, roots and rhizome. This highest organic P input coupled with low P uptake by *Miscanthus* (Cadoux et al., 2012; Magenau et al., 2022), results in an accumulation of organic P forms that can be made bioavailable by microbial activity. This is confirmed also by the increased *alkP* activity, involved in the mineralization of organic-P, which was stimulated by plant C input from decomposing litter (Fig. 2.3a-e, Tab. S2.5-2.6) (Renella et al., 2006). Despite the increment of total P (+43 kg P ha⁻¹y⁻¹) in 4 out of 6 sites remain unclear, it might be explained by external factors such as events of Saharan dust atmospheric deposition which occurred more frequently as winter storm (Gammoudi et al., 2024). The study's results showed that SOC variations were closely associated with changes in TN stock, highlighting that soil C:N ratios remain stable during *Miscanthus* cultivation (Kahle et al., 2001; Richter et al., 2015; Ruf et al., 2018). In previously managed grasslands, with land use change to *Miscanthus*, both SOC and TN were significantly reduced in the topsoil layer (Fig. S2.6). Previous studies suggested that the conversion from grasslands with C and nutrients rich soils to perennials, can accelerate the degradation of existing SOC through the priming effects (Richter et al., 2015; Zatta et al., 2014). With this accelerated soil organic matter degradation, also TN is reduced thorough SOM mineralization, by the soil microbial community, to bring nutrient and C supply close to microbial element demand (Bastida et al., 2019; Kirkby et al., 2014, 2013). However, the increase of TN stock in PAC1 and PAC2 sites (on average + 150 kg ha⁻¹ in 0-30 cm), where SOC stock was reduced, can be explained by N₂ fixation under *Miscanthus* cultivation. The hypothesis of N₂ fixation has only recently been confirmed to occur in *Miscanthus* (Cadoux et al., 2012; Martani et al., 2020) where the presence of potentially N₂-fixing bacteria was detected (Chen et al., 2020; Davis et al., 2010; Pidlisnyuk et al., 2014). Regarding stock variation of other plant secondary macronutrients such as Ca and Mg, in 4 years of *Miscanthus* cultivation, Ca offtake was higher than Mg as found also by (Dzeletovic and Glamoclija, 2015). Ca offtake estimated values for *M. sacxsin* hybrids (1.30 Mg Ca ha⁻¹) followed by *M. sinxsin* (0.87 Mg Ca ha⁻¹) and *Mxg* (respectively 0.44 Mg Ca ha⁻¹) confirmed that across locations, that soil nutrient depletion is affected by yield x initial level interaction. The greatest Ca depletion that was found at

PAC1 site was the consequence of the highest biomass production ($2.4 \text{ Mg Ca ha}^{-1}$), and the highest availability of this nutrients in soil among sites. In contrast, Mg offtake was similar across *Miscanthus* hybrids with an average value of 13 kg ha^{-1} . These Ca and Mg offtake values, their ratio and their correlation with soil stock variations confirmed the values found for *Miscanthus* by (Dzeletovic and Glamoclija, 2015; Pisani et al., 2024) and (Hillel and Hatfield, 2005).

2.4.2 Soil Organic Carbon sequestration and its drivers

Our findings show that after 4 years of marginal land conversion to *Miscanthus* cultivation, SOC was sequestered with an annual rate of $+0.4 \text{ Mg C ha}^{-1}\text{y}^{-1}$, but this rate was highly influenced by previous land uses. In former arable lands, SOC did not vary significantly ($-0.2 \text{ Mg C ha}^{-1}\text{y}^{-1}$), while a significant SOC sequestration was found in former grasslands ($+1.0 \text{ Mg C ha}^{-1}\text{y}^{-1}$), but with different behaviors in the upper and the lower layer. *Miscanthus* establishment in two managed old grasslands, led to a significant reduction of SOC stock in the 0-10 cm layer ($-1.3 \text{ Mg C ha}^{-1}\text{y}^{-1}$) and enhanced in the 10-30 cm by $+2.2 \text{ Mg ha}^{-1}\text{y}^{-1}$. These changes are presumably due to redistribution of the soil in the profile by the inversion tillage occurred before *Miscanthus* plantation, where the decomposition of plant (grass) biomass has boosted SOM formation in 10-30 cm layer. Several studies throughout Europe have reported that the potential of soil C sequestration after land conversion to *Miscanthus* cultivation in marginal sites is still uncertain, showing rates of SOC loss/sequestration between -2.9 and $+4.5 \text{ Mg C ha}^{-1} \text{ year}^{-1}$ (Agostini et al., 2015; Dondini et al., 2009; Ouattara et al., 2021; Poeplau and Don, 2014; Richter et al., 2015; Rowe et al., 2016; Zimmermann et al., 2013). These contrasting results have been ascribed to site-specific factors like mean annual temperature (Emmerling et al., 2017; Poeplau and Don, 2014), soil texture (Brami et al., 2020; Emmerling et al., 2017; Kahle et al., 2001; Ruf et al., 2018), and on previous land uses (Emmerling et al., 2017; Poeplau and Don, 2014; Rowe et al., 2016; Ruf et al., 2018; Zatta et al., 2014; Zimmermann et al., 2013). Don et al. (2012) performed a meta-analysis of published literature and calculated an average SOC accumulation of $+0.66 \text{ Mg C ha}^{-1}\text{y}^{-1}$ for *Miscanthus* cultivated on former croplands, while there was no or even negative SOC stock change ($-0.09 \text{ Mg C ha}^{-1}\text{y}^{-1}$) if grasslands were converted to *Miscanthus*. Moreover, Wu et al. (2024) identified crop age as the most important driving factors of the SOC sequestration rate, which was estimated reaching a peak in 9 years.

Most of the experiments on land use change to *Miscanthus* compared SOC stock value after a certain number of years of cultivation with those of time-zero before the land use transition.

However, it is noteworthy that our study is the first one comparing SOC stock changes after *Miscanthus* establishment among different European sites using the equivalent soil mass (ESM) of Wendt & Hauser (2013) approach to calculate SOC stock variation. This approach provides a more accurate estimate of SOC stock variations by reducing errors deriving from a fixed depth sampling on sites showing changes on BD between sampling time points. Indeed, the reduction or the increase of BD over time can respectively results in an underestimation or overestimation of SOC stock changes (Fowler et al., 2023). The adoption of this ESM approach could hence explain the non-significant changes of SOC stock after croplands conversion to *Miscanthus* cultivation differently to other studies where SOC stock is calculated from fixed depth sampling (Lee et al., 2009).

To better understand the mechanisms of SOC sequestration under perennial crops, plant C input to soil from different plant organs need to be calculated (Agostini et al., 2015). In *Miscanthus*, a great quantity of photosynthesized C is allocated belowground in rhizomes, roots and roots exudates (Briones et al., 2023; Martani et al., 2020). Martani et al. (2020) reported that during 11 years of cultivation, *Miscanthus* (*M. x giganteus*) allocated in the belowground biomass 5.7 Mg C ha⁻¹ of which 38% was allocated in rhizomes and 28% in fine roots. A recent study (Briones et al., 2023), found that *Miscanthus* hybrids with contrasting phenotypic and physiological traits divide different quantities of photo assimilated C in plant organs that ranged between 16% to 22% in rhizomes and from 6% to 8% in roots. However, no simple allometric equations are available in literature to estimate C input from *Miscanthus* belowground organs to replace the time-consuming work of in-field below ground sampling. In this study, a novel approach was proposed to estimate C input to soil from different *Miscanthus* organs (stubble, roots, root exudates and rhizomes). An allometric function to estimate RB from cumulative plant yields over the growing seasons was derived using published data. The average estimate of *Miscanthus* carbon input to the topsoil across all sites over four years was 3.93 Mg C ha⁻¹ (0.98 Mg C ha⁻¹ y⁻¹). We identified significant differences in plant C input among hybrids as affected by the yield data (Tab. 2.1). In Southern Europe, novel *M. sacxsin* hybrids (GRC14 and GRC15) were the most productive, resulting in a plant C input to soil of 1.32 Mg C ha⁻¹ y⁻¹, while in Northern Europe, the *M. sinxsin* hybrid (GRC3) had the best yield performances and the highest C input to soil (1.36 Mg C ha⁻¹ y⁻¹). Generally, the standard clone *Mxg* (GRC9) had the lowest plant C input to soil (0.66 Mg C ha⁻¹ y⁻¹). However, despite significant differences in plant C input among hybrids, no significant differences on SOC sequestration were observed among hybrids (Tab. S2.3, 2.7 a-b). In other studies that investigated the SOC sequestration potential of *Miscanthus* hybrids over time (e.g. after 5 (Ouattara et al., 2021), 6 (Zatta

et al., 2014) and 14 years (Richter et al., 2015) after planting), no significant contributions of *Miscanthus* genotypes to SOC sequestration were found, despite differences in above- and belowground biomass allocation were observed. Results of the multiple linear stepwise regression showed that plant C input to soil was not a significant factor explaining SOC stock changes (Δ SOC stock as Mg C ha^{-1}) (Tab. 2.2), but that initial SOC content was the main driving factor of SOC stock change in all environments, especially in the 0-10 cm soil layer. The negative correlation between changes of SOC stock and the initial concentration of SOC suggested the occurrence of negative priming effect of the existing SOM in response to the input of easily decomposable organic substrates from roots and root exudates, which in our study accounted for 47% and 21% of total C input respectively. This explains the significant depletion of SOC in the topsoil layer in grassland converted to *Miscanthus* cultivation where SOC levels were higher compared to the other sites within the same climatic region. In sites where no significant changes in SOC stock were observed, newly added C to soil from plant litter, roots, and rhizomes turnover, could have compensated the losses of SOC caused by the priming effect, hence compensating the loss of initial SOC as proposed by Richter et al. (2015) and Zatta et al. (2014). However, in sites previously managed as grassland, this mechanism of C compensation requires more time to occur. According to the meta-analysis of Siddique et al. (2023), the change of SOC stock under perennial crops follows a sigmoidal curve over time. The increase of SOC with perennial crops like *Miscanthus* started after approximately five years, compensating for losses of original SOC caused by soil disturbance, soil priming and lower productivity of newly established crops. Interestingly, this study highlighted a significant positive correlation between SOC concentration and the increased activity of lignin peroxidase (*perox*). This indicates that the release of oxidative enzymes, which contribute to lignin degradation of plant organs and to the humification process, can lead to a progressive accumulation of stable organic C (Sinsabaugh, 2010) and, in the long term, newly derived plant C could replace the initial SOC loss and enhance SOC stock (Tab. S2.5). It is noteworthy to highlight that two significant predictors of SOC change were the initial nutrient content of soils undergoing land use conversion and their elemental stoichiometry ratios with C. Results from the multiple linear stepwise regression (Tab. 2.2) identified that initial availability of soil nutrients (N and P), as well as their soil stoichiometry (C:N, C:P and N:P) strongly contributed to determine SOC change trajectories. It is known that the chemical composition of plant residues and organic matter in soils affects microbial activity, thus controlling SOM cycling (Zechmeister-Boltenstern et al., 2015). This implies that microbial communities, to cope with C, N and P imbalance, mineralize SOM to bring nutrients and C supply

close to microbial element demand (Bertrand et al., 2019; Coonan et al., 2020a; Kirkby et al., 2014). In this study, in the topsoil layer, initial TN content was positively correlated to Δ SOC stock in both northern and southern climatic conditions. Several authors have reported that low nutrient availability, especially N, inhibits soil C sequestration and, on the contrary, C storage increases with N fertilization (Cadoux et al., 2014; Kirkby et al., 2014; Lemus and Lal, 2005; Tiemann and Grandy, 2015; Zhao et al., 2017). Moreover, both soil C:P and C:N stoichiometric ratios were positively correlated with Δ SOC stock. For instance, when phosphate availability is scarce, an increase of soil C:P ratio has the potential to further increase C storage (Satrio et al., 2009). However, if P inhibits the formation of persistent C, but N stimulates it, the combined effect on C sequestration of N and P can be variable (Zhao et al., 2017). Our results revealed an inverse relationship between the N:P ratio and soil SOC stock variation mainly in Southern environment. Nevertheless, the mechanisms by which P and both N and P affect soil C remains unclear. Compared to N and P, the contribution of K to soil C sequestration seems minor but is noticeable. High natural contents of soil K, or potash fertilization, generally promote plant biomass production and increase the root to shoot ratio in terrestrial ecosystems (Zhao et al., 2017). Given the high K demand of *Miscanthus* hybrids it is important to assess K content before transplanting if both biomass production and soil C storage must be optimized. These results support the general hypothesis that a limitation of one or more of the plant macronutrients (NPK) may place a ceiling on the quantity of SOC that can be stored as stable C with these high yielding novel *Miscanthus* hybrids.

2.5 Conclusion

Within the GRACE project, a number of novel rhizome- and seed-based *Miscanthus* hybrids were cultivated across six European sites characterized by different marginality factors, pedoclimatic condition and previous land use histories. This study sought to provide an early evaluation of the potential of four of these *Miscanthus* hybrids to sequester soil C and to improve chemical, biological and physical soil quality. *Miscanthus* cultivation had a slightly negative impact on soil physical quality, but it remains site-specific. In unfertilized fields, cultivation of high yielding *Miscanthus* genotypes cause a depletion of K, followed by N and P. The mechanism of nutrient recycling in the plant-soil system was ineffective for K and, to a lesser extent, for N. Hence, to secure stable *Miscanthus* feedstock in the long-term, K and N fertilization might be necessary, especially in former arable lands where initial nutrient contents might be low, while the biological turnover of the organic matter maintains or increases the P content, sustaining plant's P demand. After four years

from *Miscanthus* establishment, although not significantly different from initial SOC stock, average SOC sequestration rate was 0.43 Mg SOC ha⁻¹ y⁻¹. SOC sequestration was highly affected by previous land uses. Soil stoichiometry was a key controlling factor of SOC dynamics. Novel high-yielding *Miscanthus* hybrids provide in the short- to medium-term a secure biomass supply for the bio-based industry, but a longer period of cultivation is needed to substantially ameliorate overall soil quality and quantify the net C sink.

AUTHOR CONTRIBUTIONS

Marta Bertola: Conceptualization; data curation; formal analysis; writing – original draft; writing – review and editing. **Elena Magenau:** Data curation; investigation. **Enrico Martani:** Data curation; investigation. **Mislav Kontek:** Data curation; investigation. **Chris Ashman:** Data curation; investigation. **Vanja Jurišić:** Conceptualization; funding acquisition; methodology. **Isabelle Lamy:** Data curation; investigation. **Jason Kam: Resources.** **Flavio Fornasier:** Data curation; formal analysis; investigation. **Jon McCalmont:** Data curation; investigation. **Luisa M. Trindade:** Resources. **Stefano Amaducci:** Conceptualization; funding acquisition; methodology; writing – review and editing. **John Clifton-Brown:** Conceptualization; funding acquisition; methodology; resources; writing – review and editing. **Andreas Kiesel:** Conceptualization; funding acquisition; methodology; project administration. **Andrea Ferrarini:** Conceptualization; data curation; formal analysis; investigation; methodology; supervision; validation; writing – original draft; writing – review and editing.

ACKNOWLEDGEMENTS

The authors acknowledged INA laboratories for performing soil chemical analysis, in particular Slijepčević Boris and Rukavina Slavica.

FUNDING INFORMATION

The GRACE project has received funding from the Biobased Industries Joint Undertaking (JU) under the European Union's Horizon 2020 research and innovation program under grant agreement no. 745012. The JU receives support from the European Union's Horizon 2020 research and innovation program and the Bio-based Industries Consortium.

Supplementary data related to this chapter can be found at: <https://doi.org/10.1111/gcbb.13145>

The data that support the findings of this study are also openly available in Zenodo repository at <https://doi.org/10.5281/zenodo.10987591>.

Supplementary Material

Rhizome biomass estimation

Allometric equations are widely used for plant biomass assessment. Belowground plant biomass can be estimated based on allometric relations from aboveground biomass which can be measured and sampled more easily in the field.

In this work, an allometric equations was developed to estimate rhizome biomass (RB) of *Miscanthus*. Data on both above- and belowground biomass component of this crop were collected from 10 published research on *Miscanthus x giganteus* where aboveground biomass (AGB) and RB were measured at harvest time in late winter. The list of references collected is reported in Table S1 where 34 observations for AGB and RB and site information as well are reported. From each reference data on RB, AGB at RB sampling year, cumulative AGB (CumAGB), ratio between RB/CumAGB, textural class, site location, weather conditions (MAT and MAP) and years after establishment (YAE) were extracted or calculated when necessary. We averaged the RB and cumulative AGB data per years after establishment (Tab. S2.2) and we identified a significant allometric relationship based on a logarithmic regression function (Fig. S2.1 a) between these two variables. The equation is valid for cumulative AGB values higher than 3.7963 Mg DM ha⁻¹. This formula was used to estimate RB at 4th growing season in this study. RB for this study was calculated using cumulative AGB data for each genotype/replicate reported by Awty-Carrol et al. (2022). Interestingly we also identified a significant relationship between years after establishment and the RB/CumAGB ratio (Fig. S2.1b).

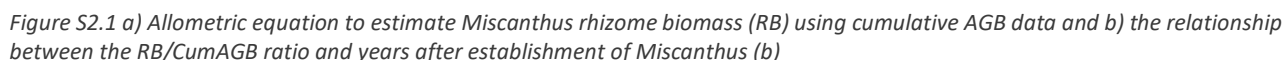
Table S2.1 Dataset collected from published literature to derive allometric equation to estimate *Miscanthus* RB.

* AGB data is the value measured at RB sampling year that correspond to value of YAE

** CumAGB has been or calculated or extracted from the corresponding reference

Reference	Country	Sand	Silt	Clay	Soil textural class	MAT	MAP	YAE	AGB *	Cum_AGB **	RB	RB/Cum AGB ratio
		%	%	%		°C	mm	years	Mg DM ha ⁻¹	Mg DM ha ⁻¹	Mg DM ha ⁻¹	
Himken et al 1997	Germany	80.4	14.8	4.8	Loamy Sand	9.3	715	4	18.1	51.1	15.1	0.30
Himken et al 1997	Germany	80.4	14.8	4.8	Loamy Sand	9.3	715	4	17.5	50.5	17.2	0.34
Himken et al 1997	Germany	80.4	14.8	4.8	Loamy Sand	9.3	715	4	15.8	48.8	15.2	0.31
Carvalho et al 2017	USA	15	70	15	Silt Loam	11.01	1042	1	1.0	1.0	0.3	0.34
Carvalho et al 2017	USA	15	70	15	Silt Loam	11.01	1042	2	3.5	4.5	1.5	0.33
Amougou et al 2011	France	7.8	72.2	19.9	Silt Loam	11.1	713	2	14.3	20.3	12.8	0.63
Amougou et al 2011	France	7.8	72.2	19.9	Silt Loam	11.1	713	2	15.3	35.6	10.2	0.29
Strullu et al. 2011	France	5	74	19	Silt Loam	10.7	625	3	20.0	33.0	16.0	0.48
Carvalho et al 2017	USA	15	70	15	Silt Loam	11.01	1042	3	11.6	44.6	4.2	0.09
Amougou et al 2011	France	7.8	72.2	19.9	Silt Loam	11.1	713	3	23.5	30.4	15.4	0.51
Amougou et al 2011	France	7.8	72.2	19.9	Silt Loam	11.1	713	3	26.1	56.5	17.3	0.31
Anderson-Teixeira et al 2013	USA	15	70	15	Silt Loam	11.01	1042	4	17.5	48.2	11.3	0.23
Strullu et al. 2011	France	5	74	19	Silt Loam	10.7	625	4	22.0	70.2	20.0	0.29
Carvalho et al 2017	USA	15	70	15	Silt Loam	11.01	1042	4	14.3	84.5	6.0	0.07
Dohleman et al. 2012	USA	10	60	30	Silty Clay Loam	12.2	962	5	44.1	129.7	23.8	0.18
Dohleman et al. 2012	USA	10	60	30	Silty Clay Loam	12.1	862	6	38.2	167.9	23.8	0.14
Dohleman et al. 2012	USA	10	60	30	Silty Clay Loam	10.2	1336	7	36.3	204.2	23.8	0.12
Kahle et al. 2001	Germany	62.1	25.4	10.4	Sandy Loam	7.9	547	4	7.5	20.8	7.7	0.37
Kahle et al. 2001	Germany	62.1	25.4	10.4	Sandy Loam	7.9	547	5	11.1	31.9	11.4	0.36
Kahle et al. 2001	Germany	62.1	25.4	10.4	Sandy Loam	7.9	547	6	12.6	44.5	12.5	0.28
Kahle et al. 2001	Germany	65.3	31	3.1	Sandy Loam	8.8	600	5	13.5	36.5	16.3	0.45
Kahle et al. 2001	Germany	65.3	31	3.1	Sandy Loam	8.8	600	6	8.8	45.3	14.6	0.32
Kahle et al. 2001	Germany	65.3	31	3.1	Sandy Loam	8.8	600	7	11.5	56.8	16.9	0.30
Kahle et al. 2001	Germany	18.6	57.6	24	Silt Loam	9.5	603	6	16.4	52.6	17.4	0.33
Kahle et al. 2001	Germany	18.6	57.6	24	Silt Loam	9.5	603	7	16.8	69.4	18.1	0.26
Kahle et al. 2001	Germany	18.6	57.6	24	Silt Loam	9.5	603	8	19.8	89.2	20.3	0.23
Himken et al. 1997	Germany	80	15	5	Sandy	9.3	715	4	16	49	16	0.33
Himken et al. 1997	Germany	80	15	5	Sandy	9.3	715	4	15	48	16	0.33
Himken et al. 1997	Germany	80	15	5	Sandy	9.3	715	4	15	48	17	0.35
Chstrian et al.2006	UK	27	50	23	Clay loam	8	600	1	6.99	6.99	4.2	0.60
Chstrian et al.2006	UK	27	50	23	Clay loam	8	600	2	10.07	17.06	6.71	0.39
Chstrian et al.2006	UK	77		23	Clay loam	8	600	3	14.03	31.09	10.23	0.33
Clifton-Brown et al. 2007	Ireland	85	10	5	Sandy Loam	9.9	873	12	10	124	20.5	0.17
Martani et al 2020	Italy	8	70	22	Silt Loam	12.2	890	11	10.7	146.8	35.0	0.24

YAE	<i>n</i> of obs	RB	Cum_AGB	RB/CumAGB ratio
years		Mg ha ⁻¹	Mg ha ⁻¹	-
2	4	7.80	19.37	0.41
3	5	12.63	39.12	0.34
4	10	14.15	51.90	0.29
5	3	17.17	66.03	0.33
6	4	17.08	77.58	0.27
7	3	19.60	110.13	0.22
8	1	20.30	89.20	0.23
11	2	26.27	209.78	0.15
12	1	20.50	124.00	0.17



Source of C	Formulas	Hybrids	Parameters Value		
			HI	R:S	β
C from stubble (<i>Cs</i>)	$0.1 \times (Y/HI) \times 0.45$	GRC 3	0.9	0.69	0.967
C in roots (<i>Cr</i>)	$\sum_{y1-y4} [(Y/HI) \times R:S \times \text{Turn} \times 0.45 \times \text{BG}_{F-\text{Depth}}]$	GRC9	0.9	0.34	0.967
C in roots exudates (<i>Ce</i>)	$\sum_{y1-y4} [0.09 \times (Y/HI) \times 0.45 \times \text{BG}_{F-\text{Depth}}]$	GRC14	0.9	0.36	0.967
C in rhizomes (<i>Crhiz</i>)	$(Y \times 0.25 \times 0.43 \times 0.7) / 3$				
C tot	$Cs + Cr + Ce + Cw + Crh$				

Y: annual yield (for Crhiz calculation, cumulative yield was used)
 HI: Harvest Index
 R:S: Root to Shoot ratio of different *Miscanthus* hybrids
 Turn: Turnover rate (0.5 for *Miscanthus* roots)
 $BGF-Depth = 1 - \beta x$, where BGF-Depth is the amount of roots (%) from the soil surface to a certain depth (x) expressed in centimeter (es: 10 cm, 30 cm). The β value (0.967) describes the shape of the vertical distribution of roots within the whole soil profile (Chimento and Amaducci, 2015).
 For Crhiz calculation, $0.25 = rate\ RB / cumAGB$; 0.43 is the C content in rhizome as C:N is lower (Beuch et al. 2000), while 0.70 is the C turnover rate. 3 are the years of plant cultivation, where the first year is not considered since in the first year of establishment RB is negligible.

Table S2.4a Analysis of variance for two soil layers of topsoil (0-10 cm and 10-30 cm): effects of site (Site), Miscanthus hybrids (Hybrid), and years of Miscanthus hybrids cultivation (Time) on measured soil physicochemical indicators.

		BD	Porosity	SOC	TN	P ₂ O ₅	K ₂ O	P	K	Ca	Mg
		g cm ⁻³	%	g kg ⁻¹	g kg ⁻¹	g kg ⁻¹	g kg ⁻¹	g kg ⁻¹	g kg ⁻¹	g kg ⁻¹	g kg ⁻¹
Layer 0-10 cm											
Site (S)	F	117.1	124.1	122.7	29.0	122.73	43.72	97.81	36.4	62.1	1179.1
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hybrid (Hy)	F	0.29	0.6	2.0	0.47	0.52	1.23	0.36	1.55	1.74	0.61
	p	0.83	0.61	0.12	0.70	0.67	0.30	0.78	0.20	0.16	0.61
Time	F	132.1	166.1	7.14	14.47	334.64	73.76	42.77	106.4	0.1	0.06
	p	<0.001	<0.001	0.09	<0.001	<0.001	<0.001	<0.001	<0.001	0.89	0.80
S x Hy	F	0.34	0.6	0.36	0.46	0.33	0.79	1.09	0.46	1.61	0.31
	p	0.98	0.87	0.98	0.94	0.98	0.67	0.38	0.94	0.1	0.99
S x Time	F	57.5	68.7	11.6	10.14	45.78	17.62	38.15	22.4	0.32	2.59
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.89	0.03
Hy x Time	F	0.30	0.6	2.01	0.47	0.51	0.30	0.74	0.45	0.18	0.611
	p	0.82	0.60	0.12	0.70	0.67	1.23	0.53	0.71	0.90	0.60
S x Hy x Time	F	0.57	0.9	0.36	0.46	0.32	0.67	1.59	0.26	0.16	0.31
	p	0.89	0.59	0.98	0.95	0.99	0.79	0.10	0.99	0.99	0.99
Layer 10-30 cm											
Site (S)	F	126.1	126.5	47.6	10.6	104.68	26.80	99.60	151.3	239.3	832.2
	p	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hybrid (Hy)	F	0.03	0.03	0.51	0.21	0.24	0.26	1.13	0.44	1.28	1.43
	p	0.99	0.99	0.67	0.88	0.86	0.85	0.35	0.72	0.29	0.24
Time	F	9.045	9.04	4.63	2.92	331.55	36.13	15.77	332.5	1.11	10.90
	p	0.003	<0.001	0.034	0.09	<0.001	<0.001	<0.001	<0.001	0.29	0.014
S x Hy	F	0.6	0.60	0.21	0.44	0.30	0.54	0.95	1.07	1.12	0.67
	p	0.8	0.85	0.99	0.95	0.99	0.90	0.52	0.40	0.35	0.80
S x Time	F	10.6	10.4	8.10	8.65	46.07	28.10	23.32	55.5	0.14	5.76
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.97	0.002
Hy x Time	F	0.03	0.03	0.99	0.21	0.24	0.26	1.57	0.18	0.18	1.41
	p	0.99	0.99	0.40	0.88	0.86	0.84	0.20	0.90	0.90	0.24
S x Hy x Time	F	0.64	0.60	0.25	0.44	0.30	0.54	1.21	0.62	0.21	0.67
	p	0.85	0.85	0.99	0.95	0.99	0.90	0.28	0.84	0.99	0.80

Table S2.4b Analysis of variance for two soil layers of topsoil (0-10 cm and 10-30 cm): effects of site (Site), Miscanthus hybrids (Hybrid), and years of Miscanthus hybrids cultivation (Time) on measured soil biological indicators such as microbial biomass (dsDNA in ng g⁻¹ dry soil) and enzyme activities (nM 4-MUF, 7-AMC g⁻¹ dry soil h⁻¹)

		C- acquiring enzymes			N- acquiring enzymes				P- acquiring enzymes					S- acq	Esterases		Redox	Microbial
		betaG	Cell	Xilo	Aprot	Argi	Chit	Leu	acP	alkP	bisP	inositP	piroP	aryS	Butir	Fda	Perox	biomass
Layer 0-10 cm																		
Site (S)	F	24.99	22.84	23.82	19.09	17.40	23.91	16.62	96.56	25.82	32.60	17.98	73.44	35.97	13.02	39.09	14.46	51.72
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hybrid (Hy)	F	0.96	0.98	1.51	0.59	0.79	0.91	0.64	1.81	0.67	0.51	0.75	0.66	0.54	3.08	0.67	0.30	1.57
	p	0.42	0.41	0.22	0.62	0.50	0.44	0.59	0.16	0.57	0.67	0.53	0.58	0.65	0.03	0.57	0.83	0.21
Time	F	31.58	41.27	125.04	52.91	1.72	24.56	25.41	0.06	4.21	2.81	11.82	21.44	57.55	72.37	59.25	103.34	30.78
	p	<0.001	<0.001	<0.001	<0.001	0.19	<0.001	<0.001	0.80	0.04	0.09	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
S x Hy	F	0.61	0.73	0.72	0.56	0.70	1.22	0.48	0.80	0.53	0.32	0.32	0.51	0.58	0.62	0.89	0.32	0.72
	p	0.85	0.74	0.75	0.89	0.77	0.29	0.94	0.67	0.91	0.99	0.99	0.93	0.87	0.84	0.58	0.99	0.76
S x Time	F	30.35	34.74	39.27	13.18	39.97	43.31	31.56	16.40	26.49	4.44	9.37	12.558	33.74	8.08	39.60	17.99	62.63
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hy x Time	F	0.96	0.98	1.51	0.59	0.79	0.91	0.64	1.81	0.67	0.51	0.75	0.66	0.54	3.08	0.67	0.30	1.57
	p	0.42	0.41	0.22	0.62	0.50	0.44	0.59	0.15	0.57	0.67	0.53	0.58	0.65	0.03	0.57	0.83	0.20
S x Hy x Time	F	0.61	0.73	0.72	0.56	0.70	1.22	0.48	0.80	0.53	0.32	0.32	0.51	0.58	0.62	0.89	0.32	0.72
	p	0.85	0.75	0.75	0.89	0.77	0.27	0.94	0.66	0.91	0.99	0.99	0.93	0.87	0.84	0.58	0.99	0.76
Layer 10-30 cm																		
Site (S)	F	29.48	9.61	25.79	10.98	12.60	23.24	14.31	130.62	52.22	121.67	47.68	294.038	77.95	95.54	35.21	73.65	43.55
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hybrid (Hy)	F	0.95	1.13	0.48	0.02	1.17	0.09	0.71	0.96	0.34	0.64	0.56	0.30	0.77	2.07	0.02	1.53	0.50
	p	0.42	0.34	0.69	0.99	0.32	0.96	0.55	0.42	0.79	0.59	0.64	0.82	0.52	0.11	0.99	0.22	0.69
Time	F	28.40	19.95	90.57	7.79	15.63	0.01	23.19	13.38	66.65	0.76	23.17	16.76	80.05	175.19	10.60	96.52	70.33
	p	<0.001	<0.001	<0.001	0.007	<0.001	0.92	<0.001	<0.001	<0.001	0.38	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001
S x Hy	F	0.65	0.58	0.69	0.73	0.72	0.55	0.69	1.27	1.01	0.65	0.59	0.82	0.89	1.41	0.49	2.01	0.39
	p	0.81	0.87	0.78	0.74	0.75	0.89	0.78	0.25	0.46	0.81	0.87	0.65	0.57	0.18	0.93	0.03	0.97
S x Time	F	12.45	5.47	9.16	2.67	9.03	4.70	12.46	2.41	14.56	26.86	24.66	58.66	14.27	9.58	1.04	6.37	10.25
	p	<0.001	<0.001	<0.001	0.03	<0.001	<0.001	<0.001	0.04	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.40	<0.001	<0.001
Hy x Time	F	0.95	1.13	0.48	0.02	1.17	0.09	0.71	0.96	0.34	0.64	0.57	0.30	0.77	2.07	0.02	1.53	0.50
	p	0.42	0.34	0.69	0.99	0.32	0.96	0.55	0.42	0.79	0.58	0.64	0.82	0.52	0.11	0.99	0.21	0.69
S x Hy x Time	F	0.65	0.58	0.69	0.73	0.72	0.55	0.69	1.26	1.01	0.66	0.59	0.82	0.89	1.41	0.46	2.01	0.39
	p	0.81	0.88	0.79	0.74	0.76	0.90	0.79	0.25	0.46	0.81	0.88	0.65	0.57	0.17	0.93	0.03	0.97

Table S2.5 Correlation matrix between variations of SOC concentration (Δ SOC g kg⁻¹) and shift of EA (Δ EA nanomoles g⁻¹ dry soil h⁻¹) during four years of Miscanthus cultivation.

	SOC_conc	dsDNA	aryS	betaG	cell	xilo	argi	chit	aprot	leu	acP	bisP	piroP	alkP	inositP	butir	fda	perox
SOC_conc	1	0.6	0.22	0.29	0.39	0.3	0.39	0.37	0.34	0.33	0.43	-0.16	-0.12	0.28	0.01	0.26	0.41	0.46
dsDNA	0.6	1	0.42	0.57	0.57	0.58	0.63	0.45	0.31	0.59	0.5	-0.01	0.04	0.4	0.18	0.37	0.52	0.46
aryS	0.22	0.42	1	0.77	0.7	0.77	0.74	0.36	0.35	0.84	0.66	0.65	0.51	0.63	0.66	0.52	0.54	0.51
betaG	0.29	0.57	0.77	1	0.88	0.96	0.87	0.53	0.29	0.89	0.4	0.47	0.48	0.74	0.61	0.38	0.56	0.29
cell	0.39	0.57	0.7	0.88	1	0.88	0.85	0.48	0.31	0.85	0.34	0.34	0.32	0.74	0.53	0.43	0.54	0.42
xilo	0.3	0.58	0.77	0.96	0.88	1	0.86	0.45	0.34	0.88	0.4	0.46	0.49	0.76	0.6	0.43	0.5	0.37
argi	0.39	0.63	0.74	0.87	0.85	0.86	1	0.54	0.34	0.92	0.4	0.4	0.4	0.81	0.55	0.39	0.59	0.42
chit	0.37	0.45	0.36	0.53	0.48	0.45	0.54	1	0.08	0.51	0.34	0.15	0.12	0.31	0.31	-0.04	0.76	0.2
aprot	0.34	0.31	0.35	0.29	0.31	0.34	0.34	0.08	1	0.34	0.29	0.19	0.21	0.38	0.34	0.32	0.16	0.32
leu	0.33	0.59	0.84	0.89	0.85	0.88	0.92	0.51	0.34	1	0.44	0.52	0.47	0.79	0.62	0.42	0.59	0.43
acP	0.43	0.5	0.66	0.4	0.34	0.4	0.4	0.34	0.29	0.44	1	0.22	0.12	0.16	0.21	0.51	0.58	0.66
bisP	-0.16	-0.01	0.65	0.47	0.34	0.46	0.4	0.15	0.19	0.52	0.22	1	0.79	0.58	0.78	0.21	0.16	0.11
piroP	-0.12	0.04	0.51	0.48	0.32	0.49	0.4	0.12	0.21	0.47	0.12	0.79	1	0.56	0.75	0.1	0.03	-0.01
alkP	0.28	0.4	0.63	0.74	0.74	0.76	0.81	0.31	0.38	0.79	0.16	0.58	0.56	1	0.71	0.31	0.3	0.31
inositP	0.01	0.18	0.66	0.61	0.53	0.6	0.55	0.31	0.34	0.62	0.21	0.78	0.75	0.71	1	0.19	0.25	0.15
butir	0.26	0.37	0.52	0.38	0.43	0.43	0.39	-0.04	0.32	0.42	0.51	0.21	0.1	0.31	0.19	1	0.22	0.56
fda	0.41	0.52	0.54	0.56	0.54	0.5	0.59	0.76	0.16	0.59	0.58	0.16	0.03	0.3	0.25	0.22	1	0.38
perox	0.46	0.46	0.51	0.29	0.42	0.37	0.42	0.2	0.32	0.43	0.66	0.11	-0.01	0.31	0.15	0.56	0.38	1

Table S2.6 Variations of EA from T_0 to T_4 sampling time expressed as Δ EA nanomoles g^{-1} dry soil h^{-1} .

Site	Depth	dsDNA	aryS	betaG	cell	xilo	argi	chit	aprot	leu	acP	bisP	piroP	alkP	inositP	butir	fda	perox
CHV	0-10 cm	15.99	2.20	0.01	0.29	0.45	4.82	0.08	-2.59	8.42	2.93	4.02	1.47	24.39	0.11	133.80	-0.24	2.92
	10-30 cm	9.61	0.51	0.75	0.07	0.20	1.85	0.15	-11.67	2.91	0.51	0.05	0.00	10.97	0.00	54.37	-0.11	0.95
OLI	0-10 cm	-6.87	-1.91	0.21	0.11	0.26	0.38	-1.22	-7.90	-3.14	-12.46	-1.41	1.71	23.47	0.09	22.76	-1.45	0.54
	10-30 cm	-1.75	4.04	1.69	0.12	0.51	2.76	-0.32	-1.94	6.36	1.21	7.82	8.85	35.64	0.94	61.67	-0.65	0.93
PAC 1	0-10 cm	17.76	3.47	6.77	0.41	1.24	6.69	1.34	-19.37	13.90	4.64	2.83	5.32	18.38	0.21	139.20	0.17	0.89
	10-30 cm	14.93	2.52	4.11	0.13	0.69	3.78	0.71	0.24	10.01	2.91	1.42	4.00	8.25	0.12	74.24	0.02	0.88
PAC 2	0-10 cm	-29.13	-5.37	-11.76	-1.26	-1.83	-20.57	-3.98	-35.44	-24.55	-4.99	-2.42	-3.01	-57.90	-0.62	-12.53	-1.43	-0.47
	10-30 cm	2.70	0.61	0.82	-0.02	0.16	-1.27	-0.02	-9.71	-0.07	0.45	0.33	0.32	-0.23	0.04	28.97	-0.03	1.07
TWS	0-10 cm	6.28	3.09	0.91	0.12	0.58	-2.22	-6.46	1.13	3.70	-0.21	3.86	4.03	19.20	0.21	239.92	-2.05	1.97
	10-30 cm	26.63	-1.73	-1.97	-0.10	-0.01	-1.31	-0.95	4.59	-4.40	8.10	-5.37	-1.92	3.91	-0.28	158.29	-0.42	3.16
ZAG	0-10 cm	18.80	2.83	3.73	0.33	0.83	5.69	0.30	-3.48	9.60	8.94	-1.17	-0.06	3.50	0.11	158.07	0.47	2.23
	10-30 cm	11.56	1.33	1.27	0.10	0.27	0.37	-0.39	-8.25	1.94	3.53	-2.44	-4.95	7.81	0.11	119.13	-0.08	1.45

Table S2.7a Analysis of variance for two soil layers of topsoil (0-10 cm and 10-30 cm): effects of site (Site), *Miscanthus* hybrids (Hybrid), and years of *Miscanthus* hybrids cultivation (Time) on calculated ESM of SOC and soil nutrients ($Mg\ ha^{-1}$).

		SOC	TN	P ₂ O ₅	K ₂ O	P	K	Ca	Mg
		Mg ha ⁻¹	Mg ha ⁻¹	Mg ha ⁻¹	Mg ha ⁻¹	Mg ha ⁻¹	Mg ha ⁻¹	Mg ha ⁻¹	Mg ha ⁻¹
Layer 0-10 cm									
Site (S)	F	43.4	345.3	155.96	59.74	19.96	95.4	449.5	1882.3
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hybrid (Hy)	F	1.83	0.24	0.56	0.82	0.49	1.0	1.24	0.97
	p	0.14	0.86	0.65	0.49	0.69	0.39	0.30	0.41
Time	F	4.34	19.3	319.09	94.5	33.93	52.4	44.56	0.53
	p	0.04	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.46
S x Hy	F	0.28	0.54	0.35	0.65	0.779	0.41	1.27	0.70
	p	0.99	0.90	0.99	0.82	0.70	0.96	0.25	0.77
S x Time	F	12.34	13.67	44.10	18.81	33.19	17.26	15.7	1.38
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.26
Hy x Time	F	1.77	0.19	0.55	0.77	0.51	0.66	0.10	1.01
	p	0.15	0.90	0.65	0.51	0.68	0.57	0.95	0.39
S x Hy x Time	F	0.29	0.56	0.34	0.63	0.75	0.48	0.43	0.70
	p	0.99	0.89	0.99	0.84	0.72	0.94	0.96	0.77
Layer 10-30 cm									
Site (S)	F	25.6	12.04	144.94	47.63	72.82	171.1	674.4	1409.3
	p	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hybrid (Hy)	F	0.34	0.12	0.25	0.63	1.14	0.51	0.94	1.35
	p	0.79	0.94	0.85	0.59	0.34	0.67	0.42	0.27
Time	F	10.69	26.73	330.0995	17.44	19.72	306.6	35.94	9.83
	p	0.0017	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
S x Hy	F	0.20	0.41	0.30	0.41	1.16	0.80	0.61	0.59
	p	0.99	0.96	0.99	0.96	0.36	0.66	0.84	0.86
S x Time	F	6.19	11.13	46.50	23.66	36.14	36.95	6.55	6.43
	p	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001
Hy x Time	F	0.94	0.15	0.26	0.56	1.70	0.12	0.30	1.31
	p	0.42	0.92	0.85	0.63	0.18	0.94	0.82	0.27
S x Hy x Time	F	0.23	0.40	0.30	0.39	1.55	0.32	0.22	0.60
	p	0.99	0.97	0.99	0.97	0.11	0.99	0.99	0.85

Table S2.7b Analysis of variance for the topsoil layer (0-30 cm): effects of site (Site), Miscanthus hybrids (Hybrid), and years of Miscanthus hybrids cultivation (Time) on calculated ESM of SOC and soil nutrients (Mg ha^{-1}).

		SOC	TN	P ₂ O ₅	K ₂ O	P	K	Ca	Mg
		Mg ha^{-1}	Mg ha^{-1}	Mg ha^{-1}	Mg ha^{-1}	Mg ha^{-1}	Mg ha^{-1}	Mg ha^{-1}	Mg ha^{-1}
Layer 0-30 cm									
Site (S)	F	46.07	17.23	149.19	49.94	79.53	322.3	682.3	1861.2
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hybrid (Hy)	F	0.97	0.01	0.29	0.34	0.29	0.61	1.02	0.83
	p	0.41	0.99	0.82	0.79	0.83	0.60	0.36	0.48
Time	F	0.34	4.42	348.94	0.94	43.39	439.3	47.52	11.2
	p	0.56	0.03	<0.001	0.33	<0.001	<0.001	<0.001	0.0013
S x Hy	F	0.25	0.53	0.30	0.45	0.58	0.53	0.80	0.39
	p	0.99	0.90	0.99	0.95	0.87	0.91	0.67	0.97
S x Time	F	7.99	7.24	47.51	22.63	29.67	53.6	10.2	6.25
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001
Hy x Time	F	1.47	0.01	0.29	0.26	0.30	0.40	0.25	0.85
	p	0.23	0.99	0.82	0.85	0.82	0.74	0.85	0.47
S x Hy x Time	F	0.34	0.53	0.30	0.42	0.58	0.47	0.22	0.39
	p	0.98	0.91	0.99	0.96	0.88	0.94	0.99	0.97

Table S2.8 Analysis of variance for the topsoil layer (0-30 cm): effects of site (Site) and Miscanthus hybrids (Hybrid) on rate of SOC and soil nutrients stock variation ($\text{Mg ha}^{-1} \text{y}^{-1}$).

		SOC	TN	P ₂ O ₅	K ₂ O	P	K	Ca	Mg
		$\text{Mg ha}^{-1} \text{y}^{-1}$	$\text{Mg ha}^{-1} \text{y}^{-1}$	$\text{Mg ha}^{-1} \text{y}^{-1}$	$\text{Mg ha}^{-1} \text{y}^{-1}$	$\text{Mg ha}^{-1} \text{y}^{-1}$	$\text{Mg ha}^{-1} \text{y}^{-1}$	$\text{Mg ha}^{-1} \text{y}^{-1}$	$\text{Mg ha}^{-1} \text{y}^{-1}$
Layer 0-30 cm									
Site (S)	F	4.89	0.87	15.65	11.06	11.07	18.82	0.03	1.75
	p	0.0074	0.52	<0.001	<0.001	<0.001	<0.001	0.99	0.18
Hybrid (Hy)	F	2.01	0.03	0.31	0.47	0.23	0.50	0.35	1.02
	p	0.12	0.99	0.81	0.70	0.87	0.69	0.78	0.39
Site x Hybrid	F	0.46	1.58	1.11	1.17	0.99	1.19	0.37	0.52
	p	0.94	0.11	0.36	0.32	0.47	0.30	0.98	0.91

Table S2.9 Contribution (%) to the total C input to soil (Mg ha⁻¹) of the following *Miscanthus* organs: C from stubble (Cs), C from roots (Cr), C from root exudates (Ce), and C from rhizomes (Crhiz).

Site	Hybrid	Cs	Cr	Ce	Crh
TWS	GRC 14	19.30	41.30	20.74	18.56
	GRC 15	11.97	47.05	21.60	19.32
	GRC 3	10.49	59.99	15.55	13.91
	GRC 9	22.74	38.33	20.49	18.33
CHV	GRC 14	12.90	44.60	22.40	20.04
	GRC 15	12.48	46.78	21.47	19.21
	GRC 3	10.51	59.98	15.55	13.91
	GRC 9	19.47	0.71	42.08	37.65
OLI	GRC 14	7.81	47.22	23.72	21.22
	GRC 15	12.03	46.11	21.16	20.64
	GRC 3	9.57	59.82	15.51	15.06
	GRC 9	12.45	41.62	22.25	23.62
ZAG	GRC 14	15.65	43.18	21.69	19.40
	GRC 15	12.25	46.90	21.53	19.26
	GRC 3	10.68	59.86	15.52	13.88
	GRC 9	16.64	41.37	22.12	19.79
PAC 1	GRC 14	9.43	46.39	23.30	20.84
	GRC 15	15.01	39.72	23.85	21.34
	GRC 3	8.92	56.27	18.35	16.42
	GRC 9	11.81	47.63	21.38	19.13
PAC 2	GRC 14	9.38	57.39	17.51	15.67
	GRC 15	11.34	50.61	20.05	17.94
	GRC 3	10.09	53.57	19.15	17.14
	GRC 9	13.80	42.79	22.88	20.47
		12.11	46.63	21.57	19.63

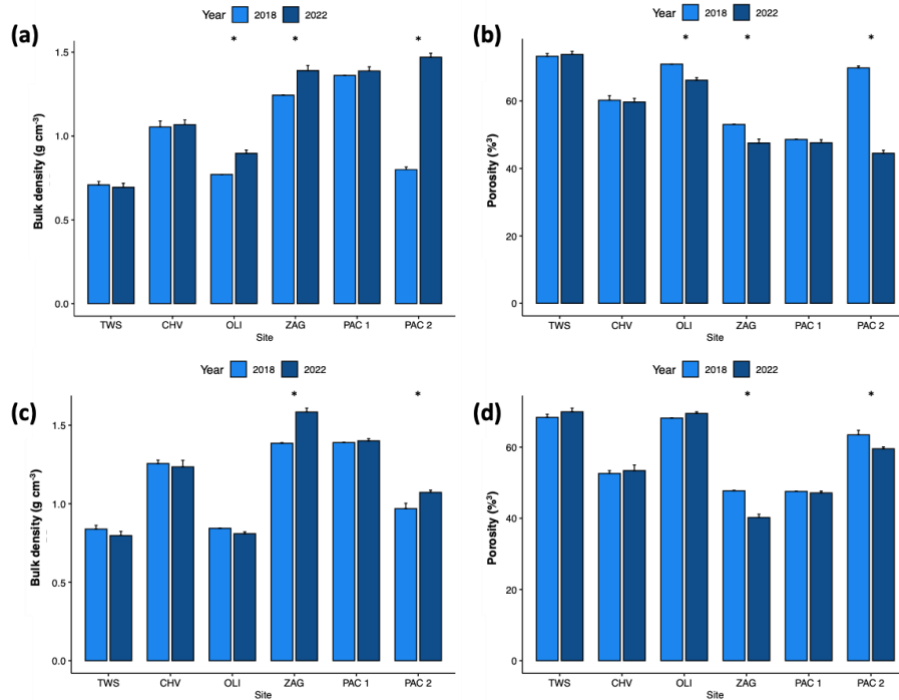


Figure S2.2 Mean values ($n=4$) of bulk density in 0-10 (a) and 10-30 (c) cm soil layers, and soil porosity in 0-10 (b) and 10-30 (d) cm soil layers among 6 European marginal sites at T_0 (light blue) and T_4 (dark blue). (*) show statistically different variations from T_0 to T_4 (Tukey's test, $P: 0.05$) within the same site.

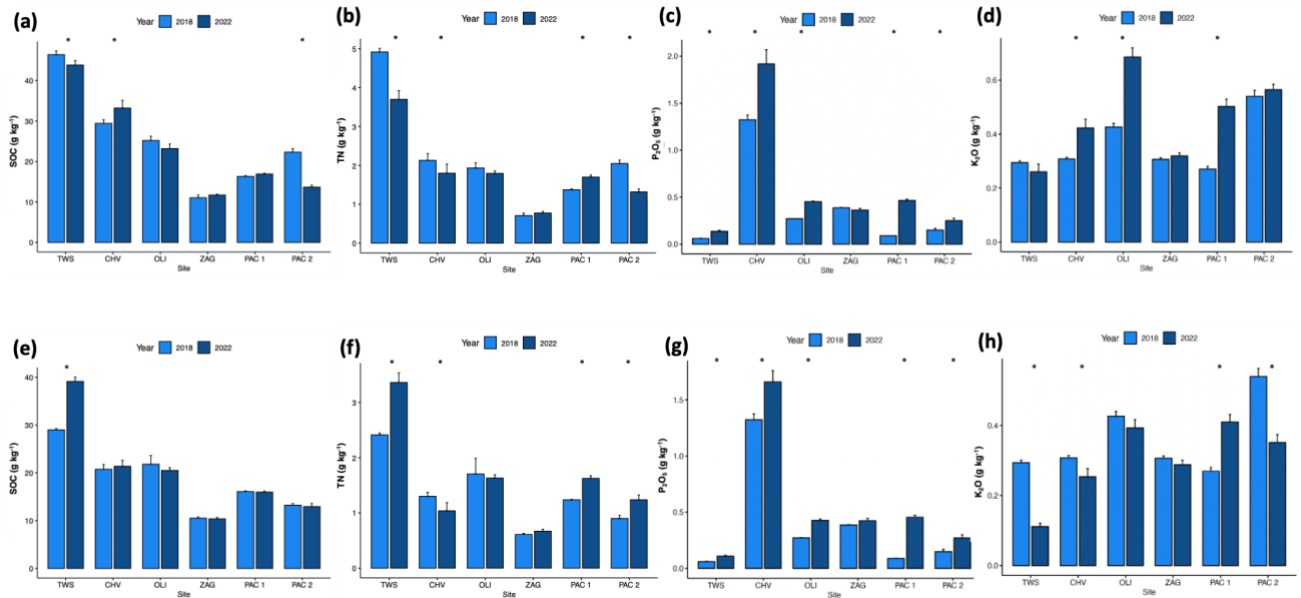


Figure S2.3 Mean values ($n=4$) of SOC, TN, P_2O_5 and K_2O concentration (g kg⁻¹) in 0-10 cm (a, b, c, d) and 10-30 cm (e, f, g, h) soil layers among 6 European marginal sites at T_0 (light blue) and T_4 (dark blue). (*) show statistically different variations from T_0 to T_4 (Tukey's test, $P: 0.05$) within the same site.

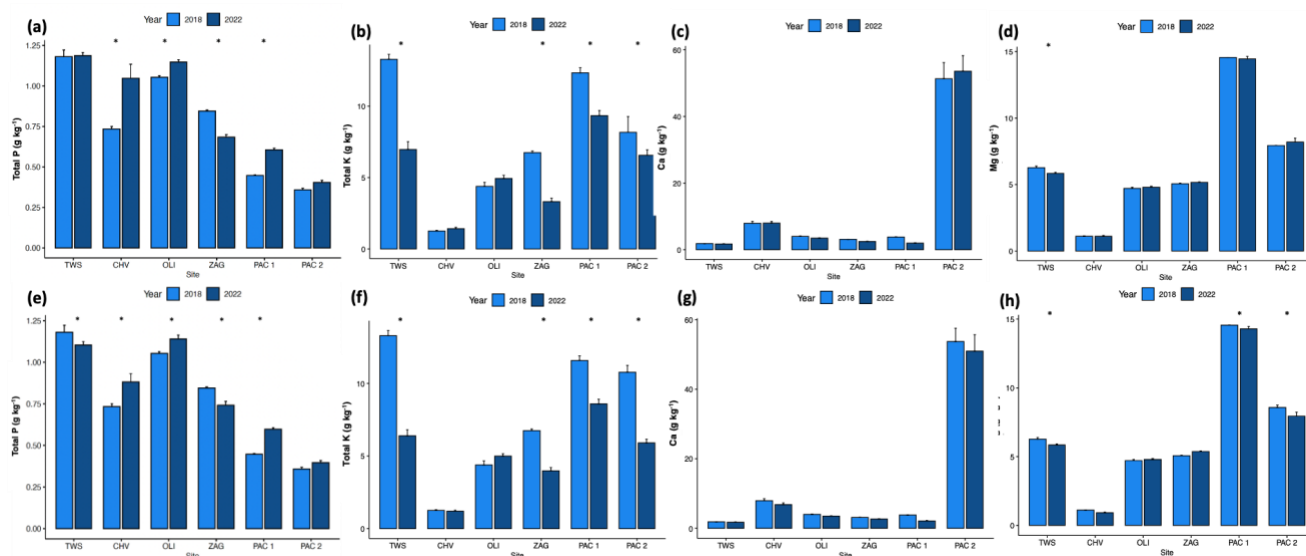


Figure S2.4 Mean values ($n=4$) of TP, TK, Ca and Mg concentration (g kg^{-1}) in 0-10 cm (a, b, c, d) and 10-30 cm (e, f, g, h) soil layers among 6 European marginal sites at T_0 (light blue) and T_4 (dark blue). (*) show statistically different variations from T_0 to T_4 (Tukey's test, $P: 0.05$) within the same site.

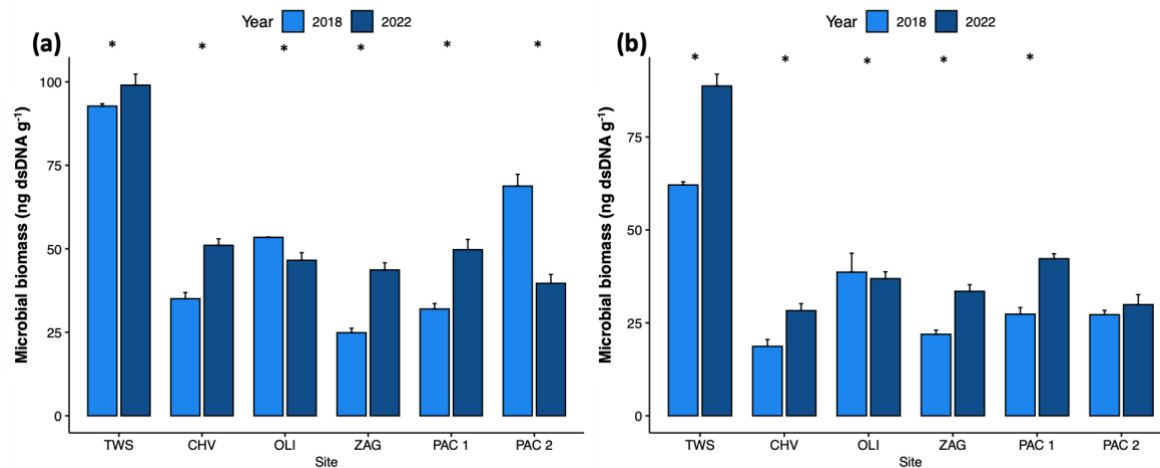


Figure S2.5 Mean values ($n=4$) of microbial biomass measured as dsDNA ($\text{ng g}^{-1}_{\text{dry soil}}$) in 0-10 cm (a) and 10-30 cm (b) soil layers among 6 European marginal sites at T_0 (light blue) and T_4 (dark blue). (*) show statistically different variations from T_0 to T_4 (Tukey's test, $P: 0.05$) within the same site.

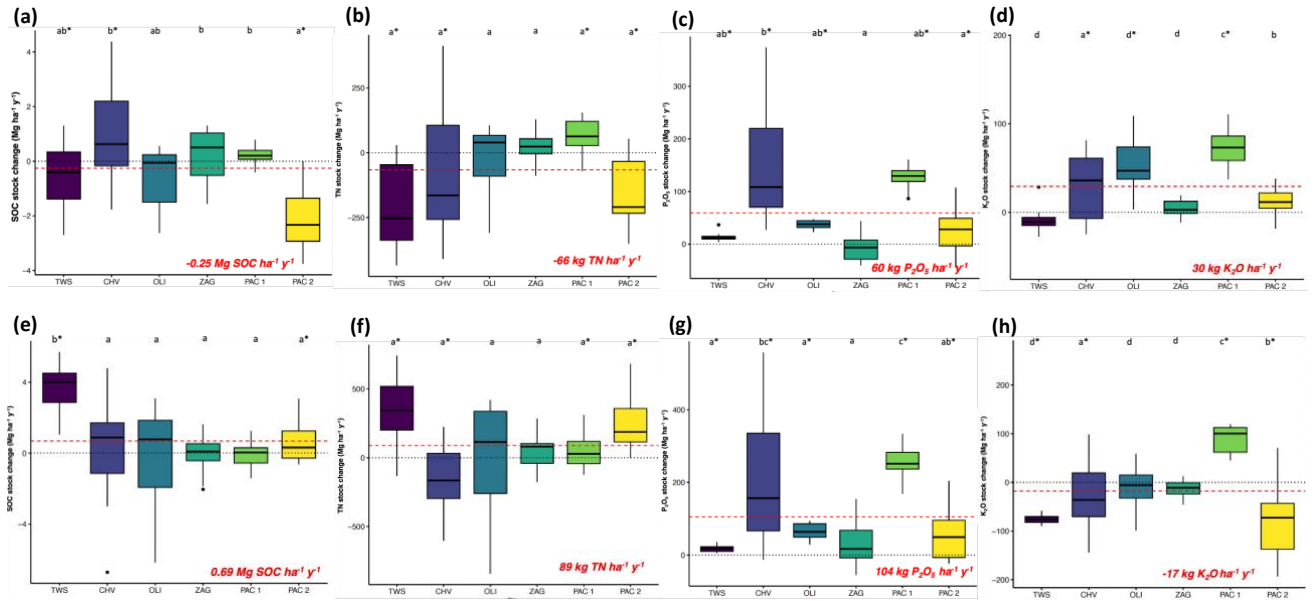


Figure S2.6 Rate of SOC and soil nutrients stock variation (Δ stock $\text{Mg ha}^{-1} \text{y}^{-1}$) in the 0-10 cm (a, b, c, d) and 10-30 cm (e, f, g, h) soil layers, in 4 years of *Miscanthus* cultivation. Different letters denote statistically different means among marginal sites (Tukey's test, $P: 0.05$), while (*) show statistically different variations from T_0 to T_4 (Tukey's test, $P: 0.05$) within the same site.

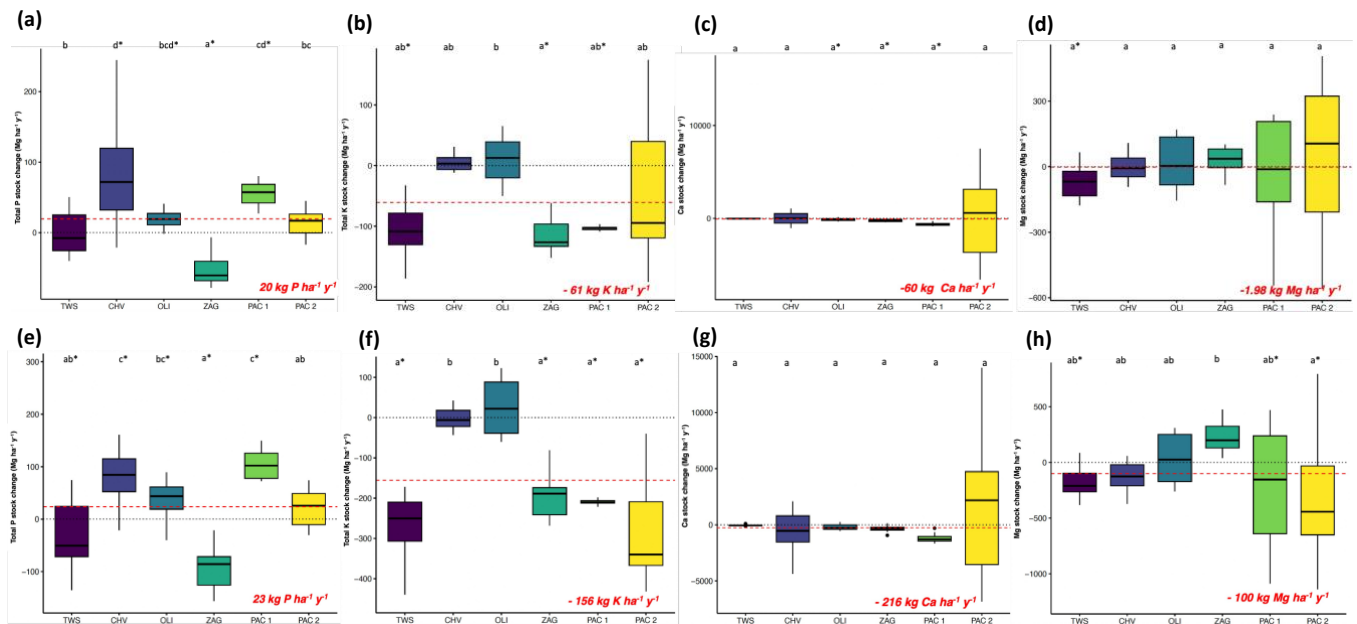


Figure S2.7 Rate of SOC and soil nutrients stock variation (Δ stock $\text{Mg ha}^{-1} \text{y}^{-1}$) in the 0-10 cm (a, b, c, d) and 10-30 cm (e, f, g, h) soil layers, in 4 years of *Miscanthus* cultivation. Different letters denote statistically different means among marginal sites (Tukey's test, $P: 0.05$), while (*) show statistically different variations from T_0 to T_4 (Tukey's test, $P: 0.05$) within the same site.

References of Table S2.1

- Amougou, N., Bertrand, I., Machet, J.-M., Recous, S., 2011. Quality and decomposition in soil of rhizome, root and senescent leaf from *Miscanthus x giganteus*, as affected by harvest date and N fertilization. *Plant Soil* 338, 83–97. <https://doi.org/10.1007/s11104-010-0443-x>
- Anderson-Teixeira, K.J., Masters, M.D., Black, C.K., Zeri, M., Hussain, M.Z., Bernacchi, C.J., DeLucia, E.H., 2013. Altered Belowground Carbon Cycling Following Land-Use Change to Perennial Bioenergy Crops. *Ecosystems* 16, 508–520. <https://doi.org/10.1007/s10021-012-9628-x>
- Carvalho, J.L.N., Hudiburg, T.W., Franco, H.C.J., DeLucia, E.H., 2017. Contribution of above- and belowground bioenergy crop residues to soil carbon. *GCB Bioenergy* 9, 1333–1343. <https://doi.org/10.1111/gcbb.12411>
- Christian, D., Poulton, P., Riche, A., Yates, N., Todd, A., 2006. The recovery over several seasons of ¹⁵N-labelled fertilizer applied to *Miscanthus x giganteus* ranging from 1 to 3 years old. *Biomass Bioenergy* 30, 125–133. <https://doi.org/10.1016/j.biombioe.2005.11.002>
- Clifton-Brown, J.C., Breuer, J., Jones, M.B., 2007. Carbon mitigation by the energy crop, *Miscanthus*. *Glob. Change Biol.* 13, 2296–2307. <https://doi.org/10.1111/j.1365-2486.2007.01438.x>
- Dohleman, F.G., Heaton, E.A., Arundale, R.A., Long, S.P., 2012. Seasonal dynamics of above- and below-ground biomass and nitrogen partitioning in *Miscanthus x giganteus* and *Panicum virgatum* across three growing seasons. *GCB Bioenergy* 4, 534–544. <https://doi.org/10.1111/j.1757-1707.2011.01153.x>
- Himken, M., Lammel, J., Neukirchen, D., Czipionka-Krause, U., 1997. Cultivation of *Miscanthus* under West European conditions: Seasonal changes in dry matter production, nutrient uptake and remobilization. *Plant Soil* 189, 117–126.
- Kahle, P., Beuch, S., Boelcke, B., Leinweber, P., Schulten, H.-R., 2001. Cropping of *Miscanthus* in Central Europe: biomass production and influence on nutrients and soil organic matter. *Eur. J. Agron.* 15, 171–184. [https://doi.org/10.1016/S1161-0301\(01\)00102-2](https://doi.org/10.1016/S1161-0301(01)00102-2)
- Martani, E., Ferrarini, A., Serra, P., Pilla, M., Marcone, A., Amaducci, S., 2020. Belowground biomass C outweighs soil organic C of perennial energy crops: Insights from a long-term multispecies trial. *GCB Bioenergy* 00, 1–14. <https://doi.org/10.1111/gcbb.12785>
- Strullu, L., Cadoux, S., Preudhomme, M., Jeuffroy, M.-H., Beaudoin, N., 2011. Biomass production and nitrogen accumulation and remobilisation by *Miscanthus x giganteus* as influenced by nitrogen stocks in belowground organs. *Field Crops Res.* 121, 381–391. <https://doi.org/10.1016/j.fcr.2011.01.005>

Chapter 3

Carbon farming solutions for the industrial tomato supply chain: evaluating agronomic practices eligibility and MRV tools accuracy on lead commercial farms in northwestern Italy.

Marta Bertola¹ | Lorenzo Cremonesi^{1,2} | Manuele Ragazzi¹ | Michele Croci,^{1,3} | Stefano Amaducci^{1,3,4} | Andrea Ferrarini¹

¹Department of Sustainable Crop Production – Università Cattolica del Sacro Cuore, Piacenza, Italy

²Consorzio Agrario Terrepadane, Piacenza, Italy

³Remote Sensing and Spatial Analysis Research Center (CRAST), Università Cattolica del Sacro Cuore, 29122 Piacenza, Italy

⁴Centro per l'Innovazione nell'Impiego del Telerilevamento nell'Industria Meccanica per l'Agricoltura di Precisione (CITIMAP), via Castellarino, 12, San Bonico, PC 29122, Italy

3.1 Introduction

The growing concern over climate change and the urgent need to mitigate its impacts have spurred interest in innovative strategies to reduce atmospheric carbon (C) levels. Soils are the planet's largest reservoir of organic carbon (SOC), but widespread degradation due to human activities has reduced their capacity to store C (Lal, 2004b; Smith, 2012). This gap between soils' potential C storage and current levels is now viewed as an opportunity to enhance SOC sequestration, positioning soils as vital C sinks capable of helping mitigate climate change (Bruni et al., 2021; Martin et al., 2021; Minasny et al., 2023).

Restoring C in agricultural soils through carbon farming (CF) has thus emerged as a promising approach within the broader framework of climate-smart agriculture. CF is a business model designed to reward land managers for adopting agricultural practices specifically designed to capture and store CO₂ in soils, plants, and ecosystems, thereby reducing greenhouse gas (GHG) emissions and sequestering C (COWI et al., 2021). Various CF practices have been tailored to different land uses, including agroforestry systems, peatlands, grasslands, and mineral soils. Among these, croplands on mineral soils have been the primary focus of CF research, largely due to their widespread global distribution and significant C sequestration potential (Bruni et al., 2021; Peralta et al., 2022). A key component of CF is the adoption of regenerative agriculture principles at the farm level. These principles include minimizing soil disturbance, maximizing crop diversity, keeping the soil covered, maintaining living roots year-round, and integrating livestock or biogas systems where feasible to create closed nutrient loops. Regenerative practices that are commonly eligible as CF practices are, perennial and agroforestry systems, cover cropping, improved crop rotation, no-till or reduced tillage, smart fertilizer management, incorporation of crop residues, and the application of organic amendments (Brummitt et al., 2024; Paul et al., 2023; Paustian et al., 2016). These practices foster C capture and minimize C losses by promoting SOM accumulation, increasing C input to the soil, and improving soil structure, which enhances the soil's capacity to store C over time (Lal, 2009; Paustian et al., 2016). In addition to C sequestration, these practices provide multiple co-benefits, particularly by enhancing soil functions and fertility through the contributions of soil organic matter (SOM). These include improved nutrient cycling to sustain soil fertility and crop productivity, increased plant-available water-holding capacity, greater aggregate stability to mitigate soil compaction and runoff risks, and enhanced soil biodiversity (Lal, 2004a; Paustian et al., 2016). These agronomic benefits also translate into economic advantages, such as reduced costs for nutrient replacement, enhanced productivity, and lower fuel consumption for tillage.

Although CF has gained considerable attention as a climate mitigation strategy, it has also faced various criticisms and challenges (Baveye, 2020; Moinet et al., 2023; Oldfield et al., 2021). One of the primary concerns is the uncertainty around the permanence of C sequestration in agricultural soils, as the risk of re-releasing stored C due to land-use changes or disturbances undermines the long-term impact of CF initiatives (Dynarski et al., 2020). Other challenges involve biophysical limitations, such as the limited availability of C input to soil from roots, exudates or crop residues, and the potential for SOC storage to reach saturation (Bruni et al., 2021; Martin et al., 2021; Moinet et al., 2023). Additionally, social and economic barriers, including the high costs of implementation and the technical feasibility of agronomic practices aimed at sequestering C, may hinder the widespread adoption of these practices (Baveye, 2020; Oldfield et al., 2021). To address this challenge, various private and public entities, along with carbon registries, have launched programs to generate agricultural carbon credits, recognizing the potential of CF practices to sequester C in agricultural soils and incentivizing farmers for their adoption. To fully unlock the potential of CF, scaling up the implementation of these practices through expanded programs is crucial, ensuring broader adoption and maximizing their impact on soil C storage and climate mitigation.

CF programs typically involve defining specific practices for C sequestration, setting measurable performance standards, and establishing verification and monitoring systems to ensure the accurate C credits generation and credible reporting (Batjes et al., 2024). The successful implementation of CF programs relies on the ability of program developers to issue transparent, credible certified units of CO₂ removal, which can be sold in voluntary carbon markets (offsetting) or claimed by agri-food companies within supply chains (insetting). Accurate, scalable, and cost-effective Measurement, Reporting, and Verification (MRV) approaches are therefore crucial for ensuring the credibility and effectiveness of CF programs, enabling stakeholders to track C sequestration outcomes, verify emissions reductions, and qualify for carbon credits or financial incentives (Batjes et al., 2024; Brummitt et al., 2024; Guan et al., 2023; Jackson Hammond et al., 2021). Depending on the CF practice, project scale, and available technology, various MRV methods are used, including field-based measurement (e.g. soil and plant biomass sampling), remote or proximal sensing technologies, process-based modeling, carbon accounting tools, and hybrids approaches (Guan et al., 2023; Smith et al., 2020). Each approach involves a trade-off between cost and accuracy, with the chosen solution needing to balance cost-effectiveness and the required accuracy for scalability, ensuring the generation of certified units across large areas enrolled in the program (Guan et al., 2023). However, accurate MRV of C removals faces significant challenges due to variability in soil

properties, management practices, and environmental conditions, which necessitate harmonized approaches to enhance accuracy and reduce uncertainty (Batjes et al., 2024; Lavallee et al., 2024; Oldfield et al., 2021). Disparities in modeling workflows, uncertainty quantification methods, and practices between different projects further exacerbate inconsistencies in results. Additionally, the lack of standardization in emerging carbon markets increases financial uncertainties, while inconsistent policy frameworks and the absence of unified protocols hinder the widespread adoption and integration of CF practices into broader climate strategies (Oldfield et al., 2021).

To address these challenges, the European Carbon Removal Certification Framework (CRCF) seeks to establish a robust and transparent system for certifying C removals in CF initiatives (E.C., 2022). The framework prioritizes high-quality carbon credits by ensuring stored C is measurable, verifiable, and permanent (Paul et al., 2023; Smith, 2012). The EU has thus proposed quality standards based on four criteria under the Q.U.A.L.I.T.Y acronym: Quantification (accurate measurement), Additionality (ensures that C removals result directly from specific interventions and represent new, measurable reductions in CO₂, rather than outcomes that would have occurred under "business-as-usual" practices.), Long-term storage (ensuring stability of sequestered C over time), and Sustainability (aligning projects with environmental and socio-economic goals).

The CRCF emphasizes the adoption of Tier-3 methodologies, which require generating modeled baselines and project scenarios using hybrid MRV approaches. These approaches integrate field-based measurements, remote sensing (RS) technologies, and process-based modeling to achieve accurate, scalable, and cost-effective monitoring of C removals (Batjes et al., 2024). Baselines in CF serve as critical reference points, representing the Business-as-Usual (BAU) scenario, or what would happen to SOC without intervention. They can be categorized as: standardized (i.e. derived from average trends in databases), specific (i.e. based on concrete data from agricultural practices and environmental conditions, often including field measurements), static (i.e. established at the project's start and unchanged throughout) or dynamic (i.e. periodically updated to account for changing conditions over the project duration). Activity-specific and dynamic baselines are preferred over standardized ones, as they better capture temporal variations and the impacts of management interventions, providing a more accurate assessment of C sequestration outcomes (Batjes et al., 2024).

The CRCF regulation addresses two main types of certified units: i) the Net Carbon Removals (NCR) that represent the additional C sequestered in soils compared to a baseline scenario and ii)

Associated Emission Reductions (AER) that include reductions in GHG emissions achieved through improved land management practices, compared to a baseline scenario.

Industrial tomato production in the Emilia-Romagna region is a key supply chain facing significant declines in SOM. Intensive agricultural practices in the area have accelerated SOM depletion, threatening soil health, and the long-term sustainability of these systems. To counter these challenges and enhance SOC sequestration, regenerative agricultural practices are being implemented on lead commercial farms (LCFs) in the region through the TomaTO-C living lab initiative. This network, established over the past four years, has emerged from a series of EIP-AGRI and EU-funded projects, including ClieNFarms (<https://clienfarms.eu/>) and Farms4Climate (<https://www.farms4climate.eu/>). For this study, five LCFs participating in the ClieNFarms project were selected. On these farms, a series of CF practices were co-designed through a collaborative process involving farmers, industry stakeholders, agronomists, and researchers, ensuring practical and scalable solutions tailored to regional conditions and supply chain demands. These practices include: (i) diversifying crop rotations by adding maize to the traditional wheat-tomato rotation to add C-rich biomass (Burney et al., 2010), (ii) integrating winter cover crops that protect the soil from erosion and provide additional C input (Poeplau and Don, 2015), (iii) adopting reduced tillage to maintain soil structure and prevent the rapid decomposition of SOM (Abid and Lal, 2009), and (iv) implementing integrated residue and organic nutrient management, which also considers the “hidden costs” of nutrient imbalances within carbon trading schemes (Richardson et al., 2014). These practices aim to simultaneously increase plant-derived C inputs to the soil, reduce SOC losses (Lal, 2004b), and activate the “microbial carbon pump” (Kallenbach et al., 2019; Liang et al., 2017), enhancing the soil’s ability to retain SOC.

To date, much of the literature on SOC sequestration has focused on experimental plots where sequestration rates are quantified under small scales. However, SOC sequestration at the farm level has rarely been monitored, reported, or verified as net C removal against an actual farm baseline (Brummitt et al., 2024; Mathers et al., 2023). Furthermore, the generated net C removal units have seldom been assessed for compliance with certification standards, such as those outlined by FAO, Verra, or the Q.U.A.L.I.T.Y criteria specified in the CRCF regulation (Brummitt et al., 2024; Mathers et al., 2023). This lack of scientific evidence regarding the accuracy and scalability of MRV approaches, as well as the real potential of CF practices to generate certifiable C removal units under the CRCF, limits project developers in two key ways: 1) it hinders their ability to implement MRV tools effectively in real-world CF programs, and 2) it restricts their capacity to engage farmers proactively

through ex-ante simulations of CF practices' potential against accurate baselines. There is an urgent need to validate hybrid MRV approaches at the farm scale, evaluate the eligibility of practices against CRCF quality criteria, and verify modeled estimates of C removals at scale. Only through this validation can certified units be issued for use in Voluntary Carbon Markets (VCM) or within insetting programs, such as Scope 3 reporting aligned with SBTi and GHG LS&R protocols. These efforts are critical to scaling up CF practices and enhancing their credibility in the global carbon markets.

This paper aims to evaluate the eligibility of CF practices for future crediting in Northern Italy, assess their associated co-benefits, integrate MRV methodologies to ensure the scalability and reliability in CF programs, and testing the potential of such practices to generate carbon credits under the CRCF. The eligibility criteria for testing CF practices are aligned with the CRCF regulation, while the criteria for verification and validation of MRV tools are tested to conform with the VERRA methodology (VM0042). Specifically, for the "Quantification" criteria, a dynamic, activity-specific baseline was established, and NCR from CF activities were estimated using a hybrid modeling approach. This approach integrates remote sensing technology to estimate crop biomass with the RothC biogeochemical soil model to simulate SOC dynamics. A smart soil sampling method was also employed to identify sample locations across heterogeneous areas, both to initialize and validate the model. Once the effectiveness of CF practices in removing C compared to the baselines was confirmed, AER in terms of direct and indirect CO₂ equivalent emissions, were calculated using the Cool Farm Tool (Hillier et al., 2011). The final certified units were then computed based on these estimates. The "Additionality" criterion was discussed in this context by evaluating whether the adoption of these CF practices would result in C removals that surpass those expected under business-as-usual scenarios. For "Long-term storage," the durability of C sequestration was evaluated by measuring changes in mineral-associated organic carbon (MAOM-C), which represents a stable pool of SOC less susceptible to rapid decomposition (Cotrufo et al., 2019). To address potential leakage, crop productivity was monitored using remote sensing, while organic amendments applied in the project are produced within the project area. This ensures that CF practices do not inadvertently shift emissions to other parts of the system or threat food security, thus maintaining the integrity of claimed C benefits. Finally, to meet the "Sustainability" criterion, the study also reports on voluntary co-benefits, which encompass improvements in soil health, nutrient cycling, aggregate stability, and water retention. These co-benefits are crucial as they highlight the broader ecological advantages of regenerative practices, beyond C sequestration, fostering multifunctional agroecosystems that are more resilient to climate change.

3.2 Materials and Methods

3.2.1 Experimental sites

The data for this study were collected from five LCFs (1-5) located in the Piacenza Province in northwestern Italy, covering a total of 30 hectares. For each LCF, two adjacent fields were selected (Fig. S3.1): one served as a reference plot for establishing a dynamic baseline, representing the typical agricultural practices adopted on the entire farm (baseline scenario – Bsl), while the other was managed using regenerative agricultural practices as part of the Carbon Farming project (CFp) over a span of three consecutive years. In this region the climate is continental with an average annual rainfall of 863 mm (1992-2022), and rainfall peaks occurring in autumn and spring. The location of LCFs and their characteristics are presented in Figure S3.1 and Table 3.1.

Table 3.1 Characterization of the selected Lead Commercial Farms (LCFs) for the study, with soil sampling conducted at a depth of 0–30 cm.

Site	LCF1	LCF2	LCF3	LCF4	LCF5
Hectares in Bsl CFp fields	3.14 3.19	1.22 3.85	2.98 2.51	2.64 1.35	5.11 4.93
Latitude	44.98283	45.04313	45.05865	45.03166	45.02377
Longitude	9.70153	9.9273	9.96232	9.88822	9.82473
Soil textural class	Silty Clay Loam	Silt loam	Silty Clay	Silty Clay Loam	Clay
Initial SOC stocks (Mg C ha⁻¹)	60.4	21.9	53.9	50.5	54.4
Soil C:N	7.3	6.9	7.5	8.8	7.4
Soil P (ppm P₂O₅)	34	45	30	32	34
Soil K (ppm K₂O)	177	144	257	275	345
Soil CaCO₃ (%)	1.3	0	2.4	17.4	7.8
Soil pH	6.3	6.1	7.8	7.8	7.8

The fields selected in the LCFs had been subjected to a decade-long narrow crop rotation, primarily involving winter wheat (*Triticum aestivum* L.) and tomatoes (*Solanum lycopersicum* L.), with occasional inclusion of silage maize in the rotation. The management practices included the application of mineral fertilizers and deep ploughing as the main soil tillage method. However, the field of LCF1 selected for the Bsl was cultivated for five years (2012-2017) with alpha alpha (*Medicago sativa*). The regenerative practices applied in the CFp differed from the Bsl scenario in the following ways (Table 3.2): (i) an enriched crop rotation incorporating maize alongside the conventional wheat-tomato rotation, (ii) the integration of winter cover crops, (iii) residues incorporation with the stoichiometric balanced addition of organic fertilizers to foster the humification efficiency, and (iv) the adoption of minimum tillage. Detailed information on the

agricultural management is presented in the supplementary Dataset S3.1. While reduced tillage, cover crops sowing, and residue incorporation with organic fertilizers were applied on all five farms, the enriched crop rotation was implemented on only two of the farms, with the remaining three maintaining a conventional main-crop rotation (Table 3.2). Among cover crops, white mustard (*Sinapis alba* L.) was sown in September 2022 and selected for its high biomass productivity and larger root system compared to other species (Blanco-Canqui and Ruis, 2020) in order to account for high C-input. In addition, a mixture of cover crops, consisting of 42% oats (*Avena sativa*), 18% tillage radish (*Raphanus sativus*), 24% pea (*Pisum sativum*), 9% crimson clover (*Trifolium incarnatum*) and 7% squarrose clover (*Trifolium squarrosum*), was sown in October 2023 with the aim of improving soil structure and aeration.

Table 3.2 Summary of the experimental design illustrating the main differences in management practices between the Baseline scenarios (Bsl) and the Carbon Farming program scenario (CFp) in each Lead Commercial Farm (LCF).

Note: Exp. = exported; Inc. = incorporated

LCFs	Years	Crop rotation		Tillage		Residues management	
		Bsl	CFp	Bsl	CFp	Bsl	CFp
LCF1	2022	Wheat	Wheat – white	Deep plowing	Minimum tillage	Exp. for wheat and not inc. in maize	Wheat and maize inc with balanced nutrient addition + inc of white mustard and mix of cover crops
	2023	Maize	mustard				
	2024	(Tomato)	Maize - mix cover crop (Tomato)				
LCF2	2022	Wheat	Wheat - white	Deep plowing	Minimum tillage	Exp. for wheat and not inc in maize	Wheat and maize inc with balanced nutrient addition + inc of white mustard and mix of cover crops
	2023	Maize	mustard				
	2024	(Tomato)	Maize - mix cover crop (Tomato)				
LCF3	2022	Wheat	Wheat - white	Deep plowing	Minimum tillage	Exp. for wheat	Wheat inc with balanced nutrient addition + inc of white mustard and mix of cover crops
	2023	Tomato	mustard				
	2024	(Maize)	Tomato - mix cover crop - (Maize)				
LCF4	2022	Wheat	Wheat - white	Deep plowing	Minimum tillage	Exp. for wheat	Wheat inc with balanced nutrient addition + inc of white mustard and mix of cover crops
	2023	Tomato	mustard				
	2024	(Maize)	Tomato - mix cover crop - (Maize)				
LCF5	2022	Wheat	Wheat - white	Deep plowing	Minimum tillage	Exp. for wheat	Wheat inc with balanced nutrient addition + inc of white mustard and mix of cover crops
	2023	Tomato	mustard				
	2024	(Maize)	Tomato - mix cover crop - (Maize)				

3.2.2 Soil sampling and samples preparation

Soil samples were collected five times throughout the experiment, for a total monitoring period of 29 months. The first sampling was conducted before wheat sowing (T_0 - October 2021), while the second occurred a few weeks after the incorporation of wheat residues (T_1 - September 2022). The subsequent samples were taken following the incorporation of white mustard (T_2 - March 2023), harvest of maize/tomato (T_3 - August 2023), and incorporation of the cover crop mixture (T_4 - April 2024). Soil samples at T_0 were used to initialize the model, while samples taken at T_1 , T_2 , T_3 and T_4 were used to verify and validate the model. Soil sample locations at T_0 and T_1 were identified by applying the conditioned latin hypercube sampling (*clhs*) algorithms (Minasny and McBratney, 2006) based on the monthly mean values of vegetation indices (NDVI) from Sentinel-2 over the last three cropping seasons. This approach allowed for the definition of $k = 3$ strata per field. Within each stratum, one sampling point was selected via *clhs*. At T_0 and T_1 , two soil cores were collected from a depth of 0–30 cm for each stratum and bulked together, resulting in a total of $n = 30$ samples. At T_2 , T_3 , and T_4 , soil samples were collected using a grid-based sampling strategy (30 x 30 m) to validate the spatial field SOC mean estimated by the model. All soil samples were air-dried and uniformly ground ($< 53 \mu\text{m}$) for analysis of total SOC and soil N using an elemental analyzer (Primacs100, Skalar Analytical B.V). In addition, three subsamples per field ($n = 30$) were frozen for microbiological and chemical analysis, ensuring consistency in sampling locations for each sampling time point. Another set of three subsamples, collected from the same locations, was air-dried, ground, and sieved at 2 mm for soil physical analysis. Multiple bulk density (BD) measurements were performed over time at all experimental sites. Significant changes of BD with time and between treatments were found. SOC stocks were thus calculated by multiplying SOC concentration by the equivalent soil mass (ESM) for a reference soil mass layers of 3860 Mg ha^{-1} (Wendt & Hauser, 2013). ESM's (Mg ha^{-1}) were calculated using a cubic spline function to provide estimates that correspond to 0–30 cm soil depth. SOC sequestration was expressed as SOC stock variation ($\Delta\text{SOC stock in Mg ha}^{-1}$), calculated as the difference between SOC stock measured at the end and the beginning of the experiment, while yearly SOC sequestration rate ($\text{Mg ha}^{-1} \text{ year}^{-1}$) was calculated dividing the $\Delta\text{SOC stock Mg ha}^{-1}$ by the years of the experiment.

3.2.3 “Quantification” criteria

3.2.3.1 Application of the RothC model for quantification of C removals

SOC dynamics were simulated by applying a modified version of the Rothamsted soil carbon model (RothC) that account for diverse exogenous organic matter (EOM) inputs (Ferrarini et al., 2022; Mondini et al., 2017). A detailed description of the original version of the model can be found in (Coleman and Jenkinson, 1996). The model was run for each field, using field-specific input parameters. Information on input parameters required by the model (i.e. land use cover, monthly C inputs from plant material and EOM, weather data, clay content (%), initial SOC stock (Mg C ha^{-1}), soil layer depth considered (cm), SOC stock distribution between C pools (%), and DPM/RPM ratio) can be found in Supplementary files (Databases S2). Land use cover and amount of EOM (digestate) were retrieved from farmer crop declaration using the Land-parcel identification system (LPIS) and field notebooks. Weather data, that includes monthly values of temperature ($^{\circ}\text{C}$), precipitation (mm), and potential evapotranspiration (mm), were measured by a nearby automated Watchdog 2000 Series (Spectrum Technologies, 3600 Thayer Court, Aurora, IL, USA) weather station installed in LCF1 and LCF2. Crop yields used to calculate plant C input were estimated from remote sensing technology by applying a Light Use Efficiency (LUE) model, which methodology is presented in Supplementary Material. Plant inputs to 0-30 cm soil layer were then transformed to C input via allometric functions, following the method of Bolinder et al. (2007). The harvest index (HI) and the shoot:root ratio (S:R) used in the formulas derive from in-situ field data collection as described in Supplementary Material. Initial soil data were retrieved from analysis of soil samples at T_0 and use as average of three sampling point per field. Differently to the original version of the model, temperature rate modifier factor was adjusted to 40 and RPM rate constant was lowered to 0.2 compared to the parameters proposed by Farina et al. (2013). These adjustments were made through trial-and-error calibration using 30% of the sampling points to better align with SOM degradation rates observed in Northern Italy. Moreover, a DPM/RPM of 0.69 was considered when cover crops were incorporated, as suggested by Dechow et al. (2019) for green manure. In fields where CF practices were applied, a modified factor for reduced tillage, with a value of 0.95 was applied according to Jordon & Smith (2022). Wheat and maize residues, as well as bio-digestate, were considered EOM and their partitioning factor (f) and decomposition rate (k) identified by Mondini et al. (2017) are included in each database.

RothC model was initialized for 100 years by assuming that the soil is at a steady state under the initial land use at the start of the simulation. An historical averaged farm crop yield declared by

farmers relative to the last 5 years was used for the model initialization. Weather data from the last 30 years were downloaded from JRC Agri4cast Resource Portal using weather station close to the experimental sites (<https://agri4cast.jrc.ec.europa.eu/DataPortal/Index.aspx>). To account for the different crop rotations over the 10 years prior to the experiment's start in Bsl fields of LCF1, the RothC model was initialized according to the previously described methodology up to 2012. From 2012 to 2022, the model simulated five consecutive years of alfalfa cultivation, followed by a conventional wheat-tomato rotation to reflect the historical crop sequence. The simulated SOC stock at this stage was then used as the starting point for model projections beginning in 2022.

3.2.3.2 Statistical analysis, evaluation of model performances, and uncertainty calculation

Changes of SOC stocks (Mg C ha^{-1}) were analyzed using a difference-in-differences (DiD) approach, with the R package *p/m*. This method, commonly applied in quasi-experimental studies to estimate causal effects, was chosen here to account for dynamic, activity-specific baselines, as also suggested by (Bradford et al., 2023). The DiD approach was used to compare SOC stock changes between treatment (CFp) and control (Bsl) groups across two time periods: before project initiation (T_0) and at project completion (T_4). SOC stock changes were considered significant when the interaction term (*treatment*time*) had a p-value of <0.05 . Model accuracy and performance were assessed according to the *VCS Module (VMD0053, version 2.0)* for both scenarios (Bsl and CFp) and for the whole project, by calculating the root mean square error (RMSE) to measure total error, relative error (RE%) to evaluate bias, the regression coefficient (R^2) to assess correlation between measured and modeled data, and model efficiency (ME) (Tab. 3.3). Observed SOC stock values (both at each sampling time point and as ΔSOC stock) computed as the mean of selected sampling points were used as the verification dataset for the model, while SOC stock values averaged from grid sampling served as the validation dataset. To assess whether the proposed approach meets the requirements for independent validation by a third-party certification body (with the VM0042 Verra protocol as a reference), the model's mean bias (calculated as the unweighted average of all biases) was compared to the pooled measurement uncertainty (PMU). This comparison was made using the five LCFs as a CF program, where each study pair consisted of a CF plot and a Bsl plot. Additionally, scatterplots comparing observed vs. model-predicted values for time series SOC stocks (Mg C ha^{-1}) (Fig. S3.2 a-b) and ΔSOC stocks (Mg C ha^{-1}) (Fig. 3.3 a-b) were generated. 90% confidence intervals for predicted ΔSOC stocks (Mg C ha^{-1}) compared with observed values were also produced (Fig. 3.3 c-d). The approach was deemed “successfully validated” if the absolute model bias was less

than the PMU value and if no more than 10% of observed values fell outside the model's confidence intervals.

Finally, uncertainty was calculated by combining estimates of sampling and modeling error as outlined in the VM0042 Verra protocol. The impact of measurement errors was assumed to be captured in model prediction error and/or sampling error. Errors were computed for each LCF and for the whole project area, expressed as the difference between simulated and observed ΔSOC stocks. The errors were calculated both on a per-hectare basis ($t\ CO_2e\ ha^{-1}$)² and for the entire project area ($t\ CO_2e$)². Model prediction error was quantified using the statistical verification dataset for both the CFp and the Bsl scenario, comparing model outputs with ground-truth measurements of SOC stock changes at the end of the CFp period as follow:

$$S_{model,\Delta SOC_{project}}^2 = 2 * S^2[(\Delta SOC_{pred} - \Delta SOC_{obs})_{CFp} - (\Delta SOC_{pred} - \Delta SOC_{obs})_{Bsl} * (1 - \rho)] \quad (eq. 1)$$

$$S_{model,\Delta SOC_{LCF}}^2 = 2 * S^2[(\Delta SOC_{pred} - \Delta SOC_{obs})_{LCF,CFp} - (\Delta SOC_{pred} - \Delta SOC_{obs})_{LCF,Bsl} * (1 - \rho)] \quad (eq. 2)$$

where

$$\rho = corr [(\Delta SOC_{pred} - \Delta SOC_{obs})_{CFp}(\Delta SOC_{pred} - \Delta SOC_{obs})_{Bsl}] \quad (eq. 3)$$

and:

$S_{model,\Delta SOC_{project}}^2$ is the variance of modeled estimates of ΔSOC stock across the entire project area; $S_{model,\Delta SOC_{LCF}}^2$ is the variance of modeled estimates of ΔSOC stock in each LCF; $S^2(\Delta SOC_{pred} - \Delta SOC_{obs})$ is the estimated variance of errors made by model prediction of ΔSOC , estimated from fields measurements in both the CFp and Bsl scenarios; ρ is the correlation between model prediction errors of ΔSOC in the CFp and Bsl scenarios.

Sampling error was determined by calculating the variance of ΔSOC stocks as simulated by the model for CFp and Bsl for the entire CFp period:

$$S_{sampling,\Delta SOC_{project}}^2 = \sum_{LCF=5}^H S_{sampling,\Delta SOC_{LCF}}^2 \quad (eq. 4)$$

Where:

$$S_{sampling,\Delta SOC_{LCF}}^2 = \frac{A_h^2}{n_h(n_h-1)} \sum_{ip=1}^{n_h} (\Delta SOC - \overline{\Delta SOC})^2 \quad (eq. 5)$$

and:

$S^2_{sampling,\Delta SOC,project}$ is the variance of ΔSOC ($t\ CO_2e\ ha^{-1}$)² due to sampling error across the entire project area, computed as the sum of $S^2_{model,\Delta SOC,LCF}$ in the CFp and in the Bsl scenarios; $S^2_{model,\Delta SOC,LCF}$ is the variance of estimated ΔSOC ($t\ CO_2e\ ha^{-1}$)² within the fields of an LCF (both in CFp and Bsl) due to sampling error; $\bar{\Delta SOC}$ is the areal mean of ΔSOC ($t\ CO_2e\ ha^{-1}$) within a specific LCF field (in CFp or Bsl), computed as the mean across the sample points collected in that field; ΔSOC_{ip} is the estimated ΔSOC ($t\ CO_2e\ ha^{-1}$) at a specific sample point location (ip); n_h is the number of sample points collected in a field; and A_h is the area of the field in hectares. Both $S^2_{sampling,\Delta SOC,project}$ and $S^2_{model,\Delta SOC,LCF}$ were divided by the area of the CFp and the Bsl scenario for each LCF and for the entire project area to calculate sampling errors on an area ($t\ CO_2e$).

The total variance of ΔSOC , expressed either as $t\ CO_2e\ ha^{-1}$ or $t\ CO_2e$, across the project area and for each LCF during the verification period, was estimated by combining the variances of both error sources as follow:

$$S^2_{total} = S^2_{sampling,\Delta SOC} + S^2_{model,\Delta SOC} \quad (\text{eq. 6})$$

To standardize the overall uncertainty of the MRV approach, the standard deviation was calculated from the total variance, providing the uncertainty in $t\ CO_2e\ ha^{-1}$ or $t\ CO_2e$.

3.2.3.3 Quantification of GHG emissions using the Cool Farm Tool

GHG emissions were assessed using a Tier 1 approach with the Cool Farm Tool (CFT) (Hillier et al., 2011) for both Bsl and CFp of each LCF included in this study. The CFT is a GHG calculator aiming to quantify on-farm GHG emissions from various agricultural activities, including residue management, tillage, irrigation, and the application of fertilizers and pesticides. Crop yields used in the tool were estimated through the LUE model (see Supplementary Material), while the amount of plant residues derive from allometric functions calculation (Bolinder et al. 2007). Fuel consumption per machinery operation was customized using real data provided by farmers. In addition to the model's required inputs, a default factor of $2\ kg\ N_2O\ ha^{-1}$ ($600\ kg\ CO_2e\ ha^{-1}$), deriving from measured in-fields emissions (Ardenti et al., 2022; Fiorini et al., 2020) was added to the baseline scenario when soils were left bare. GHG emissions are reported in this study as tons of CO_2 equivalent (CO_2e) per hectare. Detailed description of input data can be found in the Supplementary Material (Dataset S3.1).

3.2.3.4 Calculation of carbon credits

For the calculation of carbon credits, "Associated Emission Reduction" (AER) and "Net Carbon Removals" (NCR), which represent two main types of certified units, were calculated according to

CRCF EU regulation guidelines. NCR credits represent the net carbon that has been temporarily sequestered or stored in soil. NCR is calculated based on estimated ΔSOC stocks ($Mg\ C\ ha^{-1}$) using the RothC model, by comparing Bsl levels (ΔSOC_{Bsl}) to those achieved after CFp implementation (ΔSOC_{CFp}) for each LCF. The formula for calculating NCR is:

$$NCR = (\Delta SOC_{Bsl} - \Delta SOC_{CFp}) \quad (\text{eq. 7})$$

SOC stock values were then converted to CO_2e ($Mg\ CO_2e\ ha^{-1}$) by multiplying by a factor of 3.67, which represent the atomic mass ratio of CO_2 to C.

When a positive value of NCR was obtained (indicating effective C removal), AER credits were calculated based on the reduction of GHG emissions compared to the Bsl scenario. The difference in emissions between the Bsl scenario (E_{Bsl}) and the CFp (E_{CFp})'s activities for each LCF was expressed in CO_2e emitted ha^{-1} and calculated using the Cool Farm Tool. For the calculation of emissions, only on-site emissions were considered, including those derived from residues management, energy use for machinery operation, soil fertilizers application, and crop protection. The ER was calculated as follow:

$$AER = E_{CFp} - E_{Bsl} \quad (\text{eq. 8})$$

AER and NCR credits were then combined into a single certified unit (C.U.), as follow:

$$\frac{\sum_{LCF}(NCR - AER)}{n_{LCF}} > 0 \quad (\text{eq. 9})$$

3.2.4 Long-term storage criteria

3.2.4.1 Soil organic matter fractionation, C and N analysis of soil fractions

Air-dried and 2 mm sieved sub-samples were used for SOM fractionation and quantification of C and N in the resulting soil fractions, following the method of Zhang et al. (2021). The fractions obtained were particulate organic matter (POM) and mineral associated organic matter (MAOM). Both fractions were analyzed for C and N content using an elemental analyzer (Primacs100, Skalar Analytical B.V). The results are expressed as MAOM-C and MAOM-N, for total C and N content in MAOM, and POM-C and POM-N for total C and N content in POM, respectively.

3.2.5 Carbon Leakage issues assessment

In assessing potential leakage emissions — those unintended increases in emissions outside the CF project boundary due to project activities — this study accounted for two primary factors: use of local organic fertilization and monitoring crop productivity. Addition of organic amendment within the CF project was implemented using bio-digestate sourced from biogas plants located within the

project area. This approach minimizes the risk of potential C leakage, which could occur if organic amendments were imported from outside the project area, potentially increasing emissions elsewhere. By sourcing the bio-digestate locally, indirect emissions associated with transport and redistribution of organic matter are avoided. Therefore, this factor will not be considered further in the assessment and discussions. Crop productivity was estimated and compared between Bsl and CFp scenarios using the Light Use Efficiency (LUE) model. According to the *VCS Methodology (VM0042)*, productivity declines below 5% are considered "de minimis" (insignificant) and can therefore be ignored. This threshold helps ensure that minor productivity shifts do not trigger compensatory activities (like increased cultivation or inputs outside the project area) that could lead to leakage emissions.

3.2.6 "Sustainability" criteria: voluntary co-benefits

According to CRCF regulation, CF activities must ensure co-benefits that align with sustainability objectives, including biodiversity restoration, ecosystem protection, soil health enhancement, and land degradation prevention. These mandatory co-benefits are listed in the *EU Nature Restoration Regulation*, (2024) Article 11, which claim that Member States should aim for measurable improvements in at least two of three agricultural ecosystem indicators:

1. Organic carbon stock in cropland mineral soils.
2. Agricultural land with high-diversity landscape features.

Activities that enhance soil carbon sequestration or reduce emissions from mineral soils inherently improve organic carbon stocks, fulfilling the mandatory co-benefit requirements for soil health and land degradation prevention.

In addition to this mandatory outcome, several voluntary co-benefits were quantitatively assessed and are discussed in detail in the following sections.

3.2.6.1 Assessment of Water-Holding Capacity Improvement through Water Infiltration and Available Water Capacity

Water infiltration (ifs) was measured on-site in each field in October 2022 (T_0) and August 2024 (T_4) using the rainfall-simulation technique described by van Es & Schindelbeck (2003). A portable rainfall simulator was placed over an infiltration ring, which was inserted 75 mm into the soil, and equipped with a runoff tube at the soil surface to guide runoff water into an external beaker. The steady infiltration rate (cm min^{-1}) was calculated as the difference between the measured rainfall and runoff rates once steady infiltration was reached, following the method outlined by van Es &

Schindelbeck, 2003. The available water capacity (AWC in mm) was measured in laboratory on soil samples collected at T₀ and T₄ following the protocol described in Moebius-Clune et al. (2016).

3.2.6.2 Assessment of soil structure improvement via aggregate stability

To determine the water stability of soil aggregates, the wet-sieving method proposed by Six et al. (2000) was adapted for use with a wet sieving apparatus (Eijkkelkamp). Briefly, 4 g of air-dried soil sub-samples (> 2mm) was moistened on a 250 µm mesh sieve to reach 60% of their water-holding capacity. The samples were then immersed in deionized water and shaken for 3 minutes. The > 250 µm aggregates were collected, and sieving was repeated for > 53 µm fraction. All aggregate fractions were oven dried (65 °C) and weighed.

The mean weight diameter (MWD) was calculated to reflect the soil aggregate stability using to the following equation:

$$MWD = \frac{\sum_i^n x_i w_i}{\sum_i^n w_i} \quad (\text{eq. 10})$$

where w_i and x_i denote the proportion (%) and mean diameter (mm) of each aggregate size fraction, respectively.

3.2.6.3 Assessment of soil nutrients availability and synchrony through analyses of soil nutrients and their mineralization rate

The nitrate (NO₃⁻), ammonium (NH₄⁺) and phosphate (PO₄³⁻) content (mg kg⁻¹) in frozen soil sub-samples was quantified at each sampling time. Nutrient extraction was performed with 0.5 M K₂SO₄ after shaking for 1 hour. Nutrients were determined using the colorimetric methods of Doane & Horwath (2003) for nitrate, Lajtha et al. (1999) for phosphorous, and Rhine et al. (1998) for ammonium. Ion-exchange resin bags were placed in each field immediately after the sowing of the cover crop mixture to measure mineralization of soil nutrients. 4 g of mixed bed exchange resin (Amberlite MB- 150) were prepared according to the method proposed by Sherrod et al. (2002). Three replicates per field were installed inside of plastic tubes to isolate them from plant roots. Resin bags were left in the soil for a total of 69 (LCF2 and LCF4) and 83 (LCF1, LCF3, LCF5) days, but they were collected and replaced three times during the growing season to avoid resin saturation. The nutrient content in the resin extracts was determined by colorimetric methods: nitrate (, phosphate (Lajtha et al., 1999), and ammonium (Weatherburn, 1967). Nutrient mineralization rate (mg kg⁻¹ day⁻¹) was calculated by dividing total nutrients concentration (mg kg⁻¹) accumulated in resin bags by the days of their permanence in soil.

3.2.6.4 Assessment of soil health improvement via soil microbial biomass analyses

At T_0 , and T_4 , microbial biomass C (MBC in $\mu\text{g C g}^{-1}$) and N (MBN in $\mu\text{g N g}^{-1}$) concentrations were determined using the chloroform fumigation-extraction method (Vance et al., 1987), with 0.5 M K_2SO_4 as the extractant. MBC and MBN were measured with an elemental analyzer (TOC-L, Shimadzu) and calculated using a conversion factor of 2.22 and 1.85 respectively for MBC and MBN (Wu et al., 1990).

3.2.7 Statistical analysis

Changes in time in the CF project compared to changes in time of Bsl scenario were analyzed using a difference-in-differences (DiD) analysis as explained in section 2.3.2 for MAOM-C (Mg C ha^{-1}), and POM-C (g C kg^{-1}) as well as for the following indicators of “Sustainability”: MBC ($\mu\text{g C g}^{-1}$), Available N and P (mg kg^{-1}), AWC (mm), ifs (cm min^{-1}) and MWD. Differently, differences between Bsl scenario and CF project of N and P mineralization rate ($\text{mg kg}^{-1} \text{d}^{-1}$) and crop productivity (Mg ha^{-1}) were analyzed with a two-way mixed-model ANOVA. LCF and Field (Bsl or CFp) and their interaction (LCF x Field) were considered as fixed main effects with replicates as a random effect. Means were compared using Tukey’s honestly significant difference ($p < 0.05$). Log transformations were performed to satisfy assumptions of normality and heteroskedasticity when needed. Analyses were performed using the *nlme* and *multcomp* R packages. An Heatmap of response ratio values (RR: DiD/CFp at T_0 or CFp/Bsl) of Sustainability indicators was produced using the R package *ggplot2*.

3.3 Results

3.3.1 Quantification of C removals and GHG emissions

3.3.1.1 Quantification and evaluation of SOC variations and permanence

The impact of adopting CF practices on C sequestration measured as ΔSOC stock (Mg ha^{-1}) compared to the Bsl scenario was assessed using both verification and validation datasets, as illustrated in Figure 3.1. In the verification dataset (Figure 3.1a), by using the samples collected for verified modelled estimates, SOC stocks generally increased in CFp scenario over 2.5 years ($+2.42 \text{ Mg ha}^{-1}$). In contrast, SOC stocks decreased in 3 of the 5 LCFs under the Bsl scenario (-0.25 Mg ha^{-1}), although the changes were not statistically significant. However, a decrease in ΔSOC stock was observed in LCF3 under the CFp scenario. In the Bsl scenario, SOC stocks declined in 3 out of the 5 LCFs, although the changes were not significant. In the validation dataset (Figure 3.1b), which consists of a series of grid-sampled points within each field, SOC stocks increased across all LCFs

under the CFp scenario ($+3.62 \text{ Mg ha}^{-1}$) over 2.5 years, whereas in the Bsl scenario, they decreased in 3 of the 5 LCFs (-0.25 Mg ha^{-1}), though not significantly. The patterns of SOC stock dynamics were consistent across both verification and validation datasets, with exceptions of LCF3 and LCF5 under the Bsl scenario. To investigate these divergent trends, the normal distribution of ΔSOC stock values was compared between the Bsl and CFp scenarios for LCF2 (which showed consistent ΔSOC dynamics across datasets) and LCF3 (which showed divergent dynamics) in both datasets (Fig. S3.3). As shown in Figure S3.3, 3.2 out of 3 data points in the CFp scenario and 1 out of 3 for the Bsl scenario for the verification dataset fell within the tails of the normal distribution of the respective validation dataset, suggesting these points do not represent the average ΔSOC stock.

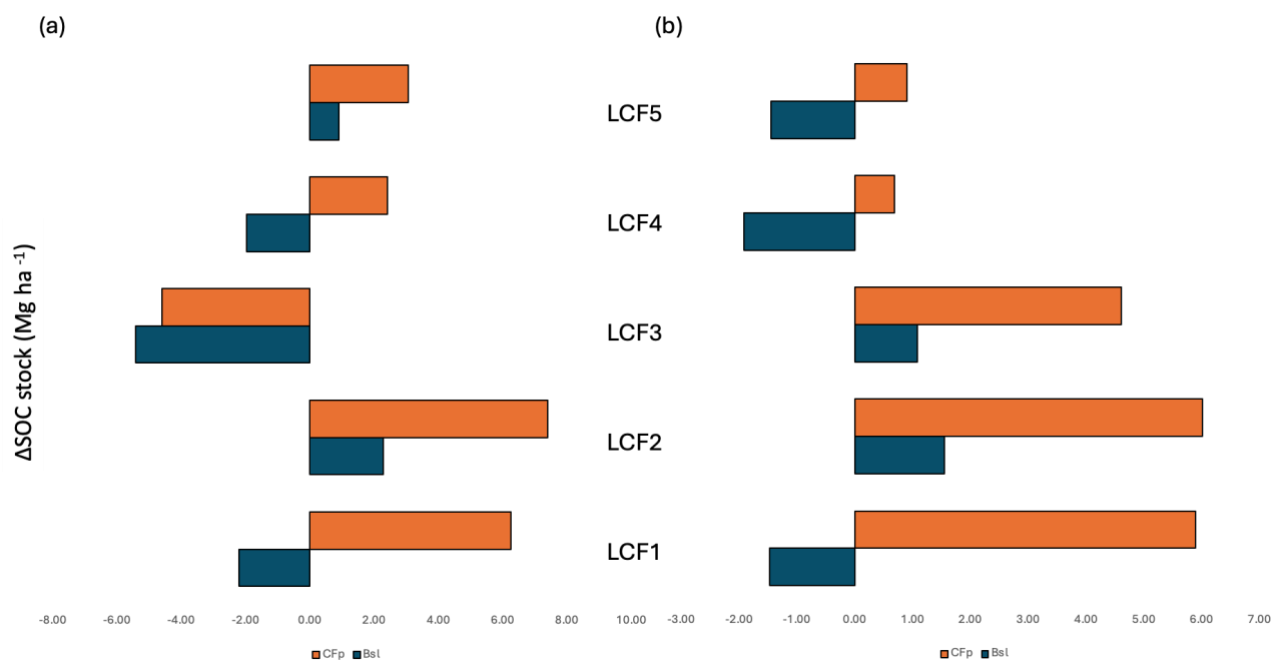


Figure 3.1 Changes in SOC stock over 2.5 years (ΔSOC in Mg ha^{-1}) across five LCFs, calculated on the verification dataset (a) and validation dataset (b). Blue bars show measured ΔSOC stock in the baseline (Bsl) scenarios, while red bars represent measured ΔSOC stock in the Carbon farming project (CFp) scenario. Stars indicate significant differences in the treatment*time interaction term from the DiD analysis ($p < .05$).

Increases in both MOAM-C stock (Mg ha^{-1}) and POM-C content (g kg^{-1}) (Fig. 3.2a-b) contributed to the overall rise in SOC stock across the 5 LCFs, except for LCF3 (for MAOM-C) and LCF1 (for POM-C). Notably, a higher, though not statistically significant, increase in MAOM-C stock was observed in LCF1 and LCF2, which have improved crop rotation through maize integration.

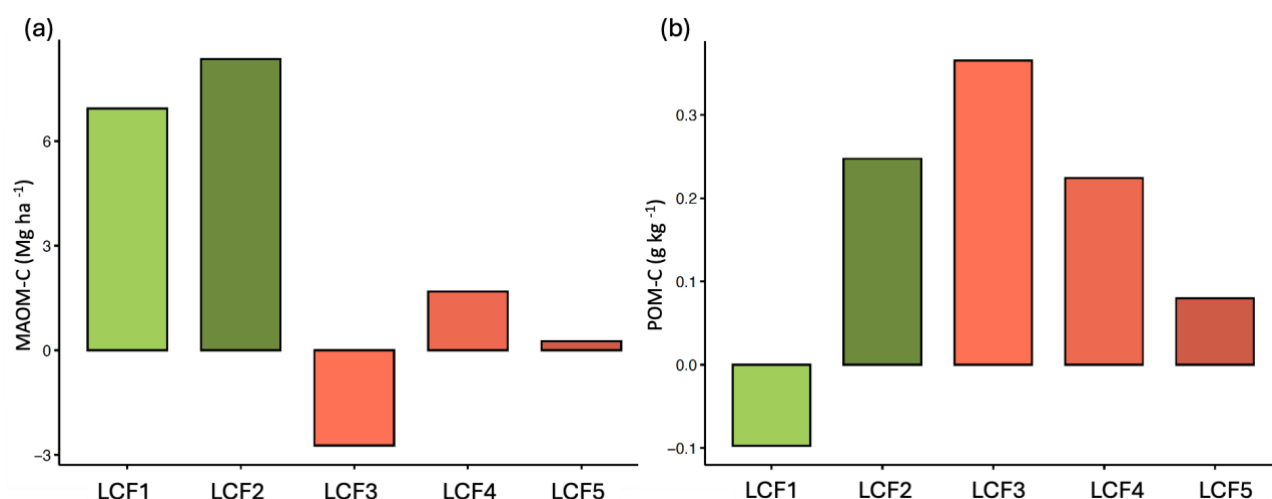


Figure 3.2 Values for the difference-in-differences (DiD) analysis of MAOM-C stock (Mg ha⁻¹) (a) and POM-C content (g kg⁻¹) (b) after 2.5 years from the start of the carbon farming project. Stars denote statistically significant differences in the treatment*time interaction term in the DiD analysis ($p < 0.05$).

3.3.1.2 Simulation of C removals using a remote sensing-based hybrid MRV approach and assessment of model accuracy

A calibrated version of the RothC model, integrated with smart sampling for model initialization and a LUE model to estimate C inputs from crops, was employed to simulate SOC dynamics under both the Bsl and CFp scenarios. The model effectively captured SOC changes for both scenarios (Figure 3.3), with an overall Δ SOC stock of -0.83 Mg ha⁻¹ for the Bsl and +3.70 Mg ha⁻¹ for the CFp scenario, over the 2.5 years. The highest modeled increase in SOC stock under CFp occurred in LCF2 (+8.90 Mg ha⁻¹), while LCF5 under the Bsl scenario showed a decrease (-3.66 Mg ha⁻¹). The model was validated for both Δ SOC stock (Mg ha⁻¹) and SOC stock (Mg ha⁻¹) over 2.5-years period from the beginning of the CF project, using both verification and validation datasets. The validation was assessed using multiple statistical indicators, as summarized in Tab. 3.3, and visually represented by scatter plots (Fig. S3.2 4a-b).

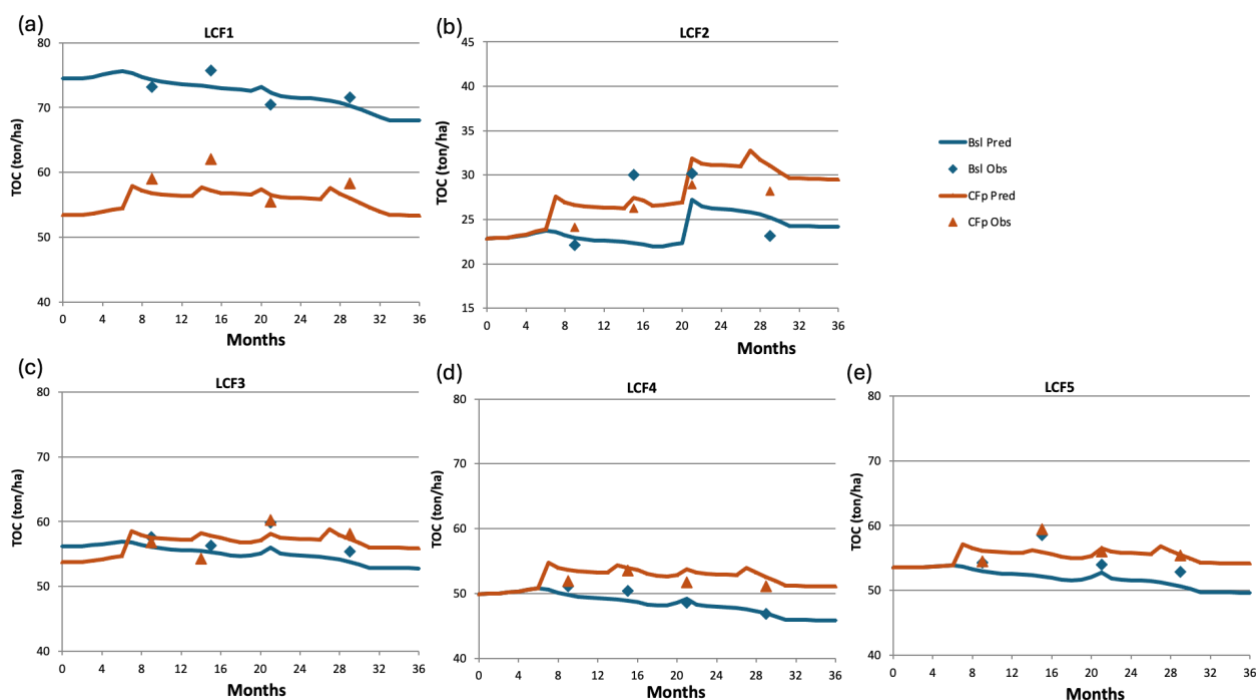


Figure 3.3 Results from the RS-based RothC model simulation of SOC stock (Mg ha^{-1}) over 2.5 years (January 2022 – May 2024) across five LCFs: LCF1 (a), LCF2 (b), LCF3 (c), LCF4 (d), and LCF5 (e). Blue lines indicate SOC stock simulations for the dynamic, activity-specific baseline scenarios, while red lines represent SOC stock for the CF project scenario. The mean SOC stock (Mg ha^{-1}), calculated on an Equivalent Soil Mass (ESM) basis from soil grid samples collected at multiple sampling intervals, is shown for both the baseline (Bsl) scenario (blue diamonds) and the CF project scenario (red triangles).

For model validation, the total RMSE for ΔSOC stock was 1.67 Mg ha^{-1} , with an RE of 44%, an ME of 0.63, and an R^2 of 0.78, indicating a moderate alignment between observed and predicted ΔSOC values. The averaged model bias was $|-0.25| \text{ Mg ha}^{-1}$, which was lower than the uncertainty (PMU of 0.91 Mg ha^{-1}). When simulating SOC stock over time, the model achieved a lower RMSE of 2.55 Mg ha^{-1} , a minimal RE of -1%, a high ME of 0.97, and an R^2 of 0.968, indicating stronger performance in capturing SOC dynamics over time compared to ΔSOC stock. However, the model bias for the SOC stock ($|-0.59 \text{ Mg ha}^{-1}|$) exceeded the PMU (0.46 Mg ha^{-1}). Analyzing by scenarios, the Bsl showed better model efficiency ($\text{ME} = 1$) and an R^2 of 0.68 in predicting ΔSOC stock, though with a relatively high RE of 71% and a bias of -0.57 Mg ha^{-1} . For the CFp, the model performed with an RMSE of 1.72 Mg ha^{-1} , a lower RE of 17%, an ME of 0.99, and an R^2 of 0.63, indicating improved prediction accuracy for ΔSOC under CFp. For SOC stock simulation over time, both scenarios achieved high R^2 values (0.97), reflecting consistent model performance across scenarios. The model bias for SOC stock was -1.48 Mg ha^{-1} for the Bsl scenario and slightly positive at 0.298 Mg ha^{-1} for CFp, both of which were below the PMU threshold.

In the verification phase, the model's performance declined compared to validation results. For total ΔSOC stock, the RMSE increased to 3.73 Mg ha^{-1} , with a negative RE of -153%, an ME of 0.20, and a lower R^2 of 0.34. For SOC stock simulation over time, the total RMSE was 3.19 Mg ha^{-1} , with a

small positive RE of 1%, an ME of 0.95, and an R^2 of 0.946, indicating good performance. Model biases remained below PMU in both cases. When analyzing by scenarios, the Bsl showed an RMSE of 3.17 Mg ha^{-1} , a substantial negative RE of -250%, an ME of 0.95, and a low R^2 of 0.12, with a model bias of $|0.98 \text{ Mg ha}^{-1}|$, which was below the PMU (1.39 Mg ha^{-1}). For the CFp, the ΔSOC stock had an RMSE of 4.23 Mg ha^{-1} , an RE of -56%, an ME of 0.98, and an R^2 of 0.17, with a positive model bias of 1.27 Mg ha^{-1} and a higher PMU of 2.13 Mg ha^{-1} . For SOC stock simulation over time, both scenarios showed high R^2 values (0.97 for Bsl, 0.93 for CFp), indicating strong model performance in tracking SOC dynamics. However, the model biases for SOC stock were -1 Mg ha^{-1} for Bsl and slightly positive (1.56 Mg ha^{-1}) for CFp, both exceeding PMU. At the end of the study, 20% of measured ΔSOC stock from the verification dataset and 10% from the validation dataset were outside the 90% confidence interval of the predicted ΔSOC stocks (Fig. 3.4 c-d).

Table 3.3 Evaluation of the remote sensing based RothC model performances in simulating (a) ΔSOC stock (Mg ha^{-1}) between T_0 and T_4 , and (b) SOC stock (Mg ha^{-1}) along sampling time points from T_0 to T_4 .

Note: Indicators are Root mean square error (RMSE), regression coefficient (R^2), relative error (RE), model efficiency (ME), absolute values of averaged model bias (Bias), and pooled measurement uncertainty (PMU).

		Δ SOC stock (Mg ha ⁻¹)						SOC stock (Mg ha ⁻¹) on time series						
		Type	RMSE	RE	ME	R ²	Bias	PMU	RMSE	RE	ME	R ²	Bias	PMU
Validation	Total	1.67	44%	0.63	0.78	-0.25	0.91	2.55	-1%	0.97	0.968	-0.59	0.46	
	Bsl	1.62	71%	1	0.68	-0.57	0.68	2.81	-3%	0.97	0.976	-1.48	0.67	
	CFp	1.72	17%	0.99	0.63	0.07	1.18	2.27	2%	0.96	0.973	0.298	0.605	
Verification	Total	3.73	-153%	0.20	0.34	1.13	1.39	3.19	1%	0.95	0.946	0.28	0.52	
	Bsl	3.17	-250%	0.95	0.12	0.98	1.39	2.94	-2%	0.96	0.968	-1.00	0.71	
	CFp	4.23	-56%	0.98	0.17	1.27	2.13	3.43	4%	0.91	0.930	1.56	0.77	

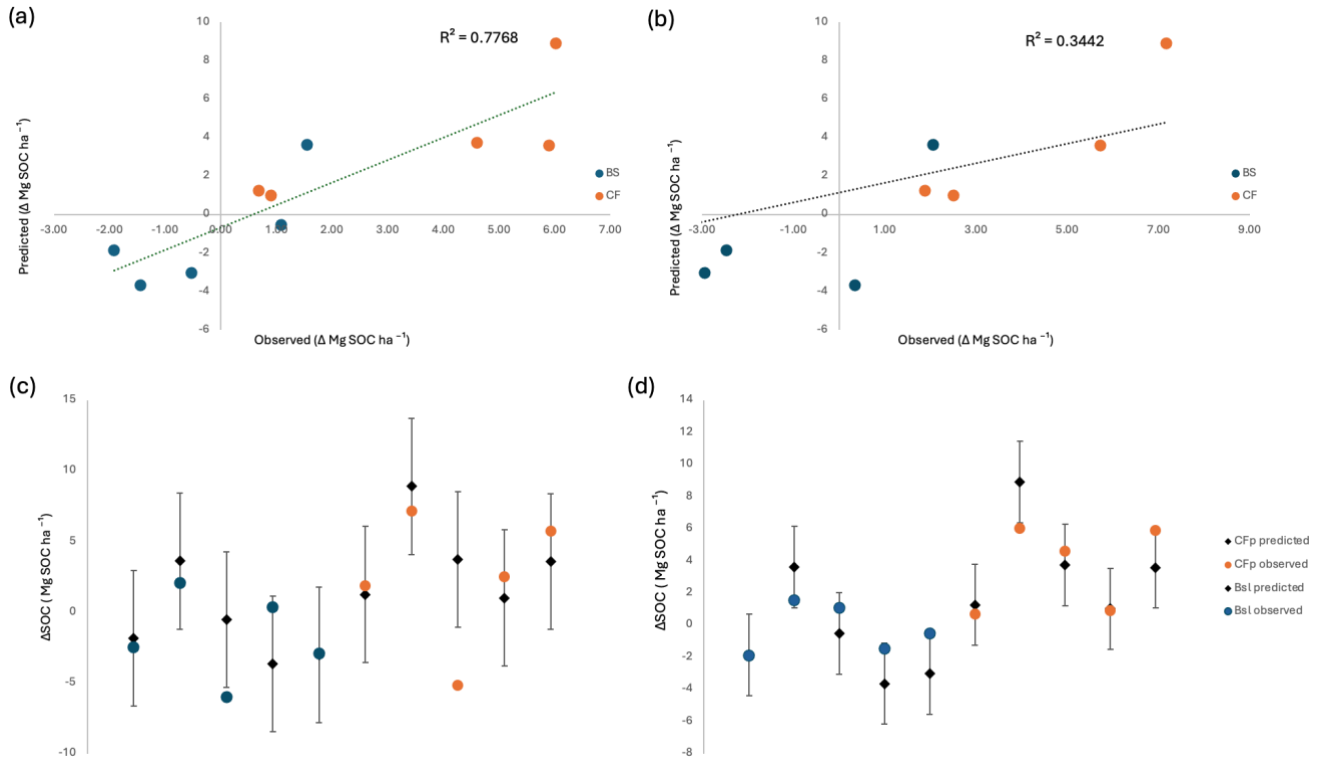


Figure 3.4 Scatterplot of model-predicted vs. observed values of Δ SOC stock (Mg ha^{-1}) for the verification dataset (a) and the validation dataset (b). Blue and orange points represent the baseline scenario and CF project, respectively. Panels (c) and (d) display 90% confidence intervals for model estimates alongside the average observed values of Δ SOC stock (Mg ha^{-1}) for the verification dataset and validation dataset, respectively, with blue and orange points indicating the baseline scenario and CF project.

3.3.1.3 Estimation of GHG emission using the Cool Farm Tool

Estimation of GHG emissions across the five LCFs, by using the Cool Farm Tool, was performed for both the Bsl and CFp scenarios. Results from this calculation, expressed in $\text{CO}_2\text{e ha}^{-1}$, reveals source-specific differences (Fig. 3.5). For residue management—which includes emissions from the decomposition of plant residues incorporated into the soil— CFp highly increased GHG emissions ($0.76\text{-ton CO}_2\text{e ha}^{-1}$) compared to the Bsl ($0.15\text{-ton CO}_2\text{e ha}^{-1}$). However, by adding $1200 \text{ kg CO}_2\text{e ha}^{-1}$ to the Bsl scenario, for the two seasons when the soil was bare, N_2O emissions in the Bsl scenario were increased to $1.35\text{-ton CO}_2\text{e ha}^{-1}$. However, emissions from energy use for machinery were higher in the CFp, at $2.77\text{-ton CO}_2\text{e ha}^{-1}$, compared to $2.34\text{-ton CO}_2\text{e ha}^{-1}$ in the Bsl. Crop fertilization emissions remained nearly the same across both scenarios, averaging $1.65\text{-ton CO}_2\text{e ha}^{-1}$, with only minor differences due to balanced organic fertilization during the incorporation of wheat and maize residues. Emissions from crop protection were minimal and nearly identical between the scenarios, averaging $0.096\text{-ton CO}_2\text{e ha}^{-1}$. Overall, total GHG emissions in the CFp scenario ($5.3\text{-ton CO}_2\text{e ha}^{-1}$) were comparable to those in the Bsl ($5.4\text{-ton CO}_2\text{e ha}^{-1}$).

3.3.1.4 Certified units generated by the LCFs and calculation of MRV uncertainty.

The calculation of C.U. revealed varying potentials for carbon credit generation across LCFs, as detailed in Table S3.3. All LCFs positively impacted *NCR*, confirming the effectiveness of CF practices in enhancing C removals compared to Bsl scenarios. Once this was stated, *AER* were calculated to capture both the potential for carbon credit gains from GHG emission reductions (negative *AER* values) or the necessity to account for increased emissions (positive *AER* values). LCF1, LCF3, and LCF5 demonstrated negative *AER* values, averaging $-0.43\text{-ton CO}_2\text{e ha}^{-1}$, indicating reduced GHG emissions under the CFp scenario. These LCFs contributed positively to carbon credit generation through both emission reductions and carbon removals, potentially achieving an average C.U. of 19.42 per hectares over 2.5 years. Conversely, LCF2 and LCF4 demonstrated positive *AER* values, averaging $+0.38\text{ tons CO}_2\text{e ha}^{-1}$, reflecting increased GHG emissions under the CFp scenario. These GHG emissions partially offset the gains from carbon removals, reducing the net carbon credit generation potential to an average C.U. of 15.03 per hectares over 2.5 years. Overall, the mean C.U. across the five LCFs was $+17.7$ per hectare, equivalent to approximately 265 C.U. for the entire project area over 2.5 years (Fig. 3.5).

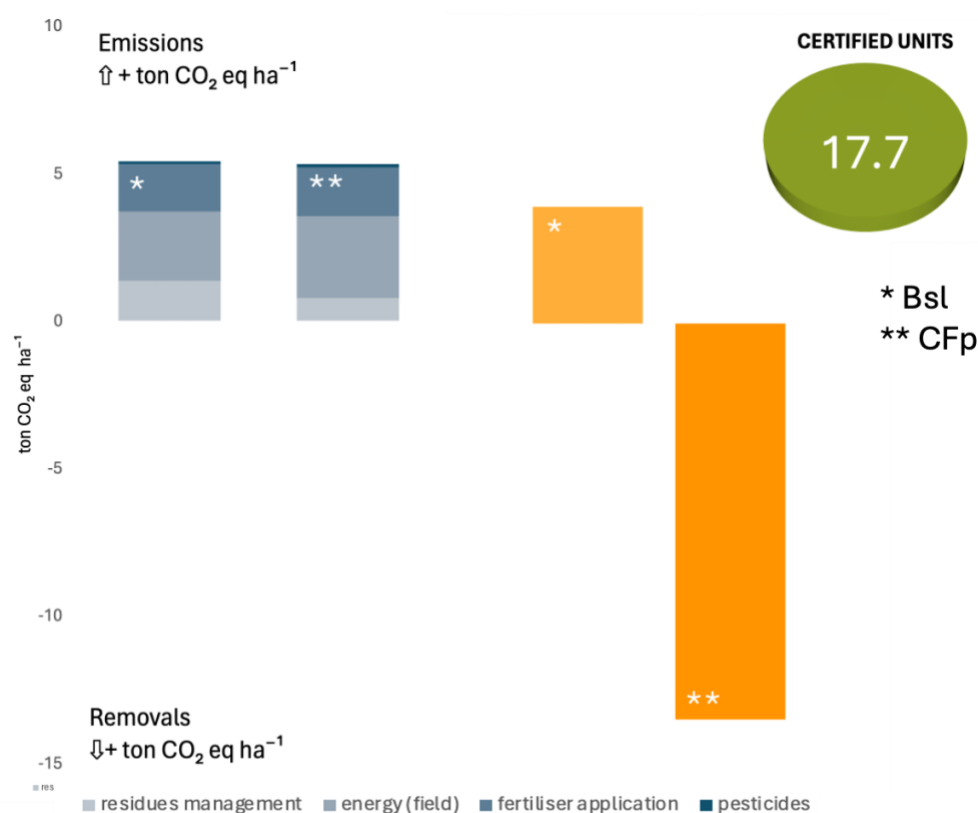


Figure 3.5 Representation of carbon credits (certified units over 2.5 years shown in the green circle) potentially generated by comparing net carbon removals (NCR) and associated emissions reductions (AER) between the baseline scenario (*) and the carbon farming project scenario (**). On-site AER ($\text{Mg CO}_2\text{e ha}^{-1}$), arising from residue management, machinery energy use, fertilizer application, and crop protection, were estimated using the Cool Farm Tool (blue bars), while NCR ($\text{Mg CO}_2\text{e ha}^{-1}$) were estimated using the RS-based RothC model (orange bars).

To ensure the reliability and robustness of carbon credit calculations, comply with verification standards, and enhance the credibility of the results for stakeholders and carbon markets, the uncertainty of the MRV approach (Tab. 3.4) was estimated by combining sampling and modeling errors. The sampling variance for the entire project was $0.15 \text{ (t CO}_2\text{e ha}^{-1})^2$ and $140.61 \text{ (t CO}_2\text{e)}^2$, while the modeling variance was $310.29 \text{ (t CO}_2\text{e ha}^{-1})^2$ and $48.45 \text{ (t CO}_2\text{e)}^2$. When summed, the total variance amounted to $310.44 \text{ (t CO}_2\text{e ha}^{-1})^2$ and $189.06 \text{ (t CO}_2\text{e)}^2$. This corresponded to a standard deviation of $17.62 \text{ t CO}_2\text{e ha}^{-1}$ and $13.75 \text{ t CO}_2\text{e}$, representing the overall uncertainty during the verification period. The relative uncertainty (calculated as the standard deviation on an area basis across the entire project, divided by the total estimated ΔSOC in $\text{t CO}_2\text{e}$) was 4.74% of the ΔSOC ($\text{t CO}_2\text{e}$) across the entire project area.

Table 3.4 Evaluation of the uncertainty of the remote sensing-based hybrid MRV approach on hectares basis as $\text{t CO}_2\text{e ha}^{-1}$ and on project area basis as $\text{t CO}_2\text{e}$. The uncertainty (S^2_{total}) expressed in $(\text{t CO}_2\text{e ha}^{-1})^2$ of C removal estimates ($\text{CO}_2\text{e ha}^{-1}$) was calculated by combining sampling error ($S^2_{\text{sampling},\Delta\text{SOCproject}}$) and modeling error ($S^2_{\text{model},\Delta\text{SOC}}$), following the VM0042 Verra protocol. The standard deviation (STD_{tot}) calculated on S^2_{total} provide the uncertainty in $\text{t CO}_2\text{e ha}^{-1}$.

	LCF	$S^2_{\text{SAMPLING},\Delta\text{SOCPROJECT}}$	$S^2_{\text{MODEL},\Delta\text{SOC}}$	S^2_{TOTAL}	STD_{TOT}
on hectares basis as $\text{t CO}_2\text{e ha}^{-1}$	LCF1	0.04	122.72	122.75	11.08
	LCF2	2.96	47.46	50.42	7.10
	LCF3	0.67	983.86	984.53	31.38
	LCF4	2.63	178.37	180.99	13.45
	LCF5	0.01	76.30	76.31	8.74
	TOT	0.15	310.29	310.44	17.62
on project area basis as $\text{t CO}_2\text{e}$	LCF1	1.42	5.14	6.56	2.56
	LCF2	76.05	1.28	77.33	8.79
	LCF3	20.29	31.02	51.31	7.16
	LCF4	41.85	2.97	44.82	6.69
	LCF5	1.00	8.04	9.05	3.01
	TOT	140.61	48.45	189.06	13.75

3.3.2 Assessment of voluntary co-benefit and potential C leakage for compliance with Sustainability criteria

To meet the "Sustainability" criteria, a heatmap (Fig. 3.6) illustrating the response ratio (RR, as DiD/CFp or CFp/Bsl) values for voluntary co-benefits—including improvements in soil health, nutrient cycling, aggregate stability, and water retention— was produced. Overall, adopting CF practices had a positive impact on all considered indicators, with significant improvement in N availability across all LCFs prior to the primary crop's cultivation. MBC ($\mu\text{g C g}^{-1}$), an indicator of soil health, increased 1.35-fold compared to the Bsl scenario, with significant gain in LCF2 and LCF4. Nutrient availability for primary crop growth (mg kg^{-1}) showed a significant higher increase for N (+0.92-fold) than for P (+0.03-fold). N availability significantly increased in LCF1 in 2023, whereas a slight, non-significant decrease in available P was observed in LCF3 and LCF4. Both N and P

mineralization rates ($\text{mg kg}^{-1} \text{ day}^{-1}$) showed moderate increases (0.31 and 0.27-fold, respectively) without statistically significant differences. AWC (mm) exhibited a moderate increase (0.15-fold), suggesting a small, enhanced soil water retention. ifs (cm min^{-1}) showed instead a negligible increase (0.02-fold). MWD of soil aggregates, reflecting aggregate stability, exhibited a modest increase (0.13-fold) and was significant in LCF2 (+0.47-fold). Crop productivity estimated via remote sensing (Mg ha^{-1}) showed a slight, non-significant decrease of -0.01, staying below the 5% threshold for productivity decline and thus avoiding potential C leakage concerns.

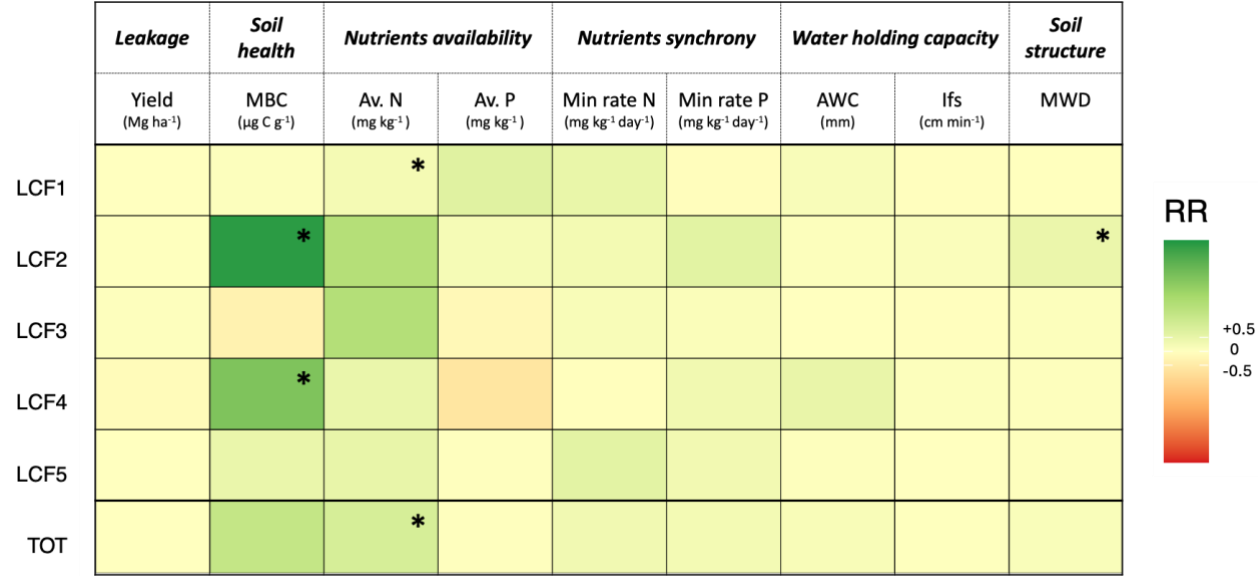


Figure 3.6 Heatmap of response ratio values for MBC ($\mu\text{g C g}^{-1}$), available N and P (mg kg^{-1}), AWC (mm), ifs (cm min^{-1}), and MWD (RR: DiD/CFp), as well as for N and P mineralization rate ($\text{mg kg}^{-1} \text{ d}^{-1}$) and crop yield (Mg ha^{-1}) (RR: CFp/Bsl). Stars denote statistically significant differences in the treatment*time interaction term in the DiD analysis ($p < 0.05$).

3.4 Discussions

3.4.1 Carbon credit generation through the quantification of carbon removals and GHG emissions

3.4.1.1 Quantification and evaluation of C removals in soil in a carbon farming project: comparison to a dynamic, activity-specific baseline and assessment of SOC long-term storage

At the European level, SOC stocks in croplands are currently near a steady-state condition (De Rosa et al., 2023; Lugato et al., 2014). However, recent estimates indicate SOC declines across Central Europe and Italy, with reported rates of -0.14 and $-0.12 \text{ Mg C ha}^{-1} \text{ y}^{-1}$, respectively (De Rosa et al., 2024). In this study, SOC stock under the Bsl scenario decreased by $-0.25 \text{ Mg C ha}^{-1}$ ($-0.10 \text{ Mg C ha}^{-1} \text{ y}^{-1}$) aligning with these observed trends. This estimate was based on calculations using the validation dataset, while a larger reduction of $-1.80 \text{ Mg C ha}^{-1}$ ($-0.74 \text{ Mg C ha}^{-1} \text{ y}^{-1}$) was observed using the verification dataset. Notably, these SOC sequestration data reflect actual measured outcomes rather than theoretical estimates, having been verified at the farm scale following

internationally recognized certification standards. This study is the first to show that scalable CF practices, including rotations with industrial tomato as the primary cash crop, can achieve measurable climate benefits. Specifically, it highlights the capacity of CF practices to enhance SOC stocks in the short term (Paustian et al., 2016; Poeplau and Don, 2015; Smith, 2012), as observed across the LCFs evaluated in this study. Under CFp scenario, SOC gains averaged $+2.42 \text{ Mg ha}^{-1}$ ($+1.00 \text{ Mg C ha}^{-1} \text{ y}^{-1}$) in the verification dataset and $+3.62 \text{ Mg ha}^{-1}$ ($+1.50 \text{ Mg C ha}^{-1} \text{ y}^{-1}$) in the validation dataset, demonstrating the positive impact of regenerative agricultural practices designed to increase C inputs and reduce losses. European studies evaluating regenerative or conservation agriculture practices, whether applied individually or in combination, have typically reported SOC sequestration rates ranging from 0.1 to $0.5 \text{ Mg C ha}^{-1} \text{ y}^{-1}$ (Aguilera et al., 2013; Lugato et al., 2014; Morari et al., 2006; Poeplau and Don, 2015). However, only a limited number of studies have calculated SOC stocks on an equivalent soil mass (ESM) basis. A meta-analysis by Petersson et al. (2025), which standardized ΔSOC stocks to an ESM basis, showed that CF practices alone could increase SOC by 0.1 to $0.4 \text{ Mg C ha}^{-1} \text{ y}^{-1}$, while combining practices could enhance this to $0.9 \text{ Mg C ha}^{-1} \text{ y}^{-1}$. Such gains can be further increased in high-productivity regions or under conditions that favor SOC accumulation. For instance, a combination of low initial SOC content and high soil clay content, as in our study (Tab. 3.1), can positively influence SOC gains (De Rosa et al., 2023).

In this experiment, a higher, though not statistically significant, increase in SOC stock was observed in LCF1 and LCF2 ($+2.44$ and $+2.50 \text{ Mg C ha}^{-1} \text{ y}^{-1}$, respectively). These increases are likely associated with enhanced crop rotations incorporating maize, along with the combined incorporation of straw and maize residues supplemented with additional nutrients. The SOC increase is further supported by a corresponding rise in the stable SOC fraction, represented by MAOM-C (Mg ha^{-1}) (Fig. 3.2a), which reflects a more persistent SOC pool (Cotrufo et al., 2019). This finding is further supported by the results presented in Chapter 3, where an incubation experiment was conducted using soil samples from LCF1 and LCF2. The study demonstrated that incorporating straw residues, particularly with organic nutrient supplementation, enhances humification efficiency and organic carbon stabilization through the activation of the “microbial-C-pump.” This is critical, as MAOM-C not only serves as a long-term carbon storage mechanism but also provides nutrients that contribute to soil fertility and sustained crop productivity over time. Additionally, similar to the findings in Chapter 3, plant-origin POM-C content (g kg^{-1}) was more readily consumed in LCF1 than in LCF2 (Fig. 3.2b), likely due to differences in soil fertility and soil structure between the two fields (Tab. 3.1 and Supplementary materials). In LCF1, higher nutrient availability, including

greater levels of SOM, N, and other essential elements, likely stimulated microbial activity and enhanced the decomposition of POM-C. Moreover, improved soil structure and porosity in LCF1 facilitated better aeration and microbial colonization, further accelerating POM-C turnover. In contrast, the comparatively lower fertility status of LCF2, characterized by limited nutrient availability and less biologically active soil, likely constrained microbial activity and slowed POM-C decomposition. These differences highlight the role of inherent soil properties and fertility status in determining the fate of SOC fractions under similar agronomic practices. While the higher turnover of POM-C in LCF1 may suggest faster cycling of labile carbon, it also underscores the importance of coupling such processes with practices that promote the stabilization of C into more persistent pools, such as MAOM-C, to achieve long-term carbon storage and soil health benefits.

Although SOC stock dynamics were consistent between the verification and validation datasets, a notable discrepancy in Δ SOC stocks in LCF3 highlights the influence of sampling strategy on SOC quantification. Soil sampling should capture field-scale variability to provide an unbiased estimate of the mean (Bradford et al., 2023; Oldfield et al., 2021). Stratification, which divides the project area into homogeneous zones, improves sampling efficiency and better represents SOC distribution across the landscape, enhancing the accuracy of SOC estimates and reducing the number of samples required to estimate mean SOC content.(Oldfield et al., 2021). In this study, a stratified random sampling approach was employed to capture SOC variability within fields, selecting three sampling locations per field at the start of the CF project, referred to as the verification dataset. In contrast, the validation dataset used a soil grid sampling approach to capture SOC's spatial and temporal variability more comprehensively. This methodological difference contributed to variability in Δ SOC estimates, particularly in LCF3. A visual representation of the normal distribution of Δ SOC stock values (Fig. S3.3) shows that 2 out of 3 data points in the CFp scenario and 1 out of 3 for the Bsl scenario for the verification dataset fell within the tails of the normal distribution of the respective validation dataset, indicating these points did not represent the average Δ SOC stock. As noted by Oldfield et al. (2021), using a stratified sampling strategy and returning to the same strata in each successive round might help reduce variability over time. However, permanent sampling locations could lead to "gaming the system," where data may be adjusted to produce favorable results. Using independent sampling within the same strata—sampling different locations within the same homogeneous area before and after the CF project, respectively for model initialization and verification/validation—addresses this issue while still capturing relevant SOC changes. By sampling independently, the risks of bias associated with permanent sampling sites are avoided, while

maintaining consistency in the data to ensure it reflects the same environmental conditions across both sampling periods.

To further improve the accuracy and efficiency of SOC monitoring, the development and integration of in-field sensors offer a promising solution (Batjes et al., 2024). These sensors enable real-time, high-resolution measurements of SOC and related properties, capturing spatial variability across large and heterogeneous landscapes. When combined with geospatial data and machine learning models, these tools can map SOC dynamics at a field scale with greater precision (See Chapter 5 for more detail). By incorporating such advancements in soil sensing alongside improved sampling designs, the accuracy, scalability, and cost-effectiveness of measurement-based SOC MRV systems can be significantly enhanced (Batjes et al., 2024; Oldfield et al., 2021). These innovations not only reduce the need for extensive physical sampling but also improve the reliability of SOC estimates, enabling the effective implementation of carbon farming projects and fostering greater trust in carbon credit markets.

3.4.1.2 Accuracy and scalability of C removals estimates using a RS-based hybrid modelling approach.

In recent years, hybrid MRV approaches have been developed to track changes in SOC stocks over time, combining soil sampling, biogeochemical modeling, and remote sensing technologies (Batjes et al., 2024; Mathers et al., 2023). In this study, the proposed MRV method integrates these tools into a systematic framework designed for scalability and efficiency.

A stratified random sampling strategy was employed to account for within-field spatial variability while reducing the number of soil samples required for model initialization. Remote sensing techniques, specifically the LUE model, were utilized to estimate crop biomass. This data was then coupled with a modified RothC biogeochemical model to simulate SOC dynamics under both CFp and Bsl scenarios across five LCFs. Additionally, farm activity data were obtained from farmer crop declarations using the Land Parcel Identification System (LPIS) and field notebooks. Finally, dynamic and activity-specific baselines were simulated for each LCFs to enable more precise accounting of additional carbon credits compared to a static baseline, as well as to allow model uncertainty calculation with side-by-side comparison. By integrating multiple data sources and methodologies, the proposed hybrid MRV approach ensures accurate SOC monitoring while enabling carbon farming projects to operate cost-effectively at larger scales, overcoming the limitations posed by the high costs of annual field sampling.

In this study, the RS-based hybrid modelling approach well predicted SOC stock changes across both scenarios (Fig. 3.3), estimating a net increase of $+3.70 \text{ Mg C ha}^{-1}$ for CFp over the study period ($+1.53 \text{ Mg C ha}^{-1} \text{ y}^{-1}$), contrasting with a net loss of $-0.83 \text{ Mg C ha}^{-1}$ ($-0.34 \text{ Mg C ha}^{-1} \text{ y}^{-1}$) in the Bsl scenario. The proposed approach was thus able to account for variations in the quality and decomposition rates EOMs applied, specifically digestates and crop residues from wheat and maize. For model estimate validation, this study followed the *VERRA VCS Module (VMD0053, version 2.0)*, which mandates the use of distinct and stringent calibration and validation datasets to ensure credible SOC sequestration estimates. Two distinct datasets were used in this validation process: the verification dataset, which evaluated the MRV approach as a cost-effective tool for assessing SOC dynamics, and the validation dataset, which provided independent data to further validate model predictions. Overall, the RS-based hybrid modelling approach demonstrated a strong agreement between measured and modeled SOC stocks, with R^2 values for SOC stock time series ranging from 0.93 to 0.98 (Tab. 3.3). However, R^2 values were moderate (0.78) for ΔSOC stock in the validation dataset and lower (0.34) in the verification dataset, indicating that the model was more accurate for predicting overall SOC stocks across the CF project period than for ΔSOC stocks, likely due to yearly SOC fluctuations. The model exhibited a mean error (ME) of 0.87 Mg ha^{-1} and a root mean square error (RMSE) of 2.77 Mg ha^{-1} on average (Tab. 3.3). Although RMSE was higher for the verification dataset, this discrepancy was likely due to sampling errors in this dataset, which impacted the model's ability to accurately predict ΔSOC stocks. These findings suggest the RS-based hybrid model has reasonable predictive accuracy, especially over time series, and underscores the importance of minimizing sampling errors in SOC modeling for CF projects.

When assessing SOC stocks over a time series, the model demonstrates a low relative error (RE), with a slight tendency to overestimate SOC increases and underestimate SOC losses (Tab. 3.3). In the context of C crediting programs, such overestimation of SOC gains and underestimation of losses are undesirable, as they could undermine the integrity and credibility of credited carbon benefits. However, the observed low RE values are minimal and unlikely to significantly impact the overall results. In contrast, the prediction of ΔSOC stock tends to significantly overestimate ΔSOC stock changes compared to the validation dataset, while underestimating ΔSOC changes in the verification dataset. This discrepancy is likely due to the inappropriate selection of sampling points for verification, where soil sampling locations were kept constant throughout the CFp period. As a result, the sampling strategy may not have fully captured the spatial variability of SOC across LCFs. Adopting a systematic random sampling approach for verification could improve the

representativeness of the sampling locations, enhance the model's accuracy in predicting SOC changes, and reduce discrepancies between the validation and verification datasets (Oldfield et al., 2021).

For a model to be considered valid for use in VERRA projects, its bias must not exceed the standard error of replicated direct soil measurements. Bias refers to the consistent over- or underestimation of predictions compared to observed data, and it is evaluated by comparing the model's predictions with validation data. Ideally, the bias should be close to zero, indicating no systematic error. If the bias exceeds a defined threshold, such as the PMU, the model is deemed inaccurate and fails validation. Minimizing bias is essential in carbon credit projects to ensure that model predictions align with actual measurements, thereby maintaining the credibility and integrity of carbon credit estimates. In this study, model bias for both CFp and Bsl scenarios was assessed by comparing model predictions to the PMU of SOC observations. Mean model biases were smaller than the PMU for all Δ SOC stock predictions but exceeded the PMU for SOC stock predictions in the verification dataset. This indicates that while the model is suitable for use in VERRA projects for Δ SOC stock predictions, it may not accurately capture SOC stock changes in the verification dataset. This could lead to inaccuracies in carbon credit calculations and undermine the model's credibility, especially if the bias significantly impacts final SOC estimates. Therefore, an accurate sampling strategy is essential to maintain the integrity of carbon credit calculations.

Furthermore, in accordance with VCS requirements, another validation criteria associated with model precision, expressed in terms of model coverage (e.g., at least 90% of validation data must fall within the 90% predictive interval) must be taken into account (Lavallee et al., 2024). In this study, 90% of the measured values from the validation dataset fell within the model's 90% prediction interval. However, only 80% of the measured Δ SOC stock values from the verification dataset fell within the model's 90% prediction interval. These results demonstrate strong performance in meeting the 90% threshold required for high-confidence carbon credit claims, while also highlighting the influence of sampling errors in the verification dataset.

3.4.1.3 Estimation of GHG emission using the Cool Farm Tool

This study integrated complementary methodologies to assess the carbon credit potential of implementing CF practices across five LCFs in the industrial tomato supply chain of northwestern Italy. The Cool Farm Tool, a widely recognized tool employing a Tier 1 approach for GHG quantification based on IPCC-aligned emission factors (Whittaker et al., 2013), was used to estimate

GHG emissions. In parallel, a RS-based hybrid model was applied to evaluate potential carbon removals. The study prioritized the quantification of net carbon removals (NCR) to verify the environmental benefits of CF practices, establishing a reliable basis for calculating any associated emissions reductions (AER). If the AER under CFp were positive (i.e., higher than the Bsl scenario), they were discounted by the NCR to ensure robust carbon accounting. Conversely, if AER under CFp were negative (i.e., lower than the Bsl), they contributed to increasing the total C.U. This dual-layered approach ensured the credibility and integrity of carbon crediting by avoiding overestimations or unverified claims.

While a Tier 3 approach—employing process-based models and site-specific activity data—offers greater accuracy in estimating emissions, particularly for soil N₂O fluxes, its implementation poses challenges (Schipanski et al., 2024). Collecting detailed input data across large-scale CF programs, often involving numerous farms and extensive landscapes, is impractical. The hybrid methodology adopted here represents a balanced solution, combining the simplicity and practicality of Tier 1 emission factors with the advanced spatial accuracy of RS-based models. This approach ensures reliable estimates of both GHG emissions and carbon removals, making it suitable for large-scale, multi-farm CF initiatives while maintaining methodological rigor.

Our findings indicate that the CFp scenario achieved modest reductions in total GHG emissions compared to the Bsl scenario (5.3 vs. 5.4-ton CO₂e ha⁻¹), with differences largely driven by specific management practices. Notably, emissions from residue management were significantly higher under CFp due to the decomposition of incorporated crop residues (0.76-ton CO₂e ha⁻¹ in CFp vs. 0.15-ton CO₂e ha⁻¹ in Bsl). This aligns with the established understanding that residue incorporation enhances short-term microbial activity in soils, leading to increased CO₂ and N₂O fluxes, as also noted in Chapter 3 and by Baggs et al. (2000). However, the Cool Farm Tool's lack of a default factor for estimating GHG emissions from bare soils may lead to underestimations of Bsl emissions, potentially misrepresenting the carbon credit potential of CFp. When emissions during fallow periods were accounted for, Bsl estimations increased to 1.35-ton CO₂e ha⁻¹, emphasizing the substantial benefits of maintaining soil cover to mitigate N₂O emissions during these periods.

Energy use for machinery, calculated from gasoline consumption, represented another trade-off, with CFp showing higher emissions compared to Bsl (2.77 vs. 2.34-ton CO₂e ha⁻¹). This increase reflects the additional field operations associated with residue incorporation, as well as the sowing and termination of cover crops. These findings emphasize the importance of optimizing minimum tillage strategies for both cover crop cultivation and residue incorporation. This optimization is

essential for reducing emissions associated with machinery use while ensuring that the seedbed conditions for main crops are properly prepared. In contrast, fertilizer-related emissions remained nearly identical between the scenarios, as no alternative smart fertilizer strategies were implemented. For instance, precision fertilization techniques—such as adjusting nitrogen inputs based on pre-sowing fertilization for summer crops, utilizing analytical nitrogen balance coupled with in-season soil analysis, or producing tailored fertilization prescriptions through variable rate maps for top dressing—were not employed. This consistency highlights an opportunity within the CFp approach to develop and adopt improved fertilizer management practices that could significantly reduce GHG emissions while maintaining or even enhancing crop productivity (Wu & Ma, 2015).

3.4.1.4 Potential Carbon credit generation in 2.5 years wheat-maize-rotation

As demonstrated in the previous sections, CFp can effectively remove atmospheric C and enhance its sequestration in soil, while contributing in some cases to the reduction of GHG emissions from agricultural activities. These practices are particularly valuable for agro-industries aiming to decarbonize their supply chains by addressing Scope 3 emissions, which refer to indirect GHG emissions that occur across the value chain. Incentivizing farmers through the generation of carbon credits within insetting programs provides agro-industries with a practical pathway to achieve decarbonization targets while enhancing the sustainability of their supply chains.

In this study, the potential for carbon credit generation among LCFs was assessed, yielding a mean C.U. value of +17.7 per hectare over 2.5 years (Fig. 3.5). Notably, LCF1, LCF3, and LCF5 showed negative AER values ($-0.43\text{-ton CO}_2\text{e ha}^{-1}$), indicating net reduction in GHG emissions under the CFp scenario (Tab S3). These systems were particularly efficient in generating carbon credits through a combination of SOC gains and emission reductions. Conversely, LCF2 and LCF4 had positive AER values ($+0.38\text{-ton CO}_2\text{e ha}^{-1}$), reflecting net increases in GHG emissions under CFp (Tab. S3.3). highlights the influence of factors like soil type and machinery use intensity, emphasizing the need to tailor carbon farming practices to local conditions for optimal sequestration and emission reduction. The overall potential for carbon credit generation across the project area, approximately 265 C.U., underscores the financial opportunities of implementing CFp. With an average farm size of 80 hectares in the region and a CO_2e price of €35 per ton, farmers adopting the proposed CFp could generate between €12,286 and €27,585 lord annually from the sale of carbon credits, assuming their credits are certified.

To ensure robust and credible carbon credit calculations that comply with verification standards and gain trust from stakeholders and carbon markets, the uncertainty of the MRV approach (Table 3.4) was assessed by combining sampling and modeling errors of the RS-based model. The project-wide relative uncertainty was 4.74% of ΔSOC (t CO₂e), which did not result in a penalty on the carbon credits generated. However, LCF3 exhibited higher model uncertainty, emphasizing the need for further refinement in sampling strategies and modeling techniques.

Integrating RS biomass estimates with spatialized SOC removal data from proximal soil sensors at the pixel level can significantly reduce uncertainties from initial sampling errors, thereby improving the accuracy of C removal estimates (see Chapter 5 for more details). These advanced technologies deliver high-resolution, spatially detailed data for SOC stock assessments, enabling more reliable quantification of C dynamics while accounting for the heterogeneity of SOC storage potential across fields. By enhancing the reliability and scalability of MRV approaches, such innovations reinforce the credibility of CF programs and their role in mitigating climate change. Furthermore, the development of high-resolution soil property maps, including C and N content, could offer valuable tools for precision agriculture practices like targeted fertilization and sowing. When integrated into CF programs, these practices can further reduce emissions associated with fertilizer application and fuel use, amplifying both environmental and economic benefits.

According to various C accounting methodologies, liability mechanisms to address C release during the monitoring period of C storage activities need to be considered (Brummitt et al., 2024; Lesschen, 2024). These mechanisms should minimize the risk of C reversal into the atmosphere and define rules for managing such risks. One potential mechanism is the discounting of C.U., where a portion of the calculated C.U. is excluded from certification to account for uncertainty and potential reversal, ensuring that these units are not available for certification. Therefore, if a 20% discount is applied and CR estimates are credited for a 5-year CF program, the CFp addressed in this study could generate on average €15805 per farm annually (€198/ha/yr) from the carbon credits sale, based on an average VCM price¹ of €35/tonCO₂ (excluding co-benefits). However, this calculation does not yet account for certification and program management costs, which are not charged to farmers.

When implementation costs of “additional” CF practices (e.g. cover crops, loss of income from crop residues sales, and minimum tillage adoption by contractors) are considered (averaging €204/ha/y) along with the inclusion of co-benefits priced at an additional €18/ton CO₂, the net

¹ <https://carboncredits.com/what-is-the-voluntary-carbon-market/>

income per farm would be €7,362 annually, equating to €92/ha/year. This net income would cover approximately 97% of the implementation costs.

3.4.2 Assessment of voluntary co-benefit and potential C leakage for compliance with Sustainability criteria

To assess the sustainability of CF practices in line with CRCF regulations, this study focused on voluntary co-benefits such as soil health, nutrient cycling, aggregate stability, and water retention. These indicators showed improvements across the LCFs, showcasing the potential of CF practices to enhance ecosystem services alongside SOC accumulation. For instance, MBC, a key soil health indicator (Bünemann et al., 2018), increased by an average of 1.35-fold in CF practices compared to the Bsl, with significant increases in LCF2 and LCF4. MBC is a sensitive indicator of short-term changes in soil management, especially with organic inputs and tillage practices (Bastida et al., 2008; Lagomarsino et al., 2009) and its increase reflects improved microbial activity that supports nutrient cycling, organic matter decomposition, and soil structure (Pulleman et al., 2012). Nutrient availability following cover crop termination, particularly N, significantly improved under CF practices, with N levels increasing by 0.92-fold compared to the Bsl scenario. This improvement can be attributed to balanced organic fertilization, the incorporation of crop residues, and maintaining year-round soil cover, which collectively enhance nutrient cycling efficiency and support the sustainability of CF practices (Bertrand et al., 2019). Numerous studies highlight the role of cover crops in increasing soil N availability and reducing the reliance on synthetic N fertilizers (Boselli et al. 2020). This effect primarily arises from net N immobilization in the cover crop biomass and the accumulation of high C residues on the soil surface, which undergo gradual mineralization, releasing N for subsequent primary crops. In this study, this mechanism was evidenced by a moderate increase in the N mineralization rate. In contrast, phosphorus (P) availability exhibited a minimal overall increase (+0.03-fold). Despite this minimal increase, previous research has demonstrated that cover crops can enhance the efficiency of P utilization from both organic and mineral fertilizers by stimulating soil biological activity, improving P uptake, and promoting the growth of primary crops (Hallama et al., 2019). Cover crops access poorly available soil P through more efficient root architecture and morphology, which allow exploration of greater soil volume. Additionally, they mobilize moderately soluble inorganic and organic P forms and contribute to the mineralization of organic P (Hallama et al., 2019). However, in this study, the moderate increase in P mineralization

rates across LCFs did not translate into higher P availability for main crop growth, likely due to P immobilization in the soil matrix.

Soil management practices that minimize disturbance and maximize SOC return to the soil, are well-known for enhancing aggregate stability (Bronick and Lal, 2005; Pagliai et al., 2004; Six et al., 2000). Reduced tillage minimizes soil disturbance, preserving existing soil aggregates and promoting soil biota activity, such as fungi and microbes, which produce binding agents like glomalin (Bronick and Lal, 2005). Similarly, practices that increase the SOC pool, such as cover cropping and residue incorporation, contribute to aggregate stability by providing organic material that acts as a binding agent for soil particles. The decomposition of residues further stimulates microbial activity, contributing to aggregate formation and stabilization (Bronick and Lal, 2005). In this study, water aggregate stability was assessed using the mean weight diameter (MWD) of soil aggregates, a parameter that integrates size class distribution into a single value to characterize overall soil structure. MWD showed a modest increase (+0.13-fold), with significant improvement observed only in LCF2 (+0.47-fold), indicating that short-term effects are more pronounced in more degraded soils. Improved soil structure enhances porosity and water-holding capacity, reduces surface crusting, and creates more continuous macropores, thereby increasing water infiltration (Six et al., 2000). In this study, the slight increase in AWC and the small rise in infiltration speed (IFS) highlight the potential of CF practices to improve water retention and soil moisture. However, these benefits are likely to become more pronounced with long-term application.

Economically, the improvements in soil health resulting from CF practices can lead to significant cost savings for farmers, particularly in reduced input requirements. For example, the enhanced nutrient cycling and increased N availability through CF practices can reduce the need for synthetic fertilizers, thereby lowering input costs. Additionally, the improvements in soil structure, such as increased aggregate stability and water retention, could reduce the need for irrigation and mitigate the risks associated with drought, which can result in more stable crop yields and reduced water costs. Furthermore, improvements in soil health may lead to higher crop productivity over time, creating an opportunity for farmers to increase their revenue. The long-term benefits of soil health improvements also contribute to the resilience of farming systems, making them more adaptable to climate change and external shocks, which can further enhance economic stability for farmers engaged in CF practices.

Within the sustainability assessment, potential C leakage resulting from the application of CF practices must be carefully evaluated and mitigated. C leakage refers to unintended emissions or

environmental impacts outside the CF project boundary that could offset the C gains achieved within the project. According to the *VCS Methodology (VM0042)*, crop productivity in the project scenario must demonstrate that it has not declined by more than 5% compared to baseline productivity. This is determined by comparing the average productivity within the project (excluding years with extreme weather events) during the project period to the average baseline productivity from the historical look-back period (e.g., 10 years). In contrast to the proposed protocol, this study assessed potential carbon leakage by annually comparing the productivity of the CF project with the Bsl. Carbon leakage was found to be minimal, with remote sensing data indicating only a slight reduction in crop productivity (-0.01 Mg ha^{-1}) under CF practices, remaining well below the 5% threshold that typically raises concerns about productivity-related carbon leakage. This suggests that CF practices did not significantly impact crop productivity, thus preventing carbon losses due to reduced yields that could otherwise lead to increased land use or intensification elsewhere.

In summary, the findings demonstrate that CF practices are aligned with sustainability and leakage criteria, as defined by Verra and CRCF EU regulation, by slightly improving soil quality indicators in the short-term. Minimal productivity losses and reductions in potential leakage further validate the role of CF practices in enhancing agricultural sustainability while sequestering carbon.

3.5 Conclusion

This study demonstrates the potential of C) practices to unlock the soil carbon economy and enhance the sustainability of agro-industrial supply chains, with a focus on industrial tomato production in northwestern Italy. By simulating a potential CF program in the region, regenerative agricultural practices were co-designed through a collaborative process involving farmers, industry stakeholders, agronomists, and researchers, ensuring that the solutions developed were both practical and scalable to regional conditions and supply chain demands. The study also employed remote sensing (RS)-based modeling MRV tools, a smart soil sampling strategy, and a dynamic, activity-specific baseline to create a robust framework for assessing carbon credit potential. These tools were assessed for compliance with certification standards, such as those outlined by Verra and the Q.U.A.L.I.T.Y criteria specified in the CRCF regulation, providing a pathway for effective MRV implementation in real-world CF programs.

Key aspects, including the climate benefit of CF practices such as C sequestration in soils and GHG emission reduction, MRV accuracy and uncertainty, associated co-benefits, long-term C storage, and potential C leakage, were all thoroughly evaluated. The implementation of CF practices across five

LCFs demonstrated their ability to increase SOC stocks compared to baseline scenarios and to slightly reduce GHG emissions. However, the impacts varied depending on management practices, soil characteristics, and operational constraints. Overall, CF practices led to net increases in SOC, which were further supported by a rise in the stable SOC fraction (MAOM-C), representing long-term carbon storage that sustains soil fertility and crop productivity. While carbon sequestration benefits were significant, modest reductions in GHG emissions were observed, primarily driven by direct in-field emissions caused by incorporation of crop residues and cover crops, and indirect emissions due to increased machinery use. These findings underscore the need for optimized tillage and residue management strategies to minimize the trade-offs associated with machinery use and maximize carbon sequestration benefits.

The carbon credit generation potential of CF practices was substantial, with an average C.U. of +17.7 tons of CO₂e ha⁻¹ over a 2.5-year wheat-maize rotation. Despite uncertainties in estimating SOC dynamics, the integration of RS-based models reduced measurement errors, enhancing the reliability and credibility of the MRV system. Furthermore, high-resolution spatial data from proximal soil sensors could offer additional opportunities, providing strata that can be used to apply a smart soil sampling strategy for more accurate model initialization and validation. These technologies can also be leveraged for precision agriculture practices, such as targeted fertilization, further amplifying the environmental and economic benefits of CF practices.

Economic assessments demonstrated the financial feasibility of CF practices, with potential annual revenues from carbon credits ranging from €21,120 to €61,920 lord annually per 80-hectare farm, assuming a CO₂e price of €60 per ton. However, to calculate the net revenue, potential risks of carbon release during the monitoring period and the costs associated with certification and CF program management must be subtracted.

In addition to the climatic and economic benefits, this research evaluated the broader environmental impacts of CF practices, focusing on soil health and ecosystem services. Key indicators, such as MBC, nutrient cycling, aggregate stability, and water retention, showed improvements under CF practices, particularly in degraded soils. Increased MBC and nutrient availability not only enhance soil health but also lead to cost savings for farmers, particularly through reduced input requirements.

The study also addressed the potential risk of carbon leakage, ensuring that CF practices met the sustainability criteria by demonstrating that productivity losses remained below 5% of baseline levels. The minimal carbon leakage observed further aligned CF practices with regulatory standards

such as the Verra Carbon Standard and the EU CRCF regulations, ensuring that these practices contribute to climate goals without undermining agricultural productivity.

AUTHORS CONTRIBUTION

Marta Bertola, Andrea Ferrarini: manuscript editing. **Marta Bertola, Lorenzo Cremonesi, Manuele Ragazzi:** field data collection. **Marta Bertola:** Laboratory analysis. **Marta Bertola, Lorenzo cremonesi, Manuele Ragazzi, Michele Croci, Andrea Ferrarini:** data analysis. **Marta Bertola, Andrea Ferrarini:** conceptualization and study design. **Andrea Ferrarini, Stefano Amaducci:** funding acquisition and supervision.

ACKNOWLEDGEMENTS

The authors acknowledged UCSC field crop group collaborators for helping in field campaign and laboratory analysis, in particular Edoardo Volpato, Levyn Eric Perez Alvarado, and Luis Brayan Lopez Lazo.

FUNDING INFORMATION

The co-design, data collection of LCF and CFT runs was performed within the ClieNFarm project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement no. 101036822. The development and validation of the C removals MRV digital components are part of the project Farms4Climate, co-funded by the Partnership for Research and Innovation in the Mediterranean Area (PRIMA) and the European Union.

Supplementary Material

Materials and Methods

Plant biomass sampling and samples preparation

In each field, plant biomass sampling was conducted prior to harvesting both primary and winter cover crops to estimate total crop biomass and yield of primary crops. Sampling took place within homogeneous areas in each field, where $n = 3$ elementary sampling units (ESUs) were identified for each growing season, following the methodology outlined in Croci et al. (2022). Within each ESU, four sub-samples were collected from a 1 m^2 area (positioned 4 meters from each corner of the $20 \times 20 \text{ m}$ ESU, along the diagonal toward the centroid) at ground level. These sub-samples were weighed in the field to determine fresh biomass weight. For each 1 m^2 sample, fresh yields were measured for wheat, maize, and tomato as follows: grain yield for wheat and maize was calculated by separating the grains from the spikes using a mechanical thresher. For tomatoes, red berries were manually separated from the plant and weighed in the field. Dry matter yield and biomass were then obtained by oven-drying sub-samples at 105°C until they reached a constant weight. Plant biomass C content was calculated by multiplying an averaged C content of 0.45% by the measured biomass. Harvest index (HI) was calculated by dividing crop yield by the total biomass. Additionally, for tomato, white mustard, and the cover crop mixture, 4 random samples of above-ground biomass (AGB) and below-ground biomass (BGB) were collected from a 0.25 m^2 area in each field. AGB was weighed in the field and again after drying at 105°C until reaching a constant weight, while BGB was cleaned of soil, weighed, and dried at 105°C to a constant weight.

Application of the Light-Use efficiency model for quantification of plant C inputs used in Roth

To estimate plant carbon input for the Roth-C model, a Light Use Efficiency (LUE) model was developed and calibrated. A detailed description of the model can be found in (Pei et al., 2022). This semi-empirical model calculates daily Net Primary Productivity (NPP, expressed as $\text{g C m}^{-2} \text{ d}^{-1}$) by integrating absorbed photosynthetically active radiation (APAR) with the theoretical maximum light use efficiency for PAR conversion to biomass (ϵ_{Max}). The model is further modified by temperature (T_s) and water (W_s) stressors, following the methodology of Prince and Goward (1995) and (Wellington et al., 2022) respectively, which constrain NPP according to environmental conditions. Input parameters required by the model were derived as follow: Field data for model calibration was obtained according to the method described above. Measured dry matter of AGB sub-samples

were averaged to provide a single representative value for each ESU. The Normalized Difference Vegetation Index (NDVI) time series for each field was obtained from Sentinel-2 imagery, part of the Copernicus mission, for each year of the simulation period. NDVI data served as a proxy for AGB quantification and was used to estimate the Fraction of Absorbed Photosynthetically Active Radiation (FAPAR), a critical input for the LUE model. Daily meteorological variables were sourced from the ECMWF ERA-5 reanalysis dataset (<https://www.ecmwf.int/en/era5-land>), which provided essential parameters for modelling plant productivity. These variables included mean air temperature at 2 meters, dewpoint temperature (both expressed in K) and surface solar radiation downwards (J m^{-2}). This data was integrated into the model to simulate environmental conditions affecting plant growth and productivity. Planting and harvest dates were obtained by farmers and did not require further derivation. The model was calibrated at the ESU level, on four key parameters: minimum, optimum, and maximum temperatures ($T_{\min}$, T_{opt} , and $T_{\max}$) and $\epsilon_{\max}$. Calibration was performed using an iterative process aimed at minimizing the normalized root mean square error (nRMSE) between observed carbon content and model simulated NPP. First, a grid of plausible values from literature (a priori distribution) for each parameter was established. Each parameter combination was tested by calculating the nRMSE between predicted and observed biomass values. The parameter set that minimized the nRMSE was then refined by testing narrower parameter ranges around this optimal set to improve calibration precision. The list of calibrated parameters obtained is reported in Table S3.1.

The calibrated LUE model was applied to the experimental fields. For each field, NDVI median values were retrieved from Sentinel-2 for the period between sowing and harvest, allowing calculation of FAPAR. ERA-5 meteorological data were merged with Sentinel-2 data, and crop-specific parameters were applied to calculate W_s , T_s , and $\epsilon_{\max}$. The resulting NPP estimates (g C m^{-2}) were converted in Mg ha^{-1} of dry matter by considered an averaged C content in plants of 0.45%.

Table S3.1 List of calibrated parameters used in the Light Use Efficiency (LUE) model simulations.

Crop	$T_{\min}$ (°C)	T_{opt} (°C)	$T_{\max}$ (°C)	$\epsilon_{\max}$ (g C MJ ⁻¹)
Winter wheat	-1.5	18.5	36	0.9
Maize	6	22	39	1.4
Tomato	3	25	38	1.4
White mustard	6	21	25	0.7
Mix cover crops	6	21	25	0.7

Table S3.2 Observed and predicted yields for a 2.5-year crop rotation under baseline (Bsl) and carbon farming practice (CFp) scenarios across different Lead Commercial Farms (LCFs). The harvest index (HI) and shoot-to-root ratio (S:R), used to calculate plant carbon input following the method of Bolinder, are also included. The Light Use Efficiency (LUE) model performance metrics are presented, including root mean square error (RMSE), relative error (RE), mean error (ME), and regression coefficient (R^2).

LCFs	Year	Scenario	Crops	Obs Yield (Mg ha ⁻¹)	Pred Yield (Mg ha ⁻¹)	HI	S:R	RMSE	RE	ME	R ²
LCF1	2022	Bsl	Durum wheat	5	6	0.4	3	3.34	14%	0.55	0.56
LCF1	2023	Bsl	Silage maize	22.7	14	0.9	4.8				
LCF2	2022	Bsl	Winter wheat	5.7	5.4	0.4	3				
LCF2	2023	Bsl	Grain Maize	4.8	4.6	0.3	4.8				
LCF3	2022	Bsl	Winter wheat	5.6	6.1	0.4	3				
LCF3	2023	Bsl	Tomato	4.9	7.8	0.5	3.4				
LCF4	2022	Bsl	Durum wheat	5.5	6.2	0.4	3				
LCF4	2023	Bsl	Tomato	7.8	8.1	0.5	3.4				
LCF5	2022	Bsl	Durum wheat	4.7	4.5	0.4	3				
LCF5	2023	Bsl	Tomato	3.3	8.8	0.5	3.4				
LCF1	2022	CFp	Durum wheat	5	5.5	0.4	3				
LCF1	2022	CFp	White mustard	5.4	2.6	1	7.4				
LCF1	2023	CFp	Silage Maize	24.6	13.9	0.9	4.8				
LCF1	2023	CFp	Cover crop mixture	1.4	3.1	1	3.6				
LCF2	2022	CFp	Durum wheat	5.7	5.5	0.4	3				
LCF2	2022	CFp	White mustard	3.8	2.4	1	7.4				
LCF2	2023	CFp	Seed Maize	5	4.7	0.3	4.8				
LCF2	2023	CFp	Cover crop mixture	2.1	3.3	1	3.6				
LCF3	2022	CFp	Durum wheat	5.6	6.3	0.4	3				
LCF3	2022	CFp	White mustard	5.6	2.1	1	7.4				
LCF3	2023	CFp	Tomato	4.1	8.2	0.5	3.4				
LCF3	2023	CFp	Cover crop mixture	1.7	3.1	1	3.6				
LCF4	2022	CFp	Durum wheat	5.5	6.2	0.4	3				
LCF4	2022	CFp	White mustard	5.3	2.4	1	7.4				
LCF4	2023	CFp	Tomato	4.8	7.4	0.5	3.4				
LCF4	2023	CFp	Cover crop mixture	2.8	2.1	1	3.6				
LCF5	2022	CFp	Durum wheat	4.7	4.7	0.4	3				
LCF5	2022	CFp	White mustard	5.2	0.9	1	7.4				
LCF5	2023	CFp	Tomato	3.9	8.7	0.5	3.4				
LCF5	2023	CFp	Cover crop mixture	2.8	2.3	1	3.6				

Table S3.3 Components for Carbon Unit (C.U.) calculation: Associated Emissions Reductions (AER) were determined as the difference between GHG emissions under the CFp scenario (E_{CFp} , expressed in CO₂e ha⁻¹) and those under the Bsl scenario (E_{Bsl} , expressed in CO₂e ha⁻¹). Net Carbon Removal (NCR) was calculated based on changes in SOC stocks (reported in CO₂e ha⁻¹), by comparing SOC changes under the baseline scenario (ΔSOC_{Bsl}) to those achieved following the implementation of CFp (ΔSOC_{CFp}) for each LCF.

LCFs	E_{Bsl}	E_{CFp}	AER	NCR	C.U.
LCF1	5.01	4.59	-0.43	24.2	24.63
LCF2	5.39	5.7	0.31	19.4	19.09
LCF3	4.28	3.97	-0.3	15.66	15.96
LCF4	6.50	6.96	0.46	11.43	10.97
LCF5	5.85	5.29	-0.56	17.12	17.68



Figure S3.1 Map of the five lead commercial farms (LCF1-5) selected for the study. Farms in order of increasing longitude: LCF1, LCF5, LCF4, LCF2, LCF3. On each farm, the fields managed using conventional practices (Baseline) and the ones using regenerative agricultural practices (CF project) are presented.

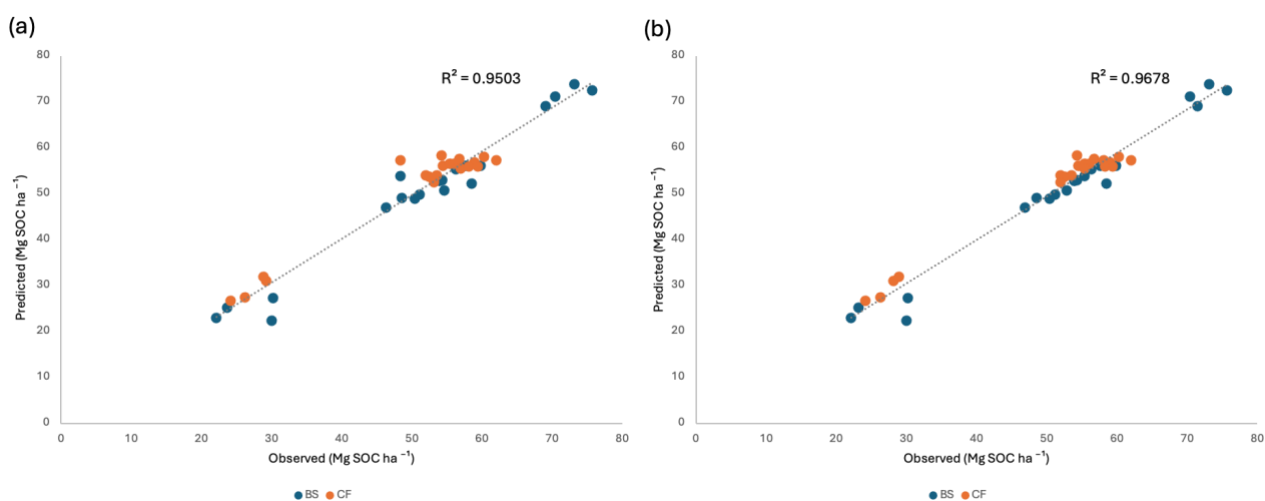


Figure S3.2 Scatterplot of model-predicted vs. observed values of SOC stock (Mg ha^{-1}) over 2.5 years (January 2022 – May 2024) for the verification dataset (a) and validation dataset (b). Blue and orange points represent the baseline scenario and CF project, respectively.

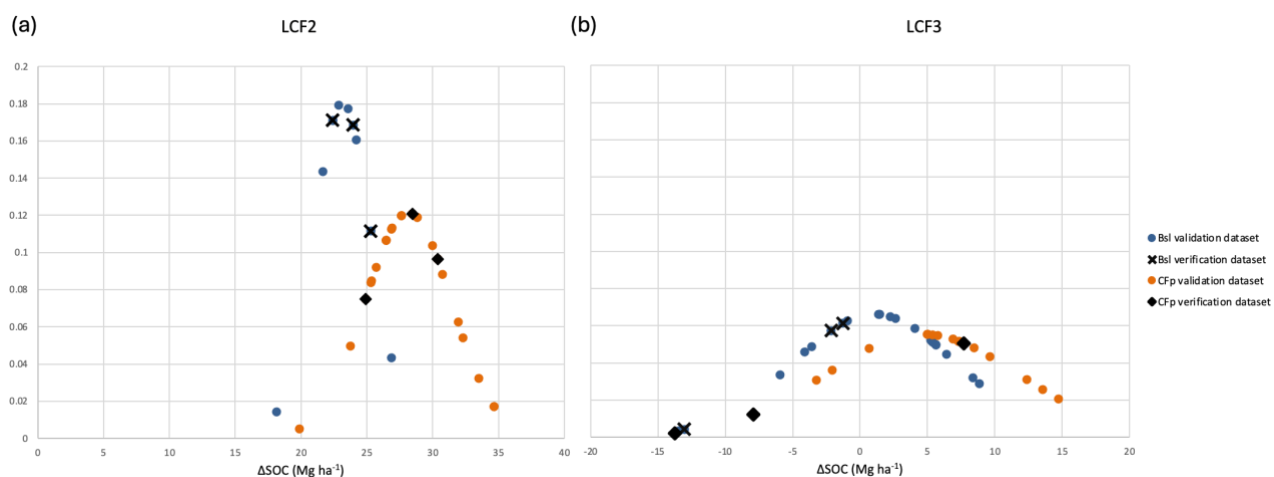


Figure S3.3 Normal distribution of ΔSOC Stock (Mg ha^{-1}) in Baseline (Bsl) and Carbon Farming project (CFp) scenarios for Validation and Verification datasets of LCF2 (a) and LCF3 (b). Blue and orange points represent the Bsl and CFp scenarios of the validation dataset, respectively, while cross and diamond markers represent the Bsl and CFp scenarios of the verification dataset, respectively.

Chapter 4

Targeted supplementation of mineral and organic nutrients enhances carbon incorporation from wheat residue into mineral-associated soil organic matter fraction

Marta Bertola¹ | Cornelia Rottensteiner² | Flavio Fornasier^{3a,b} | Stefano Amaducci¹ | Andreas Richter² | Andrea Ferrarini¹

¹ *Department of Sustainable Crop Production – Università Cattolica del Sacro Cuore, Piacenza, Italy*

² *Division of Terrestrial Ecosystem Research, Centre for Microbiology and Environmental Systems Science, University of Vienna, Austria*

^{3a} *CREA, Research Centre for Viticulture and Enology, Unit of Gorizia, Italy*

^{3b} *SOLIOMICS srl, Via del Cotonificio, 129/B 33100 Udine, Italy*

4.1 Introduction

There is a strong global interest in enhancing soil carbon (C) stocks across various agricultural systems worldwide to both increase soil health and mitigate climate changes (Lal, 2004b; Smith, 2012). Soils are the largest reservoir for terrestrial C, but their ability to retain stabilized C over time is significantly reduced in many managed systems (E.C. DG Environment, 2011). This is due to practices that either hasten the loss of soil organic matter (SOM) or hinder its formation and retention (Lal, 2004b). Nowadays, restoring lost soil C is becoming a priority for agricultural policymakers and practitioners. This effort not only contributes to climate change mitigation, but also supports the delivery of multiple co-benefits (Schmidt et al., 2011). C-rich crop residues, such as wheat, rice stubble or maize stalks, represent a global resource with significant potential for enhancing soil C levels. However, multiple studies have shown that soil C content does not always increase in response to repeated addition of large quantities of crop residues to soil (Richardson et al., 2014). Microorganisms play a key role in two contrasting mechanisms that drive SOM dynamics: they reduce soil organic carbon (SOC) stocks through decomposition, but also increase SOC stocks through the formation of microbial biomass and stabilization of its residues associated with minerals or within soil aggregates (Bhattacharyya et al., 2022; Sokol et al., 2022). Recently, the need to balance these opposing microbial functions toward greater SOC stabilization has been emphasized, notably through the conceptual framework of the “microbial carbon pump” proposed by Liang et al. (2017). This concept highlights the role of microbial processes in promoting long-term C storage in soils.

The efficiency of the transformation of exogenous organic matter into stable SOC depends heavily on the balance of C and the availability of essential nutrients, including nitrogen, phosphorous, and sulfur (Fang et al., 2019; Kirkby et al., 2014, 2013; Liu et al., 2023; Richardson et al., 2014; Zhao et al., 2024). Indeed, both the chemical composition of plant residues and organic matter in soils affects microbial growth and activity, thus controlling SOM cycling (Zechmeister-Boltenstern et al., 2015). When microorganisms feed on a substrate with an unfavorable stoichiometric ratio ($C:N:P:S \neq 10,000:833:200:143$), they must invest extra energy to acquire the missing elements by producing exoenzymes (Abay et al., 2024; Bertrand et al., 2019; Coonan et al., 2020a; Sinsabaugh et al., 2009). This additional energy allocation helps them sustain their biomass but likely causes them to partition more C taken up into respiration, leading to a decrease in microbial carbon use efficiency (CUE) (Spohn et al., 2016). Therefore, addition of organic material with high C:nutrient ratio can increase the mineralization of existing SOM through a phenomenon

known as the “positive priming effect” (Song et al., 2022), leading to even greater SOC losses. Conversely, the stoichiometrically balanced addition of exogenous nutrients increases microbial utilization of crop residue-derived labile C sources, hence boosting microbial biomass, accelerating microbial growth and turnover, and increasing CUE, thus improving the formation of SOM (Fang et al., 2019; Feng et al., 2022; Kirkby et al., 2013, 2014; Poeplau et al., 2019; Spohn et al., 2016; J. Wu et al., 2022; Zhao et al., 2024, 2017). In particular, accelerated microbial growth and turnover is associated with increased microbial necromass, which becomes part of the stable SOM pool and can reside in soils for longer periods (Prommer et al., 2020; Sokol et al., 2022; Spohn et al., 2016). Estimates of the proportion of microbially derived C transformed into non-living SOC range from 40% to 80% (Prommer et al., 2020). This is confirmed by the similarity between the stoichiometry of C, N, P, and S in SOM and the nutrient ratios of soil microorganisms, suggesting that stable SOM primarily derives from microbial necromass and by-product rather than from the direct deposition of resistant plant materials (Prommer et al., 2020). Studies across various soil types have demonstrated that adding plant residues increases the formation of stable carbon in the fine fraction of soil organic matter (ff-SOM, <0.4 mm; synonymous with heavy fraction SOM >1.4 g cm⁻³), with a 1.2 to 2-fold rise when nutrients are added (Fang et al., 2019; Kirkby et al., 2013, 2014; J. Wu et al., 2022). Higher nutrient inputs also boost “native” SOC consumption through the priming effect and promote stable carbon production by improving residue humification efficiency. While typically only 10% of added plant residues remain in the soil after mineralization, nutrient supplementation can increase residue carbon conversion into SOC by 2 to 8 times, depending on soil type (Fang et al., 2019; Kirkby et al., 2013, 2014; J. Wu et al., 2022). These studies also emphasized that the impact of added nutrients on microbial biomass, growth, turnover, and CUE is crucial for increasing the conversion of C-rich residues into ff-SOM.

Mineral-associated organic carbon (MAOM-C) is crucial for long-term carbon sequestration in soils, as it forms stable complexes with soil minerals that protect organic matter from decomposition, thereby enhancing carbon permanence and contributing to climate change mitigation (Cotrufo et al., 2019). However, recent studies suggest that MAOM-C, due to its low C:N ratio and lower chemical complexity, can also be mineralized by microbes, making its turnover an important source of nitrogen and a key contributor to short-term soil fertility (Lavallee et al., 2020; Villarino et al., 2023).

Long-term C sequestration from crop residues into SOM thus necessitates the concurrent incorporation of substantial amounts of nutrients to fulfill the stoichiometric needs of soil microbial

biomass and the newly formed SOM (Richardson et al., 2014). This process is critical not only for mitigating climate change, but also for enhancing soil fertility, and improving agricultural productivity. However, the application of nutrients to maintain and increase crop yield and simultaneously maintain or generate new and stabilized SOM in the long term implies additional costs for and emissions from fertilizers application. It is therefore important to find and maximize the use of alternative sources of nutrients to synthetic fertilizers. **Nutrient valorization** of organic amendments produced from circular processes such as anaerobic digestion offers a sustainable solution. Bio-digestate, a co-product of biogas production, has a high fertilizer value by bringing both labile carbon and essential nutrients into soil. By integrating these organic amendments into the farming system, the nutrients present in agricultural and organic waste are **recycled** back into the soil, closing the nutrient loop. This circular approach reduces the need for synthetic fertilizers, while improving soil health, enhancing C sequestration, as well as minimizing environmental impacts such as nutrient runoff and greenhouse gas emissions (Guilayn et al., 2020).

In this study, an incubation experiment was set up to test the main hypothesis that adding inorganic or organic nutrients to wheat straw residues, according to the microbial stoichiometric requirements, will increase the humification efficiency (HE) of crop-residues into the stable fraction of SOM, as well as soil fertility. The specific hypotheses tested were: (i) addition of stoichiometric balanced nutrients can rectify the C:nutrients ratio imbalance in microorganisms, increasing CUE; (ii) initial soil fertility may act as an environmental driver in determining stoichiometric imbalances in the microbial community; (iii) high CUE triggered by nutrient addition leads to greater incorporation of carbon into microbial biomass and by-products which, with microbial turnover, contributes to the formation of stable SOC as mineral-associated soil organic C (MAOM-C); (iv) the incorporation of C-rich straw residues with stoichiometrically balanced nutrients increases microbial enzymatic activity and changes their patterns; (v) introduction of labile carbon through organic fertilization can boost microbial growth and activity increasing the humification efficiency. This research ultimately aims to advance the broader goals of carbon farming by evaluating agricultural practices designed to enhance short-term carbon sequestration efficiency in soils, thereby supporting climate mitigation efforts and promoting sustainable soil management.

4.2 Materials and Methods

4.2.1 Collection and preparation of soils and wheat straw

Eight composite soil sub-samples were randomly collected in each of the two fields located in Piacenza province, Italy (Soil1, 45°04'30.61"N, 9°92'74.18"E and Soil2, 44°98'33.62"N, 9°70'11.21"E) at a depth of 0-20 cm, in June 2022. The climate is continental with an average annual rainfall of 863 mm (1992-2022), and rainfall peaks occurring in autumn and spring. These fields had been subjected to decadal narrow crop rotation of winter wheat (*Triticum aestivum* L.) and tomatoes (*Solanum lycopersicum* L.), with the application of mineral fertilizers. These soils were chosen for their different levels of soil fertility representing the province area: Soil2 has higher chemical fertility and is richer in C compared to Soil1, with higher microbial biomass carbon (MBC) relative to SOC, and greater nitrogen availability. Detailed information about soil properties is presented in Table 1. Soil samples were air-dried, milled to break down aggregates, and passed through a 2 mm sieve. They were then stored in sealed buckets until the start of the experiment. Before the beginning of the experiment (hereafter denoted as T_0) they were further sieved through a 0.4 mm sieve to remove most of the endogenous coarse organic materials to identify the impacts of added wheat straw on SOM dynamics. Three sub-sample for each soil type was analyzed for total C, N, P, K and for SOM fractionation as described below.

Wheat straw (*Triticum aestivum* L.) was harvested from Soil1 field site few days before the commercial harvesting (5.7-ton ha⁻¹ of grain yield) in 2022. Plant material was oven dried (65 °C) until constant weight, ground to around 5 mm in a knife mill, and stored at room temperature. A subsample of straw was analyzed for total C, N, P and S (Table 4.2).

Table 4.1 Mean values of soil properties for Soil1 and Soil2 used in the study.

Fields	Soil1	Soil2
Soil textural class	Silty loam	Silty clay loam
% sand	33.4	12.2
% silt	52.4	54.1
% clay	14.0	33.6
TOC (mg g⁻¹)	6.40	11.66
TN (mg g⁻¹)	0.74	1.55
P (mg g⁻¹)	0.53	0.78
K (mg g⁻¹)	1.55	2.38
NO₃⁻ (mg kg⁻¹)	0.28	63.56
NH₄⁺ (mg kg⁻¹)	1.07	1.50
Available P (mg kg⁻¹)	5.65	5.44
Available C:N:P (mg kg⁻¹)	9.6:0.2:1	11.7:12.0:1
MBC:MBN (mg kg⁻¹)	5.46	5.40
MBC:SOC (%)	2.65	3.54

4.2.2 Incubation experiment

Dried soil samples were spread out on aluminum trays, rewetted to 60% of maximum water holding capacity using distilled water, and pre-incubated at room temperature for a total duration of 14 days before starting the incubation experiment. This pre-incubation was performed to allow soil respiration to reach basal rates. Eight replicates, each consisting of 200g of equivalent dry soil, were set up for each treatment and soil type for a total of n = 64 samples (2 soil types x (3 treatments + 1 control) x 8 replicates).

After the pre-incubation period, three different treatments were applied as reported in detail in the paragraphs below. Treatments can be summarized as: (i) soils receiving straw addition (*Straw*), (ii) soils receiving straw and mineral fertilizer addition (*Straw+min*), (iii) soils receiving straw and organic fertilizer addition (*Straw+org*), (iv) and soils without treatment (*Control*). 150 g of pre-incubated soil (equivalent oven dry weight) were placed in a 500 mL glass Duran bottle and treatments were added as described below (4.2.2.2). The bottles were equipped with a PTFE faced silicone cap to allow CO₂ sampling over time using a syringe. All bottles were incubated in a dark, temperature-controlled incubator set to 24 °C. CO₂ emissions from soils were measured periodically on days 1, 4, 5, 7, 14, 21, 28, 35, 49, 63, and 94. For each measurement, 10 mL of air was sampled from the headspace of the bottles using a 50 mL syringe, after thoroughly mixing the air several

times. CO₂ concentration in the sampled air was then analyzed using an EGM-5 CO₂ Gas Analyzer (*PP Systems, Amesbury, U.S.A.*). The CO₂ emission rate (ng CO₂-C g⁻¹ dry soil h⁻¹) and cumulative CO₂ emissions (mg CO₂-C kg⁻¹ dry soil) were calculated based on the amount of CO₂-C produced during the incubation. The emission rate was determined by dividing the CO₂-C released over a specific measurement interval by the time elapsed and the mass of dry soil. The cumulative emissions were obtained by summing the CO₂-C produced across all measurement intervals over the incubation period, normalized to the mass of dry soil. Following CO₂ measurement, bottles were left open and ambient air was pushed into the open bottles several times using a 100 mL syringe. This was done to ensure the air in each bottle was consistent with ambient air conditions prior to resealing the bottles. Four replicates were collected after 7 days of incubation and used for microbiological analysis and analysis of available nutrients as described below (2.3). From the 7th day of incubation (hereafter denoted as T₁) and for each CO₂ sampling time, the remaining four replicates were mixed to enhance straw breakdown and improve homogeneity. Since glass bottles were sealed, all soils were maintained at 60% of maximum water holding capacity and no addition of distilled water was needed. These samples were incubated until CO₂ efflux of all soils became low and approached an asymptote (Fig. 3.1 a-b). After 94 days (hereafter denoted as T₂), half of each soil sample was air-dried, sieved at 2 mm, and stored for soil chemical analysis, while the other fresh part was used for microbiological analysis and analysis of available nutrients.

4.2.2.1 Quantification of supplementary nutrients addition

In order to maximize the humification efficiency of wheat straw, the stoichiometry of the more stabilized SOM was considered, with a C:N:P:S ratio of 10,000:833:200:143 (Table 4.2) as well as a maximum humification efficiency of 30% of the newly added straw-C (Kirkby et al., 2013). Elemental analysis of straw showed that it was nutrient poor compared to the stable SOM fraction therefore additional nutrients were required to achieve the potential 30% humification (Table 4.2). For the treatment with mineral fertilizer, nutrient addition was done by adding 25 mL kg⁻¹ soil of a nutrient solution containing 3.7, 2.7 and 0.38 g per L of N, P and S, respectively, using urea, potassium dihydrogen phosphate and ammonium sulphate. The pH of the nutrient solution was adjusted to pH 7 using a 10 M sodium hydroxide solution. Organic fertilization was carried out using biogas digestate derived from dedicated energy crops (mainly triticale/winter wheat and maize), applied in its bulk form, comprising both liquid and solid fractions. The digestate had the following characteristics: 6% dry matter, 0.41% N, 0.05% P, 0.04% S, and 0.31% K. Here, the availability of

inorganic nutrients within the soil was disregarded as the nutrients were expected to be an order of magnitude less than the addition.

Table 4.2 Calculation of supplementary nutrients (mg) required to humify 30% of the C in 1 g of straw (equivalent to 10 tons ha⁻¹ incorporated in top 15 cm soil layer).

Expected humus C:N:P:S ratios		C	N	P	S
		10 000	833	200	143
Straw (g) added per 150 g dry soil	1				
C, N, P, S concentration of straw (%)		45.0	0.501	0.039	0.158
N, P, S concentration of digestate (g kg ⁻¹)			4.1	0.5	0.4
Nutrients (mg) in 1 g straw		450	5.01	0.39	1.58
Maximum humification (30%) aimed for	30				
C (mg) that will be humified if 30% humified		135			
Total nutrients required (mg) to humify 135 mg C			11.25	2.70	1.93
Supplementary nutrients (mg) required for humification (total nutrients required for humification - nutrients in straw)			6.23	2.31	0.35

4.2.2.2 Treatments

In the Straw treatment, wheat straw was added to the soil at a rate of 6.66 g kg⁻¹ dry soil and thoroughly mixed in the glass bottles. The amount of straw added to the soil was equivalent to a field rate of 10 t ha⁻¹ of wheat residues assuming a soil depth of 15 cm. The quantity of wheat residues was approximated by dividing the averaged wheat commercial yield in the province (6.5-ton ha⁻¹) by the difference of an averaged harvest index of wheat (0.4) and the grain yield. The *Straw+min* treatment was identical to the one previously described, except that 25 mL of the mineral nutrient solution per kg of dry soil (as detailed in the paragraph above) was sprayed uniformly onto aluminum trays. The soils were then adjusted to 60% of their maximum water-holding capacity. 150 g of the fertilized soil was then mixed with wheat straw in a Duran bottle.

The *Straw+org* treatment was identical to the *Straw+min* except that digestate, rather than a mineral fertilizer, was homogenized with soil at a rate of 1.45 g kg⁻¹ dry soil¹.

4.2.3 Sample analysis

4.2.3.1 Soil physical and chemical analyses

The maximum water holding capacity of soils was determined by saturating 50 g of soil in an immersed funnel equipped with a sealed fiberglass filter. Water was then left to drain off through

the filter in a cylinder and the amount of drained water was measured after 30 min. Air-dried sub-samples collected at T_0 were analyzed for total C, N, P and K, while sub-samples collected at T_2 were analyzed for total C and N. Total organic carbon (TOC) and total nitrogen (TN) were determined by an elemental analyzer (Primacs100, Skalar Analytical B.V.) while total phosphorus (TP) and potassium (TK) were determined by microwave digestion method and read with an ICP-OES (ICP-OES 5100; Agilent Technologies). Fresh sub-samples taken at T_0 , T_1 , and T_2 , were used for nitrate (NO_3^-), ammonium (NH_4^+) and phosphate (PO_4^{3-}) quantification. Available nutrients were extracted in 0.5 M K_2SO_4 after shaking for 1 hour in a linear shaker at 250 rpm and quantified through colorimetric methods based on Doane & Horwáth (2003) for nitrate, Lajtha et al. (1999) for phosphorous and Weatherburn (1967) for ammonium.

4.2.3.2 SOM fractionation and HE calculation

Air-dried and 2 mm sieved sub-samples were used for SOM fractionation and quantification of C and N in the soil fractions obtained according to the method of Zhang et al. (2021): particulate organic matter (POM) and mineral associated organic matter (MAOM). Both fractions were analyzed for C and N content on an elemental analyzer (Primacs100, Skalar Analytical B.V) and hereafter are expressed as MAOM-C (organic C content in MAOM), MAOM-N (total N content in MAOM), POM-C (organic C content in POM), POM-N (total N content in POM). Carbon humification efficiency (HE in %) was then calculated as the ratio between MAOM-C variation ($\Delta\text{MAOM-C}_{T_2-T_0}$ in g kg^{-1}) and the total carbon input from straw (g kg^{-1}).

4.2.3.3 Determination of microbial metabolic process

From fresh sub-samples taken at T_1 and T_2 , microbial CUE, growth rate and the turnover time of soil microbial biomass were determined based on incorporation of ^{18}O from ^{18}O -labeled water into microbial genomic DNA following Spohn et al. (2016). Briefly, two replicates of 400 mg of each soil sample were weighed into 1 ml vials and transferred into 30 ml sealed vials. To one of them ^{18}O - H_2O (97.0 at%, Campro Scientific) was added with a syringe (Hamilton) to reach 20.0 atom% of ^{18}O in the final soil water. The other was amended with the same volume of non-labeled Millipore water to serve as natural abundance control. The incubation vials with the soil samples were incubated for 24h at 24°C. Before the incubation and at the end of incubation, 5 ml gas samples were taken from each vial and the CO_2 was measured using an EGM-5 CO_2 Gas Analyzer (PP Systems, Amesbury, U.S.A.). Subsequently, the soil samples were stored at -80°C until DNA extraction. DNA extract was weighed to determine extract volume and the DNA concentration was determined fluorometrically

by the Qubit assay using a kit (Qubit™ dsDNA Quantification Assay Kits, ThermoFisher Scientific). 50 μL of the DNA extract was dried in silver capsules to remove any water and subsequently the ^{18}O abundance and the total O content were measured using a Thermochemical elemental analyzer (TC/EA Thermo Fisher) coupled via a Conflo III open split system (Thermo Fisher) to an Isotope Ratio Mass Spectrometer (Delta V Advantage, Thermo Fisher).

4.2.3.4 Soil microbiological analyses

At T_0 , T_1 , and T_2 , microbial biomass C (MBC) and N (MBN) concentrations were determined by the chloroform fumigation-extraction method (Vance et al., 1987) using 0.5 M K_2SO_4 extractant. MBC and MBN were measured using an elemental analyzer (TOC_L, Shimadzu). and calculated with conversion factors of 2.22 for MBC and 1.85 for MBN (Wu et al., 1990). The C and N concentrations of the non-fumigated soils were considered as dissolved organic C (DOC) and N (DTN).

Hydrolytic and oxidative extracellular enzyme activities (EA) were measured at T_0 , T_1 , and T_2 . The assay based on the procedure of (Ferrarini et al., 2021) tested 20 enzymes involved in the principal nutrient cycles, namely: A-glucosidase (alfaG), β -glucosidase (betaG), α -galactosidase (alfaGAL), β -galactosidase (betaGAL), β -1,4-glucanase (cell), β -1,4-xylanase (xilo), involved in C cycling; β -D-glucuronidase (uroni), N-acetyl- β -D-glucosaminidase (chit), leucine amino-peptidase (leu), arginin amino-peptidase (arg) and aspecific protease (aprot) involved in N cycling; acid- (acP) and alkaline- (alkP) phosphomonoesterase, phosphodiesterase (bisP) and pyrophosphate-phosphodiesterase (piroP) involved in P cycling; arylsulfatase (aryS); butyrate esterase (butir) and fluorescein diacetate hydrolysis (fda) involved in the hydrolysis of ester bonds; oxidase (ox) and peroxidase (perox) involved in redox reactions of soil organic matter cycles. All measurements were taken in triplicates and the activities were expressed as nanomoles of MUF (or AMC) g^{-1} dry soil h^{-1} .

4.2.4 Calculations of microbial growth, turnover, microbial stoichiometric imbalance, and resource limitation

The respiration flux was calculated based on the amount of $\text{CO}_2\text{-C}$ produced during the incubation period. The amount of DNA ($\mu\text{g g}^{-1}$ dry soil) produced during the incubation with ^{18}O -labeled water addition, the flux of C allocated to biomass production, the growth rate (ng C g^{-1} dry soil h^{-1}), CUE, turnover time (days), and the metabolic quotient ($q\text{CO}_2$) were calculated following Spohn et al. 2016. Furthermore, vector analysis was used to analyze microbial resource limitation as proposed by Zhao et al., 2024 where activity of C- (betaG, cell, xilo), N- (uroni, chit, leu) and P-

(acP, alkP) acquiring enzymes were considered. The calculation of the microbial C:N stoichiometric imbalances between substances and microorganisms were calculated based on Zhao et al. (2024).

4.2.5 Statistical analysis

Before conducting analysis of variance (ANOVA), all data were tested for homogeneity of variance and normality of distribution. Analyses were performed using R: *nmle* (Pinheiro et al., 2012) and *emmeans* (Lenth et al., 2018) packages. The effects of straw and nutrient addition on microbiological parameters and nutrients were tested using a three-way mixed model ANOVA. “soil type” and “treatment” × “time” interactions were used as fixed factors, while “treatment”, “replicates” and “time” were considered as random effects to represent the experimental designs. Changes in time of SOM pools (Δ MAOM-C, Δ MAOM-N, Δ POM-C, Δ POM-N) were analyzed with a two-way mixed-model ANOVA for complete randomized block design. “Soil type” × “treatments” interaction was used as fixed factors, while “treatments” and “replicates” were considered as random effects. Log transformations or square root transformations were performed to satisfy assumptions of normality and heteroskedasticity when needed. If the transformation did not meet the criteria, “function weights” were used in the linear mixed-model effect formula to consider the nonhomogeneous variance structure introduced by the factors studied. The function *emmeans* was used to estimate the marginal means and Holm-Šidák post-hoc tests were used to assess significant differences between treatments when the F-tests indicated statistically significant effects. All test results were considered statistically significant at $p \leq 0.05$.

A distance-based Redundancy Analysis (dbRDA) based on Bray–Curtis’s distance was used to assess the effects of the overall differences in soil EA among treatments and to understand the influence of soil and microbial variables. dbRDA was performed separately for each soil type, differentiating hydrolytic EA from oxidative EA. dbRDA was run on a four-step basis as described in Ferrarini et al. (2021).

Using the function ‘dredge’ in the MuMIn package (Barton, 2023) to identify the best predictors of carbon mineralization (as cumulative CO₂ emissions expressed in mg CO₂-C kg⁻¹ dry soil) and changes in MAOM-C (g kg⁻¹ dry soil), a model selection procedure based on the corrected Akaike's information criterion (AICc) was employed. To identify predictors of carbon mineralization, all treatments including the control were considered, while only treatments with straw addition were selected to identify predictors of MAOM-C changes. This was done to isolate the effects of nutrient addition from the straw incorporation. Since multiple models were selected, model averaging was

performed based on AICc weights. To ensure the comparability of parameter estimates, we standardized all predictors and response variables using the Z-score before conducting the analyses. The relative importance of the predictors considered as drivers of carbon mineralization and sequestration were assessed by calculating the relative effect of the parameter estimates for each of the predictors compared with the effect of all parameters estimates in the model. A total of four variance fractions were examined: (1) soil nutrients availability and stoichiometry; (2) microbial stoichiometric imbalance; (3) microbial metabolic processes; (4) microbial resource limitation.

4.3 Results

4.3.1 Soil CO₂ emission, SOM pools and humification efficiency

Straw addition immediately increased soil CO₂ emission rates in all soils compared to *Control* (3 times higher) and peaked after 5 days, rapidly declined thereafter, and stabilized after 49 days (Fig. 3.1 a-b). Straw addition also significantly increased CO₂ cumulative emission compared with *Control* in both soils (Fig. 3.1 c-d). In the less fertile soil (Soil1), both the CO₂ emission rate at peak of respiration and the cumulative CO₂ emission at the end of the experiment were significantly higher under *Straw+min* (2045 ng CO₂-C g⁻¹ h⁻¹ or 495 mg C-CO₂ kg⁻¹) compared with *Straw+org* (1880 ng CO₂-C g⁻¹ h⁻¹ or 447 mg C-CO₂ kg⁻¹) and *Straw* (1540 ng CO₂-C g⁻¹ h⁻¹ or 439 mg C-CO₂ kg⁻¹) (Fig. 3.1 c).

At the end of incubation (94 days after treatment addition), straw addition with both mineral and organic fertilizers increased MAOM-C content (on average +0.68 mg C g⁻¹) in both soils to a greater extent than straw addition without fertilizer (+0.13 mg C g⁻¹), while in *Control* MAOM-C content had significantly decreased over time (-0.29 mg C g⁻¹) (Fig. 3.2a). The two soils responded differently to straw addition showing a contrasting behavior in MAOM-N changes (Fig. 3.2b). Compared to T₀, an immobilization of TN was observed in the MAOM pool in Soil2 across all treatments, with an average decrease of -0.10 mg MAOM-N g⁻¹. In contrast, in Soil1, the MAOM-N content significantly increased in all straw + nutrient treatments (on average +0.05 mg MAOM-N g⁻¹), whereas in the *Straw* treatment, nitrogen immobilization was observed instead (Fig. 3.2b). In the *Control* treatment of Soil1, “native” POM-C was significantly reduced over time (-0.23 mg C g⁻¹), while the addition of straw significantly increased POM-C in both soils (on average +0.34 mg C g⁻¹ in Soil1 and + 0.73 mg C g⁻¹ in Soil2). In Soil1, no differences in POM-C were shown within straw incorporation treatments (Fig. 3.2c). In both soils, POM-N followed the same trend of POM-C, with no significant differences within treatments (Fig. 3.2d). The humification efficiency (HE) was significantly higher under *Straw+org* (11.35%) compared with *Straw+min* (8.88%) and *Straw* (1.85%) (Tab. S3.1).

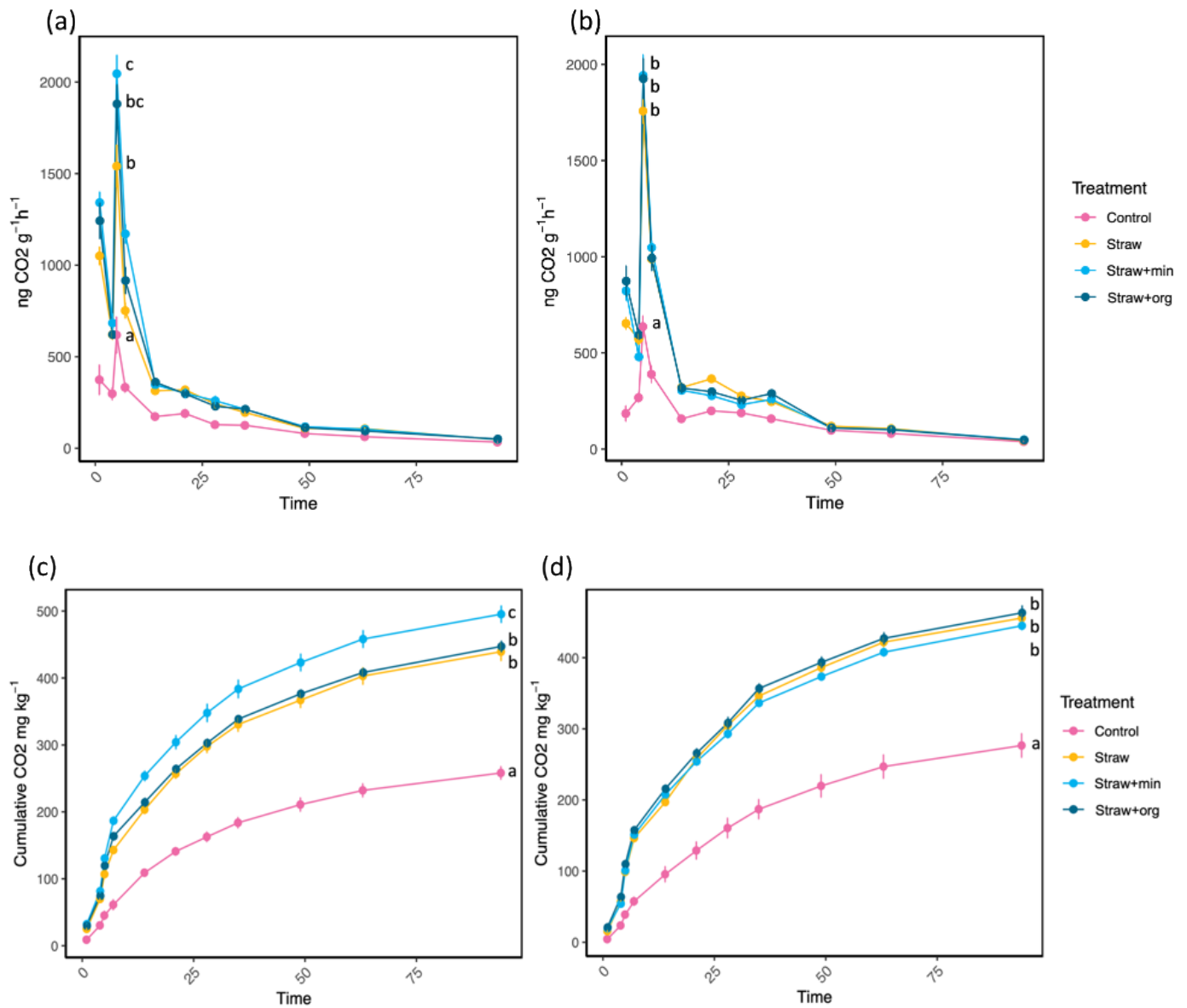


Figure 4.1 CO₂ emission rate in Soil1 (a) and Soil2 (b); cumulative CO₂ emissions in Soil1 (c) and Soil2 (d) under addition of straw and mineral or organic nutrients, along 94-days of incubation.
Note: Values are means $\pm$ standard errors (n=4).

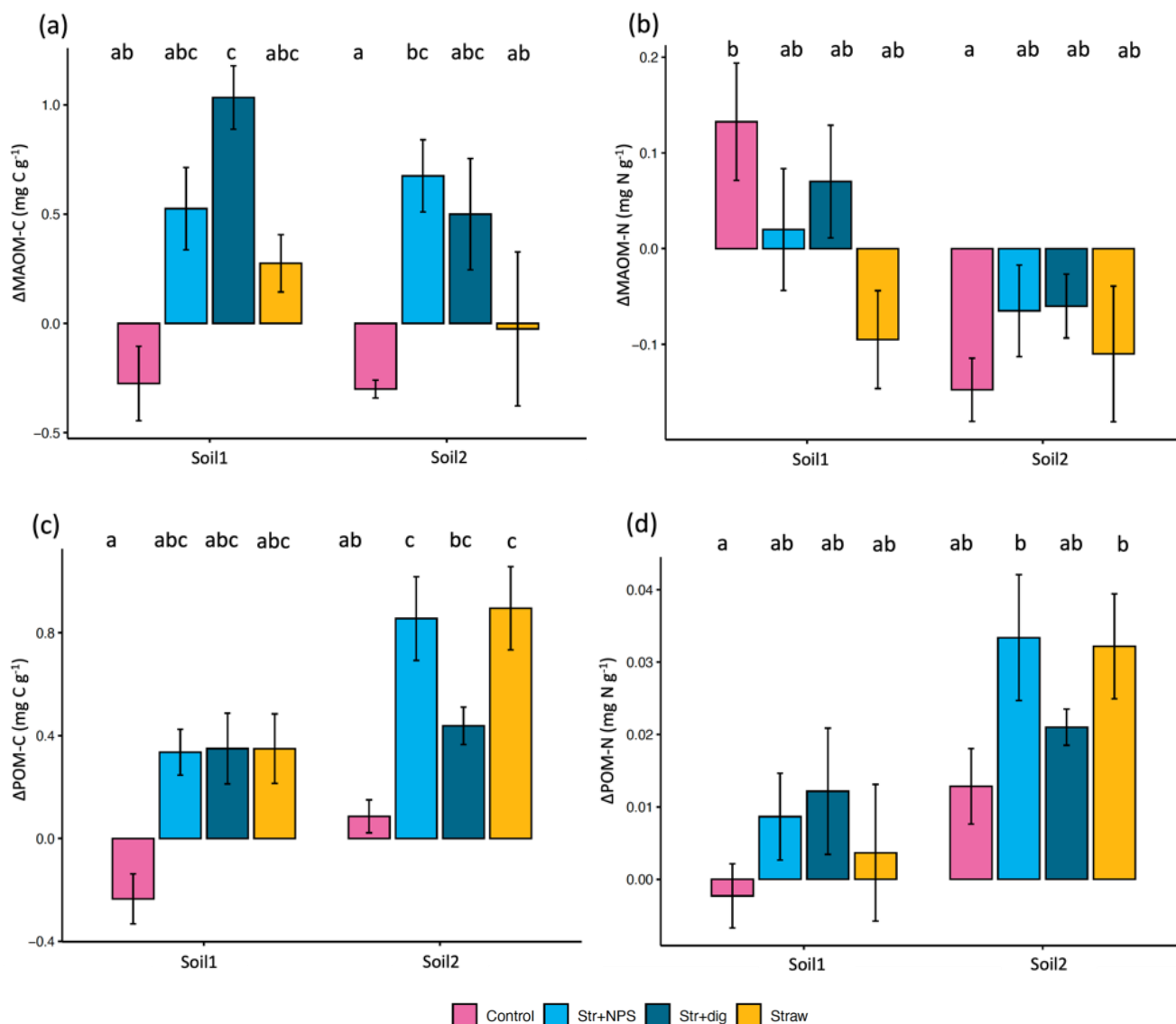


Figure 4.2 Average value and standard error of change at the end of incubation (T2 – T0) of MAOM-C (a), MAOM-N (b), POM-C (c), and POM-N (d) contents after addition of straw and both mineral and organic nutrients.

Note: Different letters denote statistically significant differences between treatments ($p < .05$) for each soil type.

4.3.2 Microbial metabolic processes

4.3.2.1 Microbial growth rate, turnover time, and CUE

The variation of microbial metabolic processes was significantly different between the less fertile Soil1 and the C-rich Soil2 during the incubation (Fig. S4.2), but they were not significantly affected by any of the straw treatments (Tab. S4.3, S4.6). At both, T₁ and T₂, MBC, growth rate, turnover time and CUE were significantly higher in the C-rich Soil2 than in Soil1, while qCO₂ was significantly higher in the less fertile Soil1 than in Soil2 (Fig. S4.2, Tab. S4.6). MBC increased at the end of incubation (T₂) with straw incorporation in both soils and showed the highest values at T₁ (Fig. S4.2, Tab. S4.6). Although with no significant differences, MBC in *Straw+min* and *Straw+org* treatments reached

higher values and remained higher compared to only *Straw* treatment till the end of incubation (T_2) (Fig. S4.2, Tab. S4.6). Growth rate increased from T_1 to T_2 , with the *Straw+org* treatment showing higher values compared to *Control* in both soils (Fig. S4.2, Tab. S4.6). CUE was not significantly affected by straw and nutrients addition, showing consistently high values across all treatments and in the control, with values of 0.5 in Soil1 and 0.6 in Soil2, respectively (Fig. S4.2, Tab. S4.6). Turnover time was significantly higher at T_1 compared to T_2 in both soils (Fig. S4.2). At T_1 turnover time showed a no significant trend where it is higher at *Control* compared with straw incorporation treatments and this difference decreased at T_2 (Tab. S4.6). qCO_2 was significantly higher at T_2 compared to T_1 (Fig S4.2).

4.3.2.2 Microbial enzymes activity and stoichiometric imbalance

The results of the dbRDA model on EA patterns and SIMPER analysis (Figs. 4.3, 4.4, S4.1 and Tab. S4.4a) showed that soil microbial activity of Soil1 and Soil2 responded differently to straw incorporation during the incubation. In Soil1, the patterns of hydrolytic EA differed significantly from the *Control*, with straw incorporation causing separation along axis 1 ($P = 0.001$) and axis 2 ($P = 0.001$) and accounting for 86.6 % of the total variance (Fig. 4.3a). In contrast, straw incorporation in Soil2 did not significantly alter the pattern of hydrolytic EA, although it differed from the EA observed in soil samples prior to any treatment application (*none*). The effect of “time”, here expressed by the *none* treatment, caused separation along axis 1 ($P = 0.001$) and axis 2 ($P = 0.001$) and accounted for 71.6 % of the total variance (Fig. 4.3b). dbRDA results indicated that the soil parameters having the highest influence ($P < 0.05$) on EA distribution were mostly available P and N in the form of MAOM-N and the MAOM-C:N in Soil1 and only NH_4^+ in Soil2 (Tab. S4.4b). Species score plots (Fig. 4.3c-d) showed that the increase in *FDA*, *aprot*, and *alkP* in Soil1 led to a significant differentiation along axis 1. In contrast, in Soil2, the increase in *FDA*, and the reduction of *aprot*, *alkP*, *betaG* and *butir* caused significant differentiations along both axis 1 and 2. This was further confirmed by the SIMPER analysis (Fig. S4.1), which showed that *FDA* made a cumulative contribution to the differentiation between the control and the straw-treated samples in both soils. In Soil1, *chit* contributed significantly and positively to the cumulative differentiation between the control and all treatments, whereas *alkP* and *butir* contributed significantly and negatively. Differently to hydrolytic EA, oxidative EA showed similar patterns across both soil types. In Soil1, straw incorporation caused a separation along axis 1 ($P = 0.001$) and axis 2 ($P = 0.13$), accounting for 90.5 % of the total variance (Fig. 4.4a). In Soil2, separation occurred along axis 1 ($P = 0.001$) and axis

2 ($P = 0.04$) explaining 85.1% of the total variance (Fig. 4.4b). Species score plots (Fig. 4.4 c-d) showed that the increase of *perox* activity was the primary driver of significant differentiation along axis 1, with a significant positive cumulative contribution in the *Straw+min* treatment (Fig. S4.1). dbRDA results from multivariate analysis on oxidative EA indicated that available P (for Soil1) and available C:P (for Soil2) were the main soil parameters that had the highest influence on oxidative EA distribution (Tab. S4.4b).

The addition of straw resulted in a shift of microbial community nutrient limitation (Tab. S4.6). Negative linear relationships between vector length and vector angle were observed in both soils at T_2 ($r = -0.51$, $p < .001$). Straw additions resulted in increased vector lengths in both soils, but with a significant increment only in Soil1. Straw addition reduced vector angle by 17% (*Straw+org*), 31% (*Straw+min*) and 35% (*Straw*), compared with no straw addition in Soil1, while is reduced by an average of 21% in Soil2.

Straw addition increased stoichiometric imbalances of C:N by 18% on average compared to the *Control* at T_1 , which was higher under mineral fertilization (Tab. S4.5). At T_2 C:N imbalances under straw addition were diminished compared to T_1 (2% higher than the *Control*), which was alleviated in *Straw+org* compared to *Straw+min* and *Straw*.

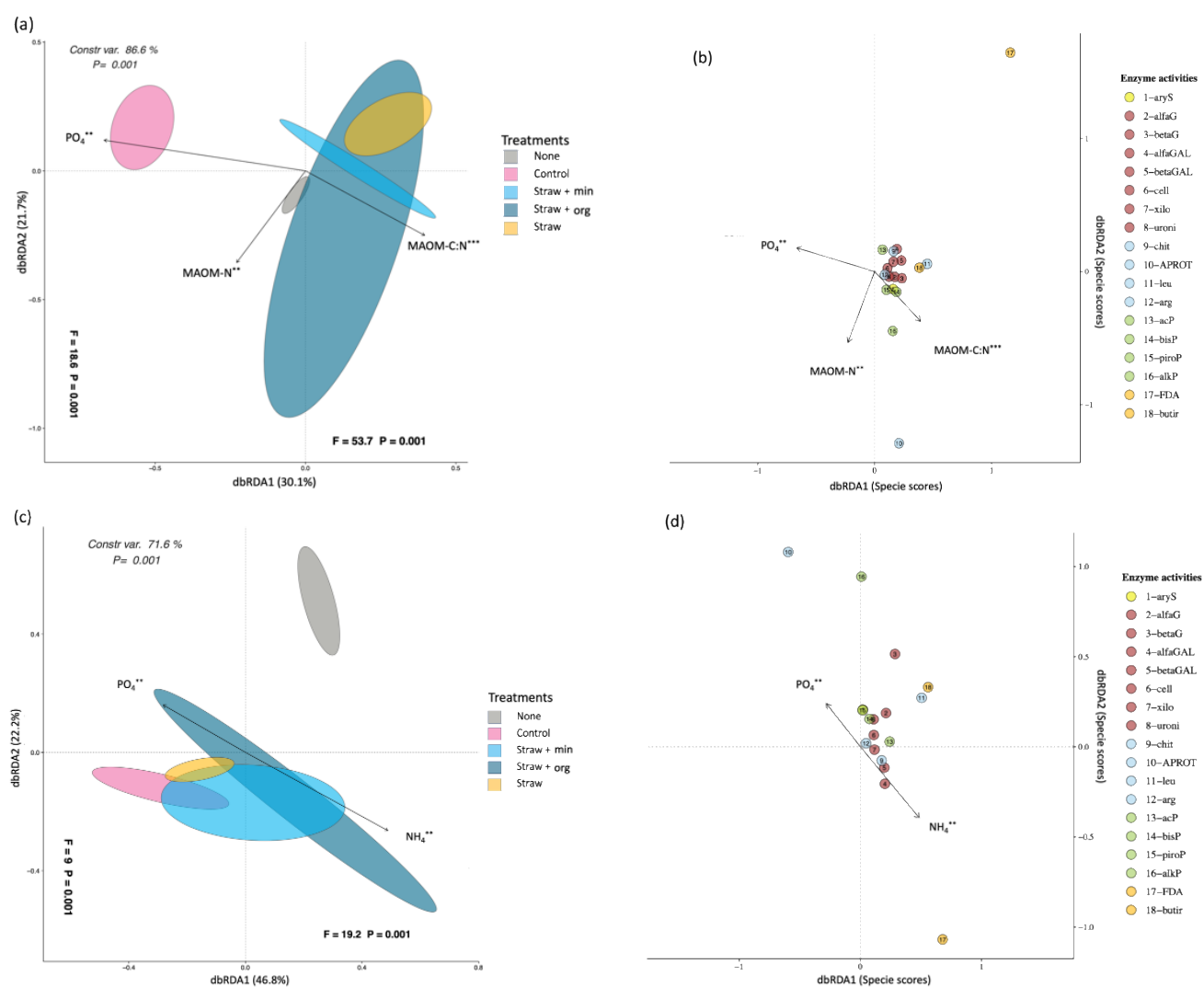


Figure 4.3 dbRDA plots of the model showing shifts in hydrolytic EA in Soil1 (a) and Soil2 (c). Species scores corresponding to the dbRDA plots (coordinates for enzymes included in model) are reported in the scatter plots (Fig. b-d for Soil1 and Soil2 respectively). Note: For enzymes abbreviations see Section 2.3.4.

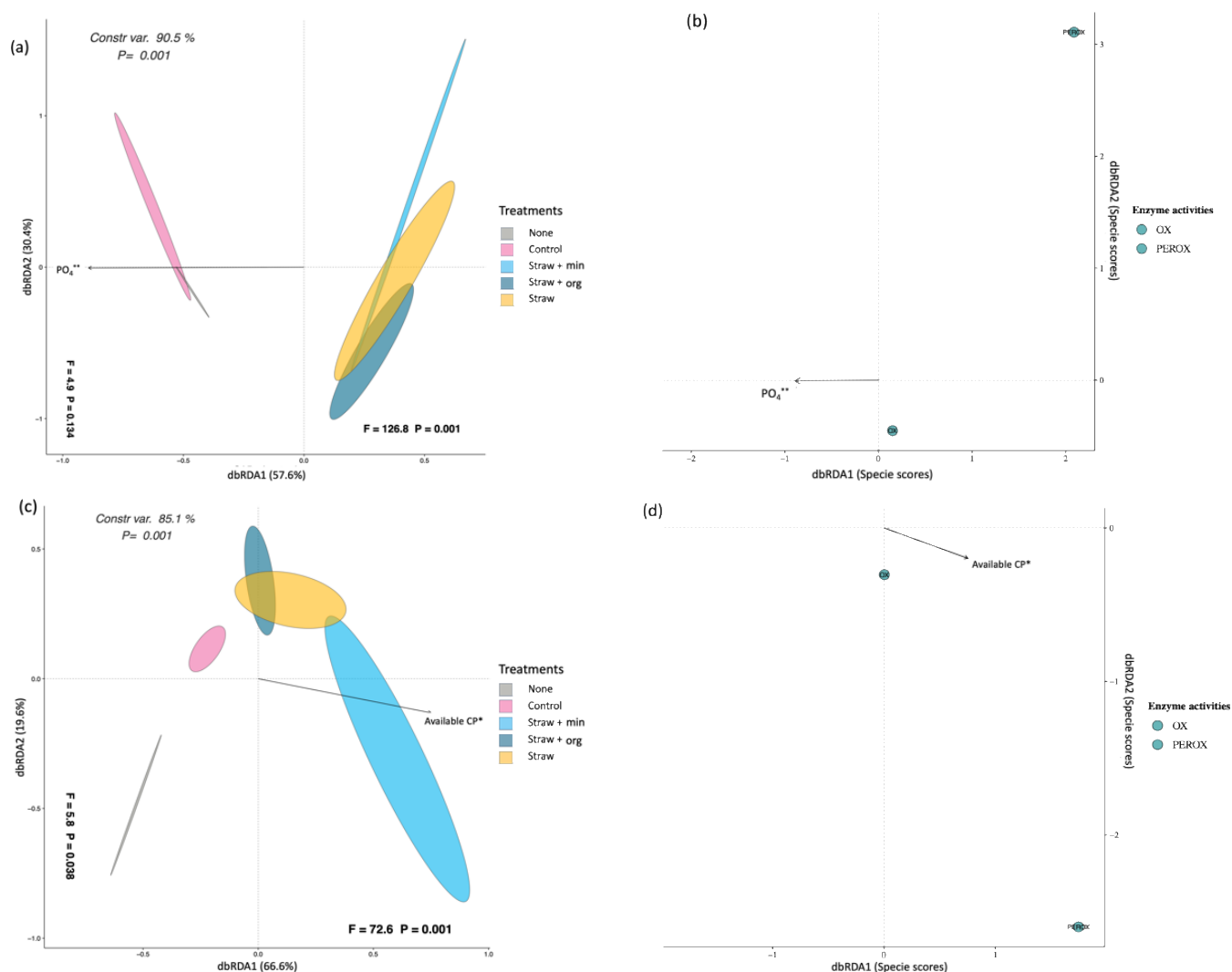


Figure 4.4 dbRDA plots showing shifts in oxidative EA in Soil1 (a) and Soil2 (c). Species scores corresponding to the dbRDA plots (coordinates for enzymes included in model) are reported in the scatter plots (Fig. b-d for Soil1 and Soil2 respectively). Note: For enzymes abbreviations see Section 2.3.4.

4.3.3 The controlling factors of SOC mineralization and MAOM-C changes

The multivariate models explained 87% of the variation in SOC mineralization and 77% of the variation in Δ MAOM-C (Figure 4.5a-b). Availability of soil nutrients at the end of the incubation explained up to 58% of the variation of SOC mineralization. Among them, available P had a significant negative effect on SOC mineralization. In addition, microbial C:N, CUE, and vector angles had significant negative effects, while MBC had a significant positive effect. Relatively to the predictors of the model explaining Δ MAOM-C, differences from T_0 to T_2 of soil nutrients availability explained up to 44% of the total variation, followed by microbial processes (39%). Available C:N, available C:P, NO_3^- , MBC and CUE had significant positive effects, while NH_4^+ , MBN, vector lengths and POM-C had significant negative effects on MAOM-C variations.

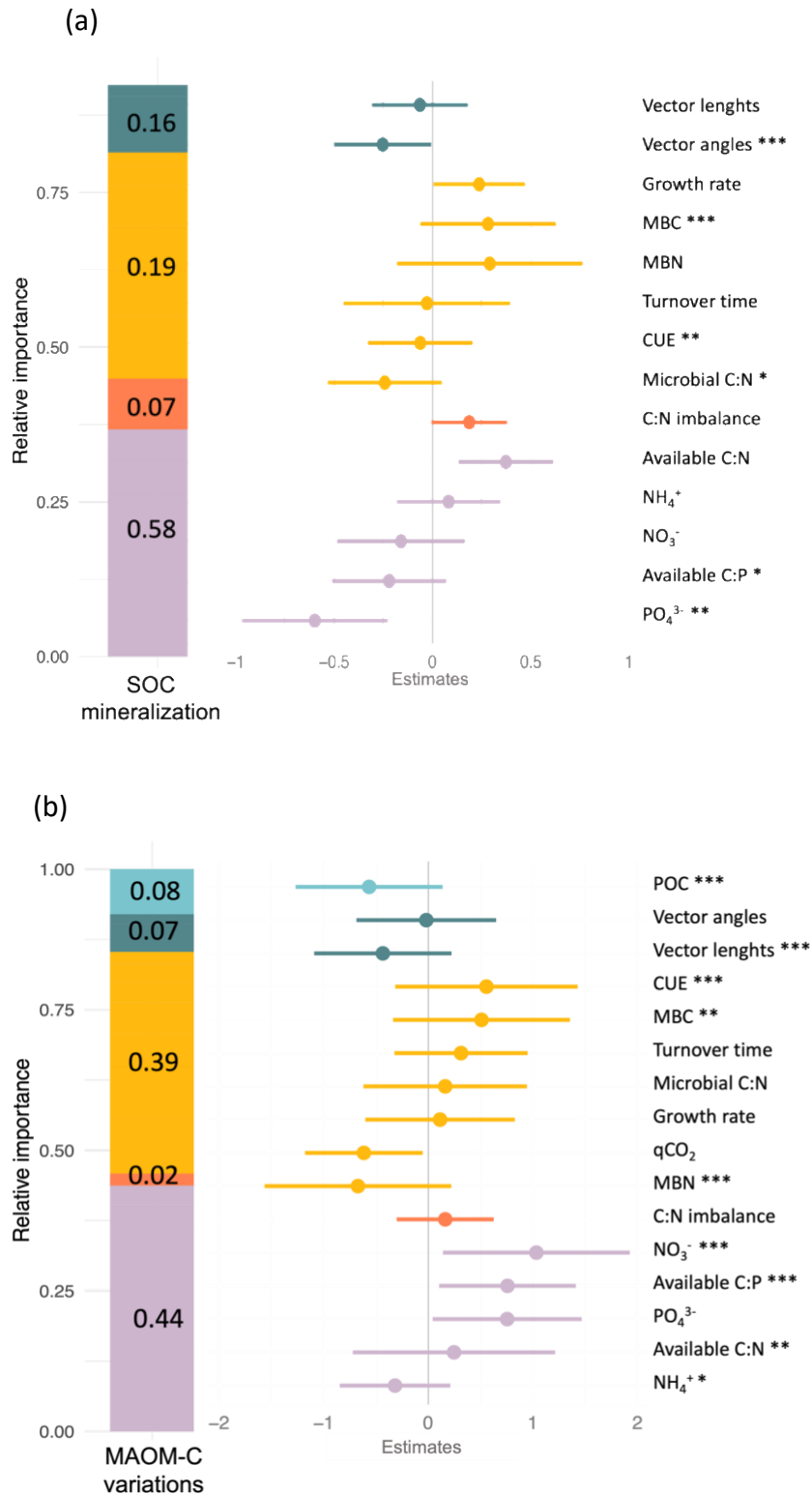


Figure 4.5 Effect of soil nutrients availability and stoichiometry (purple), microbial stoichiometric imbalance (orange); microbial processes and microbial community dynamics (yellow), microbial resource limitation (dark blue), and changes in POM-C content (g kg⁻¹ light blue) on (a) soil carbon mineralization expressed as cumulative CO₂ emissions (mg CO₂-C kg⁻¹ dry soil) and (b) changes in MAOM-C (g kg⁻¹) from T0 to T2.

Note: The p value of each predictor is given as: *: $p < .05$; **: $p < .01$; ***: $p < .001$.

4.4 Discussions

4.4.1 *Balanced nutrient addition to C-rich straw residues affect soil mineralization, variations in MAOM-C, and humification efficiency.*

When straw residues were added to soils, they underwent rapid mineralization shortly after incorporation, both with and without nutrient additions (Fig. 4.1 a-b). In the early stage of the incubation, the decomposable fraction of straw residues likely caused an intensive stimulation of the microbial activity (Fig. S4.1) as demonstrated in many incubation studies with crop residues addition (Cotrufo et al., 2019; Kirkby et al., 2016; Sokol et al., 2019; Zhao et al., 2024). These intense mineralization could have exacerbated the degradation of both the exogenous OM added as well as the native SOM (Fang et al., 2019; Kirkby et al., 2013; H. Wu et al., 2022). Mineralization of wheat straw was enhanced by nutrients addition in both soils, although without significant differences between straw-treatments (Fig. 4.1a-b). Soils that have received abundant annual mineral fertilization may already contain sufficient available nutrients to meet microbial demand when amended with crop residues alone (Fang et al., 2019). During the decomposition processes, nutrients would have been released via positive priming of native SOM (Fig. 4.2b), in addition to some direct supply of nutrients from the residues (Fang et al., 2019). In contrast, both mineral and organic fertilizers supplied the essential nutrients required for microbial growth, intensifying straw decomposition by microbial activity (Liang et al., 2022; H. Wu et al., 2022). Moreover, nutrient supplementation reduces nutrient limitation and inhibits microbes to mine more nutrients from the labile fractions in both native SOM and residues (Fig. 4.2b) (H. Wu et al., 2022). In our study, this was evident in both soils where MAOM-N was consumed more with residues addition alone. However, in Soil2, which is characterized by a more active microbial community (Tab. 4.1), MAOM-N was consumed in all treatments likely due to nutrient runoff during the incubation period. In Soil1 mineralization was significantly enhanced under incorporation of straw with mineral fertilizers compared to the other treatments (Fig 4.1a-c). Soils lacking organic carbon such as Soil1, often have an energy-starved microbial community with a high demand for easily accessible carbon sources and nutrients (Berthelin et al., 2022). When plant residues are introduced along with readily available mineral nutrients, they provide fresh energy sources that stimulate microbial growth and activity, leading to higher mineralization rates (Berthelin et al., 2022). In contrast, organic fertilizers require more microbial processing to release nutrients, resulting in lower immediate CO₂ emissions (J. Wu et al., 2022).

In this study, straw addition increased SOC in the stable form of MAOM-C by 2.4-fold (Fig 4.2a), yielding a greater amount of MAOM-C when incorporated with both mineral and organic fertilizers (4.2- fold higher compared to the addition of residues alone), without differences between soil types (Fig. 4.2a, Tab. S4.1). Different studies that consider various soil types, reported that the inputs of plant residues favor the formation of “new” stable C, measured as ff-SOC (C in the fine fraction of SOM), which was increased by 1.2- to 2.0-fold with nutrient supplementation, compared to adding residue alone (Fang et al., 2019; Kirkby et al., 2014, 2013; H. Wu et al., 2022). Moreover, by increasing the levels of nutrient inputs, the consumption of native SOC was exacerbated by the priming effect, and the production of “new” stable C increased due to the efficient humification of residue C based on a stable C:N:P:S stoichiometry (Fang et al., 2019; Kirkby et al., 2014, 2013; H. Wu et al., 2022). Generally, only about 10%, at most, of added plant residues remain in soils after mineralization by soil organisms (Berthelin et al., 2022). However, it was demonstrated that the conversion of residue carbon into soil carbon, can be increased by two- to eightfold, depending on the soil type, when inorganic nutrients are added (Fang et al., 2019; Kirkby et al., 2014, 2013; H. Wu et al., 2022). In our study, the HE was five- to sixfold higher, respectively when inorganic (8.88%) or organic (11.35%) nutrients were added compared with the incorporation of only straw (1.85%), which is consistent with other studies (Berthelin et al., 2022; Kirkby et al., 2014; H. Wu et al., 2022). Notably, in C-poorer soils like Soil1, the incorporation of organic amendments with C-rich straw residues significantly increase the HE (15.29%), favoring SOC stabilization. Labile carbon serves as an easily accessible energy source for soil microorganisms which boost microbial activity, accelerate OM decomposition, and generate large amounts of organic molecules and microbial residues that can contribute to the formation of a stable SOC fraction (Angers et al., 2022; Liang et al., 2017; Wu et al., 2023).

Despite the similar rate of MAOM-C variations between soil types, POM-C content decreased more in Soil1 compared to Soil2 (Table S4.1). This might suggest that the more fertile soil (Soil2) was a POM-dominated soil where the organic matter is typically less decomposed (Yu et al., 2022). In clay-richer soils, like Soil2, POM is usually protected from decomposition by being physically occluded within soil aggregates (Cotrufo et al., 2019). Moreover, POM-dominated soils tend to have a slower release of nutrients because the decomposition of POM occurs gradually over time (Hoffland et al., 2020; Villarino et al., 2023). This might be confirmed by the high availability of available nitrogen as NO_3^- in Soil2 both at the beginning and at the end of the experiment in the *Control* (Tab. 4.1, S4.5). In Soil2, the incorporation of straw combined with stoichiometrically

balanced organic fertilizers appears to promote POM degradation, as evidenced by a relatively smaller increase in POM-C at the end of incubation compared to the other treatments (Fig. 4.2c). The lack of labile organic carbon in mineral fertilizers means that the microbes primarily decompose the straw, resulting in a more straightforward incorporation of POM into SOM without the same level of accelerated decomposition. However, the nutrient runoff along the incubation period doesn't allow the identification of this phenomenon. This is highlighted only by a reduced immobilization of MAOM-N under nutrients addition compared to the other treatments. Organic fertilizers contain complex organic compounds that can serve as both a nutrient source and a carbon source for soil microorganisms leading to more intense microbial processing, potentially accelerating the decomposition of both the straw and existing POM in the soil. This may also suggest that the formation of MAOM-C in Soil1, having low MBC relative to SOC and high mineral surface abundance, primarily derives from the direct sorption of soluble litter components (i.e., leachates) to minerals. In contrast, in the more fertile and biologically active Soil2 (Table 4.1), MAOM-C derived mainly from stabilization of microbial residues (Cotrufo et al., 2022; Sokol et al., 2019; Zhu et al., 2024).

4.4.2 *Microbial metabolic processes underpinning SOC stabilization.*

According to the conceptual framework of the “microbial carbon pump”, microorganisms play an active role in soil C storage by degrading and assimilating SOM, which is subsequently stabilized in the soil (Liang et al., 2017; Poeplau et al., 2019). More specifically, microorganisms influence SOM formation by both releasing extracellular enzymes to degrade plant residues, resulting in the direct deposition of plant-derived C to soil minerals, and through the turnover of organic substrates via cell uptake–biosynthesis–growth–death, resulting in deposition of microbial-derived C (Sokol et al., 2022). In this study, both microbial metabolic pathways were significantly different between soil types (Tab S3, S4). Soil2 was more fertile and C-rich compared to Soil1, showing higher MBC relative to SOC (Tab. 4.1). This implies that Soil2 had more active microorganisms decomposing organic matter and that its soil environment was more favorable for microbial growth (Tab. S4.3, S4.6) (Kallenbach et al., 2015). This is indicated by an accelerated microbial growth and turnover, as well as higher MBC compared to Soil1 (Tab. S4.6). Moreover, microorganisms in Soil2 were more efficient at converting organic matter into MB rather than respiring it as CO₂, which was indicated by a significant lower qCO₂, and higher CUE compared to Soil1 (Wu et al., 2023). Together, these microbial metabolic processes are translated into a relatively higher amount of MAOM-C in Soil2.

However, this did not significantly enhance the MAOM-C accumulation after treatment application, as shown by no significant differences between Δ MAOM-C in Soil2 and Soil1 (Fig. 4.1, Tab. S4.1). In contrast, the addition of C from straw with stoichiometric balanced nutrients, both in form of mineral and organic fertilizers, resulted in a relatively higher increase of MAOM-C in both soils, compared to the addition of straw alone (Fig. 4.1).

After the application of treatments, the composition of extracellular enzymes quickly adjusted, adapting primarily to the availability of the newly introduced straw residues as a substrate (Fig. S4.1). Among the EA, oxidative EA measured as *perox* significantly increased in both soils. However, it significantly contributed to overall EA dissimilarities only with the addition of mineral fertilizers (Fig. 4.4, S4.1). The increased *perox* activity indicates intensified degradation of recalcitrant plant materials such as lignin and cellulose (Sinsabaugh, 2010), that lead to the release of labile substances readily usable by microbes (Tian and Shi, 2014). Environmental variables identified by PERMANOVA analysis (Fig. 4.4) showed that degradation of C by oxidative enzymes was regulated by P availability. In Soil2, which is P-limited (Tab. S4.5, S4.6), the addition of mineral P may have resulted in its adsorption onto soil mineral surfaces, thereby reducing P availability and increasing oxidative EA to mine for P (Sinsabaugh, 2010). Conversely, in Soil1, where P is more available, the abundance of C in the exogenous organic substrates likely stimulated microbial activity, including *perox*, while with no straw residues addition, *perox* EA was increased for nutrient mining. After *perox* EA, the hydrolytic enzymes *FDA* was the most produced enzymes in common to both soils and treatments. *FDA* is a broad indicator of total microbial activity in soil, as it reflects the activity of various hydrolytic enzymes, such as lipases, proteases, and esterase (Prosser et al., 2011). These enzymes are involved in the breakdown of organic compounds, releasing smaller molecules that can be further utilized by microbes (Schnürer & Rosswall, 1982). Both the EA of *perox* and *FDA* were previously identified to directly affecting the accumulation of MBC, which production was increased with litter nitrogen content (Gaspar et al., 2001; Sinsabaugh, 2010). In this study, although not statistically significant, the increased availability of C and nutrients following straw incorporation — particularly with organic nutrient supplementation — likely promoted microbial growth (Tab. S4.6). This enhanced microbial growth and activity, led to an accumulation of MBC in the early stage of the incubation, contributing to an increment of stable SOC (H. Wu et al., 2022).

The addition of C-rich straw residues together with nutrients, also affected the resource utilization characteristics of microbes and their extracellular enzyme stoichiometry. The rapid release of labile C from the degradation of straw residues with high C:N ratio, was reflected in an

increase of C:N imbalance between available resources and microbial biomass in the early stage of the incubation (Tab S5) (Zhao et al., 2024). This was exacerbated by a temporary N immobilization as soil microbes used available N to decompose the C-rich straw residues (Tab. S4.5). However, after three months C:N imbalance was significantly reduced, suggesting that labile C sources stimulated microbial C acquisition. Moreover, the incorporation of organic fertilizers lowered C:N imbalances compared with mineral fertilizers and straw alone by both favoring the increase of MBC (Tab. S4.5), and also by alleviating soil nitrogen deficiency (Tab. S4.5) (Kallenbach et al., 2015). With the addition of C-rich crop residues, microbial C competition (indicated by increased vector lengths, Tab. S4.6) intensified, particularly in Soil1, which was poorer in C compared to Soil2 (Tab. S4.4, S4.6). According to H. Wu et al. (2023), prolonged cultivation under conventional tillage with low C input and soil fertility decline, such as in Soil1, likely increased the microbial demand for exogenous C to form MBC, as demonstrated by increased vector length. In Soil1, this C competition was slightly alleviated at T₂ by nutrients addition, though no significant effects were observed in Soil2. This suggests that in the less fertile Soil1, where N is stored in MAOM, the microbial C competition was reduced because microorganisms did not need to mineralize SOM to obtain nutrients (Zhao et al., 2024). This is further supported by the correlation of MAOM-N and MAOM-C:N with mineral and organic fertilization, respectively (Fig. 4.3a). Additionally, in Soil1, the incorporation of residues alone or with mineral fertilizers exacerbated N limitation (reflected by reduced vector angles below 45°), whereas the addition of organic fertilizer helped maintain enzyme stoichiometry closer to equilibrium (Wu et al., 2023). This highlights the potential of organic fertilizers to support microbial growth and MBC accumulation by gradually releasing nutrients (Kallenbach et al., 2015). Conversely, in Soil2, both addition of straw alone and with nutrient supplementation reduced P limitation, bringing enzyme stoichiometry closer to equilibrium. This is also indicated by the SIMPER analysis, where *alkP* production significantly decreased (Fig. S4.1).

In conclusion, the combined input of straw and nutrients provided sufficient substrates to microbes, stimulating them to invest in more carbon and nutrient acquisition enzymes. This allowed them to maintain microbial biomass balance without depleting carbon and other nutrients stored in MAOM (Mooshammer et al., 2014).

4.4.3 The relationships of soil properties, enzymatic stoichiometry, and microbial metabolic processes with SOC mineralization and MAOM-C accumulation.

Microorganisms are central to two opposing processes in SOM dynamics: they mineralize SOC, while also contributing to its accumulation and stabilization in soil (Bhattacharyya et al., 2022; Sokol et al., 2022a). The combination of these two processes can be explained by the first-order kinetics: mineralization tends to be very intense after incorporation of OM into soil, then progressively slows down until one reaches a stage where little further mineralization occurs, and the OM that remains is stabilized in soil mainly by being incorporated into microbial biomass (Angers et al., 2022). It is widely recognized that SOC mineralization is a fundamental process for SOC stabilization but understanding what factors regulate the extent of the partitioning of C between microbial biosynthesis and mineralization is fundamental for promoting SOC sequestration to reduce the impact on climate change.

In our study, availability of soil nutrients explained most of the variation of both SOC mineralization and changes in MAOM-C (Fig. 4.5). SOC mineralization was predominantly regulated by the availability of P, where the reduced availability of P in soil leads to higher mineralization of SOC by driving microbial communities to decompose more organic matter for nutrient mining (Bertrand et al., 2019; Coonan et al., 2020a). This process lowers microbial CUE (Fig. 4.5a) since microorganisms must invest more energy to acquire nutrients (Kallenbach et al., 2019; Manzoni et al., 2010). This means that less C is allocated to growth and incorporated into MB, and more C is respired as CO₂ (Spohn et al., 2016). Conversely, both availability of N and P is significantly and positively correlated with changes in MAOM-C. When nutrients are more available, microorganisms can grow and reproduce more efficiently, thus increasing microbial CUE (Kallenbach et al., 2019; Sinsabaugh et al., 2013). Consequently, C is retained in the soil, either as part of the microbial biomass or as stabilized organic matter (Poeplau et al., 2019; Prommer et al., 2020). This means that even if in our study changes in CUE are not significant, CUE is a key factor that drive partitioning of C between microbial biosynthesis and mineralization (Angers et al., 2022). Moreover, the positive relation between the stoichiometric ratio of available C:P or C:N with changes in MAOM-C indicated that the acquisition of labile C by microbes is limited by the availability of nutrients, thereby affecting the generation of new stable C (Li et al., 2018).

In a study of H. Wu et al. (2022), it was pointed out that increased nutrient supplementation at residue incorporation promoted microbial growth and MBC accumulation, as well as microbial activity, increasing both SOC mineralization and the formation of new stable C. This is confirmed

also in our study where MBC is positively correlated to both SOC mineralization and formation of MAOM-C. Moreover, with increased nutrient supplementation the ratio of SOC that was mineralized to newly formed soil C significantly decreased, suggesting that decomposers preferred to use supplemental nutrients and/or decompose exogenous OM for nutrients rather than using native SOM (H. Wu et al. 2022). Thus, a greater proportion of residue C, along with N, P, and S was utilized by microbes and transferred into stable SOM as microbial detritus, metabolites, and secretions (Kästner et al., 2021; Prommer et al., 2020; Sokol et al., 2022). In soils with differing fertility levels, such as Soil1 (moderately fertile) and Soil2 (nutrient-rich), this process might explain the small increase of MAOM-C under straw addition alone, which was enhanced with the incorporation of both mineral and organic fertilizers. These findings highlight the potential of balanced nutrient supplementation as an effective regenerative agricultural practice that not only promotes the short-term sequestration of C into stable SOM, but also enhances soil fertility by limiting nutrient mining. The microbial C:N ratio was also identified as significant predictors of SOC mineralization. In conditions where microbes have adequate N for their metabolic needs, the MB has a relatively higher N content compared to C (Tab. 4.1) (Delgado-Baquerizo et al., 2017; Mooshammer et al., 2014). However, when C-rich crop residues are introduced into the soil, microbes encounter an excess of C relative to the N they need for growth. Hence, to balance their internal C ratio, microbes will assimilate the necessary N and respire the C in excess as CO₂ (Mooshammer et al., 2014). This process leads to increased mineralization of SOC, where the C is broken down and released as CO₂ rather than being incorporated into microbial biomass (Fang et al., 2020; Manzoni et al., 2010). However, when supplemental nutrients are incorporated with crop residues, microorganisms can take up more N, leading to an increase of MBN (Mooshammer et al., 2014). Increased N availability supports also rapid microbial growth and reproduction, allowing microbes to more efficiently assimilate the newly introduced C into their biomass rather than respire it as CO₂, thereby increasing SOC accumulation (Mooshammer et al., 2014). Moreover, N-rich microbial residues are more likely to bind with soil minerals or be protected within soil aggregates, leading to enhanced stabilization of C in the soil (Hu et al., 2023).

The addition of C and nutrients affected the resource utilization characteristics of microbes as well as their extracellular enzyme stoichiometry, significantly affecting SOC mineralization and accumulation. Specifically, low vector angles significantly induced an increment of SOC mineralization. Low vector angles suggest a greater emphasis on N-acquiring enzyme activity relative to C-acquiring enzymes, indicating N limitation in the microbial community (Agumas et al.,

2021). When N is limiting, microbes prioritize the acquisition of N to support their metabolic needs, leading to higher SOC mineralization (Abay et al., 2024). In our study this phenomenon is particularly evident in Soil1, which is characterized by low dissolved N content, mainly in the form of NO_3^- . In this condition, the addition of straw alone or with mineral fertilizers induced nutrient mining, hence increasing soil respiration (Fig. 4.1a-c) and limiting the stabilization of residue C into soil C. Moreover, nutrients addition alleviated the microbial C competition (decreased vector lengths) caused by the addition of C-rich straw residues, which means that with increased availability of nutrients, the ability of microbes to metabolize C increases (Fang et al., 2019). Therefore, the higher amounts of microbial residues can become stabilized in the soil through interactions with minerals and within soil aggregates (increased MAOM-C).

Finally, changes in soil POM are linked to changes in MAOM through processes of decomposition, microbial processing, and stabilization (Cotrufo et al., 2019). The decomposition of POM leads to the release of simpler organic compounds (e.g., amino acids, carbohydrates, organic acids) that can both bind to soil minerals and be further processed by soil microorganisms (Sokol et al., 2019). This process stabilizes the organic matter and reduces its susceptibility to further microbial breakdown. In Soil2, POM was more protected from decomposition likely by being physically occluded within soil aggregates (Fang et al., 2019).

4.5 Conclusion

In conclusion, the study underscores the pivotal role that balanced nutrient addition plays in influencing SOM mineralization, microbial activity, and SOC stabilization. The incorporation of straw residues into the soil, especially when combined with nutrient supplementation, led to rapid SOM mineralization, and enhanced microbial activity. Both mineral and organic fertilizers stimulated microbial decomposition of the added residues, while nutrient additions mitigated the microbial mining of native SOM, fostering the stabilization of C in MAOM-C. This is critical because MAOM-C not only represents a long-term C storage mechanism, but also serves as a key source of soil fertility, providing nutrients over time that sustain crop productivity. Soils with contrasting properties displayed different responses to straw incorporation. In the C-poor soil (Soil1), microbial communities greatly benefited from the combination of straw and nutrient additions, leading to a substantial increase in HE and the stabilization of organic carbon. In contrast, in the more fertile soil (Soil2), microbial activity was already efficient, with higher MBC and CUE, meaning that the immediate impact of straw residues on organic carbon stabilization was less pronounced. Microbial

metabolic processes emerged as central to SOC stabilization, regulating the balance between C mineralization and sequestration. Microbes contributed both through the decomposition of plant residues and the turnover of microbial biomass to SOC stabilization. This study emphasizes that nutrient availability, especially P and N, is crucial in determining whether microbial communities prioritize mineralization or C sequestration. Consequently, proper nutrient management is essential for optimizing C inputs into soils to maximize long-term C storage and improve soil fertility. The application of organic fertilizers, in particular, showed notable advantages in supporting microbial growth, nutrient cycling, and C stabilization compared to mineral fertilizers. Organic fertilizers offered a more balanced, slow-release nutrient supply that nurtured microbial activity without triggering excessive SOM mineralization, enhancing the build-up of MAOM-C and contributing to soil health.

Incorporating crop residues with stoichiometrically balanced nutrients is a key strategy for achieving the goals of carbon farming programs, which aim to enhance SOC sequestration and improve soil health. Although this targeted fertilization strategy may incur higher emission costs (with a slight increase in fertilizer use and overall emissions), it leads to greater carbon sequestration per unit of nitrogen input into the system in the mid-term. When integrated with other regenerative agricultural practices—such as reduced tillage, cover cropping, and diverse crop rotations—this approach can significantly improve carbon use efficiency (CUE) (Kallenbach et al., 2019), promoting greater SOC storage in agricultural systems. These practices also enhance soil structure, increase microbial biodiversity, reduce erosion, and improve water retention, all of which contribute to the long-term stabilization of SOC and support sustainable agriculture.

AUTHOR CONTRIBUTIONS

Marta Bertola, Cornelia Rottensteiner: Laboratory analysis. **Marta Bertola, Andrea Ferrarini:** incubation data analysis. **Marta Bertola, Andrea Ferrarini:** manuscript editing. **Marta Bertola, Andrea Ferrarini, Andreas Richter:** study design. **Andrea Ferrarini, Stefano Amaducci:** supervision and funding acquisition.

FUNDING INFORMATION

This study is part of the project Farms4Climate, co-funded by the Partnership for Research and Innovation in the Mediterranean Area (PRIMA) and the European Union.

Supplementary Material

Table S4.1 Analysis of variance of the effects of soil type (S) and treatments (Trt) on SOM pools variations (difference between beginning and end of incubation (T0 and T2)), cumulative CO2 emission at T2, CO2 emission rate at T1, and humification efficiency. Note: *, **, *** denote statistically significant differences for $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.

Soil parameter	Soil type		Treatment		S x Trt	
	F	P	F	P	F	P
MAOM-C	1.71	ns	12.42	***	1.24	ns
MAOM-N	11.12	**	1.60	ns	2.15	ns
POM-C	18.64	***	14.24	***	1.55	ns
POM-N	17.24	***	2.17	ns	0.93	ns
DOC	0.02	ns	3.28	*	0.30	ns
DON	5.45	*	63.68	***	20.11	***
Cum. CO2 emission	0.00	ns	194.14	***	6.03	*
CO2 emission rate at peak	0.42	ns	84.09	***	0.93	ns
HE	1.89	ns	5.39	*	1.46	ns

Table S4.2 Analysis of variance of the effects of soil type (S), treatments (Trt) and time on available nutrients. Note: *, **, *** denote statistically significant differences for $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively).

Soil parameter	Soil type		Treatment		Time		S x Trt		S x Time		Trt x Time		S x Trt x Time	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
PO ₄	4.02	ns	7.85	***	6.22	*	1.49	ns	0.11	ns	21.13	***	0.90	ns
NO ₃	17.75	***	27.81	***	48.04	***	13.45	***	1.79	ns	2.38	ns	0.39	ns
NH ₄	11.17	*	0.72	ns	15.16	***	3.42	*	1.45	ns	6.07	**	5.58	**
DIN	20.84	***	44.22	***	54.08	***	29.79	***	3.48	ns	5.27	**	4.27	**
DOC	0.02	ns	0.24	ns	0.13	ns	2.95	*	1.10	ns	3.68	*	2.57	*
DON	18.89	***	82.51	***	121.76	***	23.56	***	7.47	**	49.85	***	17.78	***
Available_C:N	0.25	ns	15.31	***	85.90	***	2.35	ns	1.30	ns	6.89	***	1.27	ns
Available_C:P	1.61	ns	4.16	*	6.55	*	1.28	ns	0.10	ns	9.72	***	2.21	ns
C:N_imbalance	0.05	ns	13.96	***	48.64	***	0.71	ns	2.28	ns	5.20	**	0.59	ns

Table S4.3 Analysis of variance of the effects of soil type (S), treatments (Trt) and time on microbial variables at the beginning and end of incubation.

Note: *, **, *** denote statistically significant differences for $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively

Soil parameter	Soil type		Treatment		Time		S x Trt		S x Time		Trt x Time		S x Trt x Time	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
MBC	190.48	***	4.41	**	70.05	***	0.15	ns	6.81	*	0.57	ns	0.33	ns
MBN	253.61	***	24.43	***	35.09	***	1.17	ns	23.40	***	1.51	ns	3.11	*
MBC:MBN	1.22	ns	12.90	***	19.5	***	2.44	ns	20.08	***	3.12	*	8.63	***
CUE	10.88	**	1.70	ns	1.23	ns	0.49	ns	0.00	ns	0.30	ns	0.07	ns
Growth rate	38.73	***	2.16	ns	0.3	ns	0.69	ns	0.79	ns	0.08	ns	0.36	ns
Turnover time	16.19	***	0.86	ns	32.12	***	1.72	ns	0.18	ns	1.57	ns	1.78	ns
qCO ₂	35.77	***	2.20	ns	15.72	**	1.88	ns	17.95	***	3.75	*	2.46	ns

Table S4.4a Analysis of variance: effects of soil type (S), treatments (Trt) and time on measured enzymes activity.

Note: *, **, *** denote statistically significant differences for $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.

Soil parameter	Soil type		Treatment		Time		S x Trt		S x Time		Trt x Time		S x Trt x Time	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
dsDNA	259.0	***	11.4	***	20.1	***	2.0	ns	20.9	***	2.5	ns	0.7	ns
aryS	309.2	***	0.7	ns	9.9	**	7.9	***	54.7	***	1.1	ns	0.8	ns
alfaG	3.4	ns	76.6	***	7.1	*	0.8	ns	3.8	ns	0.7	ns	2.2	ns
betaG	14.2	***	25.1	***	49.9	***	3.7	*	5.9	*	1.08	ns	1.7	ns
alfaGAL	82.3	***	4.5	**	1.5	ns	3.7	*	0.1	ns	1.1	ns	3.1	ns
betaGAL	29.6	***	14.8	***	0.3	ns	3.9	*	0.0	ns	2.0	ns	3.6	*
cell	4.0	*	29.7	***	41.9	***	2.2	ns	0.19	ns	7.0	***	5.0	**
xilo	7.9	**	20.2	***	0.4	ns	3.6	*	9.6	**	1.3	ns	6.0	**
uroni	16.5	***	13.4	***	12.1	**	1.9	ns	4.6	*	1.2	ns	0.4	ns
chit	57.1	***	13.4	***	0.0	ns	4.6	**	0.0	ns	1.3	ns	3.5	*
aprot	203.5	***	0.3	ns	4.5	*	1.8	ns	0.0	ns	0.2	ns	1.6	ns
leu	0.1	ns	20.7	***	21.2	***	1.1	ns	9.6	**	1.0	ns	0.9	ns
arg	67.3	***	20.1	***	0.7	ns	1.6	ns	47.0	***	4.3	**	2.2	ns
acP	207.4	***	4.7	**	18.1	***	2.1	ns	3.7	ns	0.4	ns	1.9	ns
bisP	137.8	***	22.3	***	25.4	***	2.0	ns	15.5	***	0.2	ns	0.5	ns
piroP	262.8	***	5.7	**	17.7	***	0.0	ns	48.2	***	0.9	ns	0.1	ns
alkP	315.0	***	2.6	ns	27.5	***	8.0	***	37.0	***	0.1	ns	0.4	ns
FDA	52.2	***	3.3	*	0.4	ns	3.6	*	1.0	ns	0.3	ns	3.4	*
butir	0.0	ns	13.4	***	27.1	***	0.7	ns	16.5	***	1.1	ns	0.4	ns
ox	171.6	***	4.4	**	3.8	ns	1.2	ns	24.7	***	0.1	ns	2.6	ns
perox	58.6	***	19.4	***	37.9	***	1.1	ns	1.3	ns	11.8	***	3.9	*
Vector length	19.8	***	7.5	***	0.1	ns	0.5	ns	6.1	*	2.7	ns	4.9	**
Vector angles	90.6	***	17.4	***	0.05	ns	1.8	ns	0.5	ns	3.0	*	3.3	*

Table S4.4b Results of the best dbRDA models for the two soil types (Fig. 4.3 and 4.4) resulting from multivariate backward multiple regression on C-, N-, P-, and S-cycling enzymes (2g 4-MUF,7-AMC g-1 dry soil h-1). dbRDA best models' selection was performed differentiating hydrolytic EA from oxidative EA. The starting database of explanatory soil variable was: MAOM-C, MAOM-N, MAOM C:N, POM-C, POM-N, DOC, DON, PO43-, NO3-, NH4+, available C:N, available C:P, MBC, CUE, growth rate and turnover time. (*, **, *** denote statistically significant differences for $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively).

Soil 1 hydrolytic EA			Soil 2 hydrolytic EA			Soil 1 peroxidase EA			Soil 2 peroxidase EA		
Model	F	P	Model	F	P	Model	F	P	Model	F	P
Model 11.1	0.001	***	Model 5.5	0.001	***	Model 26.6	0.001	***	Model 15.9	0.001	***
Residuals	0.03		Residuals	0.04		Residuals	0.1		Residuals	0.08	
RDA Axis			RDA Axis			RDA Axis			RDA Axis		
Axis 1	53.7	0.001 ***	Axis 1	19.2	0.001 ***	Axis 1	126.7	0.001 ***	Axis 1	72.6	0.001 ***
Axis 2	18.6	0.221 ***	Axis 2	9.0	0.001 ***	Axis 2	4.9	0.134	Axis 2	5.7	0.038 *
Axis 3	2.3	0.412	Axis 3	3.2	0.232	Axis 3	0.9	0.918	Axis 3	0.8	0.909
Residuals	0.41		Residuals	0.05		Residuals	0.1		Residuals	0.08	
Variable			Variable			Variable			Variable		
MAOM-C:N	12.1	0.001 ***	PO4 ³⁻	5.9	0.007 **	PO4 ³⁻	30.3	0.003 **	Available C:P	6.1	0.018 *
MAOM-N	7.9	0.001 ***	NH4 ⁺	6.8	0.006 **	Residuals	0.1		Residuals	0.08	
PO4 ³⁻	6.2	0.003 **	Residuals	0.05							
Residuals	0.03										

Table S4.5 Available nutrients in Soil1 and Soil2 following straw and nutrient additions at peak (T1) and end (T2) of incubation. Note: Within each column, different letters indicate significant differences among treatments and time ($p < 0.05$, Tukey's test).

Field	Treatment	Time	DOC	DTN	PO4 ³⁻	NO3 ⁻	NH4 ⁺	DIN	Available C:N	Available C:P	C:N imbalance
			µg g ⁻¹	µg g ⁻¹							
Soil1	Control	1	104.7 ^a	11.9 ^{ab}	4.8 ^{bc}	4.1 ^a	6.5 ^{ab}	10.6 ^{abc}	10.8 ^{ab}	23.9 ^{abc}	0.5 ^{ab}
Soil1	Straw+org	1	111.4 ^a	5.8 ^a	4.2 ^{ab}	6.2 ^{ab}	1.1 ^a	7.3 ^{abc}	33.0 ^{bc}	24.0 ^{abc}	3.4 ^{cd}
Soil1	Straw+min	1	46.2 ^a	6.1 ^a	4.2 ^{ab}	0.0 ^a	1.3 ^a	1.3 ^a	36.0 ^c	11.4 ^a	5.4 ^d
Soil1	Straw	1	63.7 ^a	5.6 ^a	4.7 ^{bc}	3.8 ^a	1.6 ^a	5.4 ^{ab}	30.3 ^{bc}	13.1 ^{ab}	4.4 ^{cd}
Soil1	Control	2	72.0 ^a	48.0 ^c	7.6 ^c	38.2 ^c	1.6 ^a	39.8 ^d	1.9 ^a	9.5 ^a	0.3 ^a
Soil1	Straw+org	2	54.1 ^a	12.2 ^{ab}	2.8 ^{ab}	29.2 ^{bc}	2.5 ^a	31.7 ^{cd}	6.5 ^a	28.6 ^{abc}	0.2 ^a
Soil1	Straw+min	2	90.3 ^a	22.0 ^{bc}	2.4 ^{ab}	14.9 ^{abc}	13.6 ^b	28.5 ^{bc}	6.8 ^a	55.0 ^{bc}	1.0 ^{abc}
Soil1	Straw	2	61.5 ^a	17.0 ^{ab}	1.3 ^a	1.7 ^a	12.6 ^b	14.3 ^a	9.5 ^a	70.6 ^c	0.5 ^{ab}
Soil2	Control	1	46.2 ^a	18.9 ^a	3.8 ^{ab}	51.9 ^b	1.2 ^a	53.1 ^c	0.9 ^a	12.7 ^a	0.1 ^{ab}
Soil2	Straw+org	1	79.4 ^a	10.0 ^a	4.3 ^{abc}	0.0 ^a	1.3 ^a	1.3 ^a	63.6 ^b	18.4 ^a	6.6 ^c
Soil2	Straw+min	1	78.3 ^a	6.7 ^a	5.9 ^{bc}	0.0 ^a	1.1 ^a	1.1 ^a	73.6 ^b	13.7 ^a	8.7 ^c
Soil2	Straw	1	96.7 ^a	7.3 ^a	6.4 ^{bc}	2.5 ^a	1.1 ^a	3.6 ^a	44.3 ^b	15.3 ^a	5.5 ^c
Soil2	Control	2	70.2 ^a	118.4 ^b	7.1 ^c	120.0 ^c	1.6 ^a	121.6 ^d	0.6 ^a	10.0 ^a	0.0 ^a
Soil2	Straw+org	2	73.6 ^a	21.5 ^a	4.2 ^{abc}	22.7 ^a	7.0 ^a	29.6 ^{bc}	2.6 ^a	19.7 ^a	0.4 ^{ab}
Soil2	Straw+min	2	99.1 ^a	8.1 ^a	2.3 ^a	12.7 ^a	2.4 ^a	15.1 ^{ab}	7.2 ^a	88.5 ^b	0.8 ^b
Soil2	Straw	2	69.1 ^a	10.9 ^a	2.7 ^a	11.9 ^a	1.8 ^a	13.7 ^{ab}	7.1 ^a	27.1 ^a	0.9 ^b

Table S4.6 Summary of the indicators of microbial biomass (e.g., MBC, MBN, MBC:MBN), activity (e.g., growth rate, CUE, turnover time and qCO₂) and nutrients limitation (vectors angles and vector length) in response to straw and nutrient additions.

Note: Within each column, different letters indicate significant differences among treatments and time ($p < 0.05$, Tukey's test).

Field	Treatment	Time	MBC	MBN	MBC:MBN	Vector lengths	Vector angles	CUE	Growth rate	Turnover time	qCO ₂
			µg g ⁻¹	µg g ⁻¹					ng C g _{dry soil} ⁻¹ h ⁻¹	days	mgCO ₂ -C/gCmic/h
Soil1	Control	1	195.3 ^{ab}	9.4 ^a	22.2 ^c	0.61 ^{ab}	40.5 ^a	0.45 ^a	537.4 ^a	19.6 ^b	1.9 ^a
Soil1	Straw+org	1	256.0 ^b	28.9 ^b	9.0 ^{ab}	0.66 ^b	37.6 ^a	0.53 ^a	645.5 ^a	16.5 ^{ab}	2.9 ^{ab}
Soil1	Straw+min	1	252.9 ^b	27.0 ^b	9.5 ^{ab}	0.69 ^b	38.4 ^a	0.54 ^a	563.2 ^a	16.5 ^{ab}	1.9 ^a
Soil1	Straw	1	224.4 ^{ab}	19.1 ^{ab}	11.9 ^b	0.60 ^{ab}	40.7 ^{ab}	0.64 ^a	568.0 ^a	16.4 ^{ab}	1.8 ^a
Soil1	Control	2	61.0 ^a	7.9 ^a	7.6 ^{ab}	0.54 ^a	51.5 ^b	0.45 ^a	423.5 ^a	8.0 ^a	9.5 ^c
Soil1	Straw+org	2	207.8 ^{ab}	23.2 ^{ab}	9.5 ^{ab}	0.62 ^{ab}	42.6 ^{ab}	0.50 ^a	721.0 ^a	14.2 ^{ab}	3.9 ^{ab}
Soil1	Straw+min	2	101.8 ^{ab}	22.7 ^{ab}	4.5 ^a	0.63 ^{ab}	35.5 ^a	0.51 ^a	601.1 ^a	9.6 ^{ab}	6.7 ^{cb}
Soil1	Straw	2	133.5 ^{ab}	19.6 ^{ab}	6.9 ^{ab}	0.69 ^b	33.3 ^a	0.54 ^a	694.6 ^a	8.6 ^a	4.9 ^{ab}
Soil2	Control	1	485.4 ^{bcd}	45.5 ^{bcd}	10.9 ^a	0.52 ^a	61.2 ^b	0.64 ^a	931.0 ^a	28.5 ^b	1.1 ^a
Soil2	Straw+org	1	553.8 ^d	57.7 ^{de}	9.7 ^a	0.58 ^a	47.2 ^a	0.61 ^a	1539.3 ^a	22.5 ^{ab}	1.7 ^a
Soil2	Straw+min	1	534.2 ^d	61.2 ^{de}	8.7 ^a	0.59 ^a	51.5 ^{ab}	0.61 ^a	1394.8 ^a	16.4 ^a	1.8 ^a
Soil2	Straw	1	513.7 ^{cd}	63.3 ^e	8.1 ^a	0.59 ^a	47.0 ^a	0.71 ^a	1103.0 ^a	20.2 ^{ab}	1.1 ^a
Soil2	Control	2	272.3 ^a	21.9 ^a	12.7 ^a	0.58 ^a	60.9 ^b	0.59 ^a	970.9 ^a	14.8 ^a	2.4 ^a
Soil2	Straw+org	2	358.2 ^{abc}	53.4 ^{cde}	7.2 ^a	0.60 ^a	47.1 ^a	0.62 ^a	1237.0 ^a	12.6 ^a	2.1 ^a
Soil2	Straw+min	2	333.5 ^{ab}	35.8 ^{ab}	9.3 ^a	0.61 ^a	49.3 ^a	0.58 ^a	1213.9 ^a	16.1 ^a	2.3 ^a
Soil2	Straw	2	330.0 ^{ab}	38.7 ^{bc}	8.5 ^a	0.60 ^a	47.9 ^a	0.62 ^a	1006.7 ^a	14.4 ^a	2.0 ^a

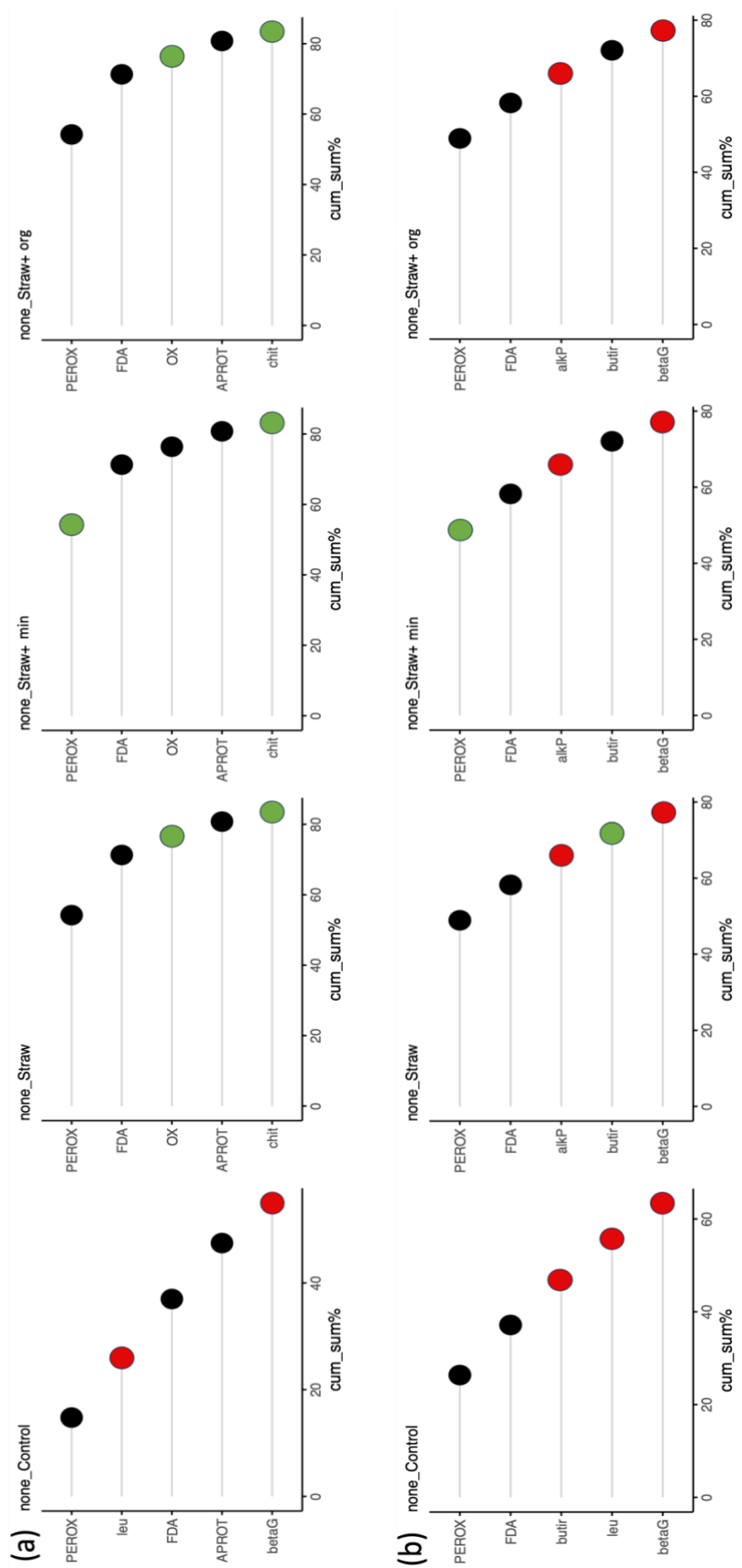


Figure S4.1. SIMPER analysis showing enzyme activities that contributed most (>80%) to the Bray-Curtis dissimilarity at the end of the incubation following straw and nutrients addition. For each soil types (Soil1(a) and Soil2(b)), enzymes activities before treatment application (none) are compared with Straw, Straw+min and Straw+org treatments. Note: Different colors indicate a significant positive (green) or negative (red) contribution to the Bray-Curtis dissimilarity.

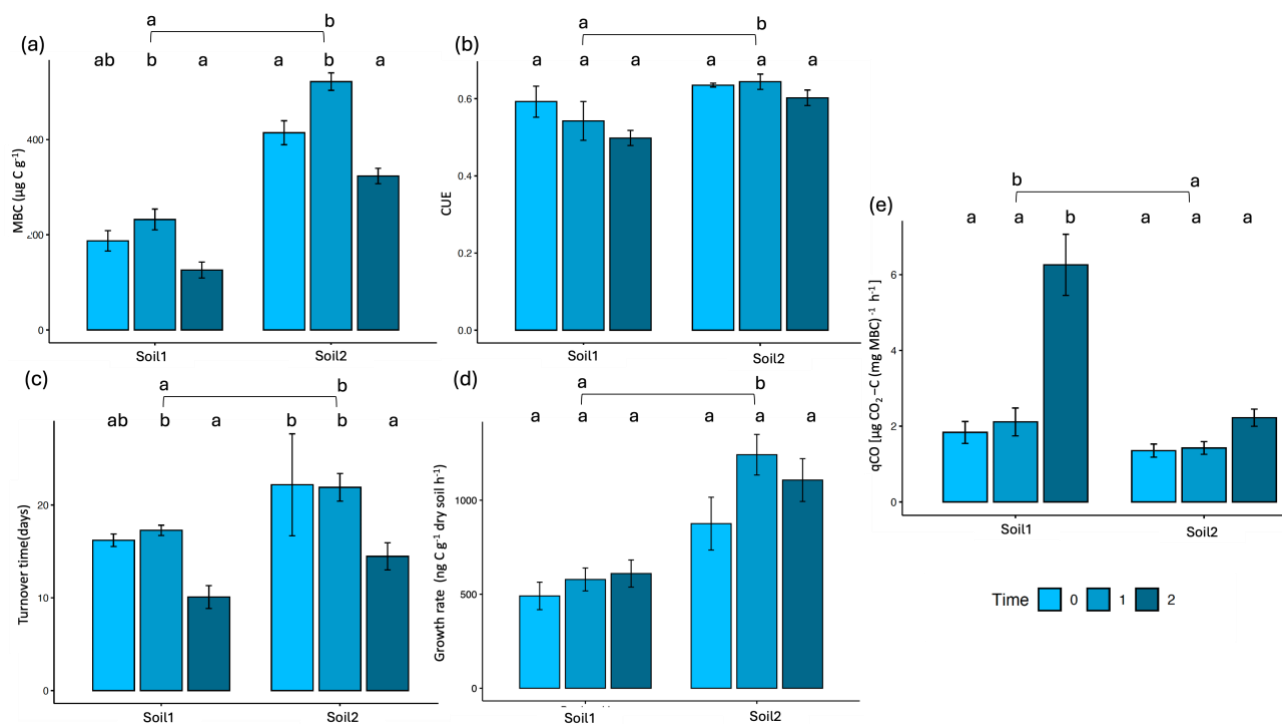


Figure S4.2 Comparison of soil types and incubation times for key indicators of microbial biomass (e.g., MBC) (a) and microbial activity (e.g., CUE, turnover time, growth rate, and $q\text{CO}_2$; b, c, d, and e, respectively). Different letters indicate significant differences among incubation times and soil types ($p < 0.05$, Tukey's test).

Chapter 5

Integrating proximal soil sensing into SOC stock accounting for carbon farming applications: a case study in Northern Italy

Irena Ymeti¹ | Marta Bertola^{1,4} | Lorenzo Cremonesi^{1,2} | Michele Croci^{1,3} | Stefano Amaducci^{1,3,4}
| Andrea Ferrarini¹

¹ *Department of Sustainable Crop Production – Università Cattolica del Sacro Cuore, Piacenza, Italy*

² *Consorzio Agrario Terrepadane, Piacenza, Italy*

³ *Remote Sensing and Spatial Analysis Research Center (CRAST), Università Cattolica del Sacro Cuore, 29122 Piacenza, Italy*

⁴ *Centro per l'Innovazione nell'Impiego del Telerilevamento nell'Industria Meccanica per l'Agricoltura di Precisione (CITIMAP), via Castellarino, 12, San Bonico, PC 29122, Italy*

5.1 Introduction

Monitoring the spatial and temporal variability of soil carbon is crucial for advancing sustainable agriculture and implementing effective carbon (C) sequestration strategies (Amelung et al., 2020; Smith et al., 2014). The success of carbon farming (CF) practices in enhancing soil organic carbon (SOC) stocks depends on the development of accurate, scalable, and cost-effective methods to track SOC changes over time (Smith et al., 2020). Monitoring, Reporting, and Verification (MRV) protocols are critical tools for tracking SOC stock dynamics, supporting farmers, policymakers, and project developers engaged in offsetting or insetting programs (FAO, 2020; VERRA VM0042, 2024). These protocols ensure the credibility of carbon credits by verifying that sequestered C is real, additional, and permanent. However, uncertainties in C removal estimates undermine the voluntary C market's ability to incentivize CF practices, discouraging both farmer participation and credit buyers (Croft et al., 2021; Dorning et al., 2021; Mattila et al., 2022). Process-based models are widely used in MRV protocols to quantify net C removal as SOC sequestration resulting from adoption of additional CF practices. While these models are invaluable for understanding SOC dynamics, their reliance on input data such as soil properties and crop biomass introduces uncertainties (Dechow et al., 2019; Gurung et al., 2020). Addressing these uncertainties requires the development and validation of field-scale SOC stock accounting approaches providing accurate and cost-efficient input data for soil models (Kyker-Snowman et al., 2022; Potash et al., 2023). Direct field measurements of soil variables, such as SOC concentration and bulk density (BD), offer high accuracy but are labor-intensive and expensive, limiting their scalability. Recent advancements in soil sensing technologies, including spectral methods like vis-NIR and MIR spectroscopy, offer rapid and cost-effective alternatives when properly calibrated, balancing cost and accuracy (England and Viscarra-Rossel, 2018; Li et al., 2022; Ma et al., 2023). Combining field measurements with spectral techniques or process-based modeling can further enhance MRV systems by integrating accuracy with scalability (Mäkipää et al., 2008; Smith, 2004). These hybrid approaches align with the quantification approaches of the EU's Carbon Removals and Carbon Farming (CRCF) regulation, which supports its use as a standard for CF programs (European Commission, 2021).

However, many MRV systems rely on the CR estimates at field-scale SOC stock changes averages, (e.g. Verra, 2024), which fail to capture soil spatial variability and its impact on CR estimate potential. To address this, protocols such as FAO's GSOC-MRV refine initial SOC stock accounting by proposing a stratification sampling approach using homogeneous zones, enabling more accurate, but discrete evaluations of initial SOC stock (FAO, 2020). Proximal soil sensing (PSS) technologies improve spatial

resolution of soil properties estimation, reducing the need for extensive sampling while maintaining cost-efficiency. These tools, including spectroscopy, dielectric sensors, or gamma-ray sensing, offer environmentally friendly and reproducible methods for soil property assessment, making them valuable for CF programs (Silvero et al., 2023). Studies have shown that PSS can achieve prediction accuracy comparable to conventional laboratory methods (Kuang et al., 2015; Morellos et al., 2016); for instance, vis-NIR spectroscopy has demonstrated strong predictive performance for SOC content and BD (Gholizadeh et al., 2021; Lobsey and Viscarra Rossel, 2016). Combining PSS data types, such as spectroscopy and gamma-ray sensing, further enhances the accuracy of predictions, making these technologies valuable for CF applications (Andrade et al., 2022; Swetha and Chakraborty, 2021). Additionally, PSS plays a vital role in precision agriculture by generating high-resolution soil data for producing fertilization prescription maps and thus optimizing fertilization of mineral and organic inputs with variable-rate technologies (Bönecke et al., 2021; Clarke et al., 2024).

Integrating proximal soil sensing (PSS) with remote sensing (RS) technologies can further enhance the accuracy and scalability of SOC stock accounting. PSS provides high-resolution, site-specific data, while RS offers broader spatial coverage, enabling the upscaling of predictions across larger areas (Stevens et al., 2010).

Vegetation indices like NDVI and EVI, along with spectral bands of bare soils from satellite imagery such as Sentinel-2, improve SOC predictions by capturing local variability and regional trends (Biney et al., 2022; Dvorakova et al., 2023; Vaudour et al., 2022; Wadoux and McBratney, 2021; Wiesmeier et al., 2019). Sentinel-2 synthetic bare soil imagery (SBSI) has shown promise in predicting SOC and clay content, with spectral resolution effective for field-scale studies and spatial resolution critical for regional or continental applications (Castaldi, 2021; Castaldi et al., 2019; Vaudour et al., 2019). Nonetheless, challenges such as soil moisture, surface roughness, and vegetation cover can limit the accuracy of RS-based SOC estimations (Abdulraheem et al., 2023). While RS methods face these challenges, combining RS with PSS addresses these limitations and improves the estimation of key soil properties such as SOC content, soil texture, and soil moisture (Wiesmeier et al., 2019). These properties are vital for SOC stock calculations and for initializing models like Roth-C to simulate SOC sequestration under CF practices.

To further refine SOC stock predictions and optimize the cost-accuracy tradeoff, integrating PSS, RS, and auxiliary data such as SOC, soil texture, soil type, topography, and land use, is crucial (Viscarra Rossel and Brus, 2018). This approach has been shown to improve SOC stock mapping accuracy by up to 10%. While auxiliary data are available at EU-wide and regional scales (e.g., NUTS

2 and 3 levels), their varying resolution and derivation methods present challenges for consistent accuracy and their usability into integrated approaches across different spatial scales.

Machine learning (ML) models are instrumental in linking PSS, RS, and auxiliary data to measured soil properties, enabling accurate and scalable SOC predictions (Canal Filho et al., 2023; Mohammedzein et al., 2023). ML models can predict BD and other soil properties (Panagos et al., 2024), complementing traditional pedotransfer functions (PTFs) (Tarocco and Aprea, 2021) with both methods impacting field-scale SOC stock quantification. Global ML models, trained on diverse datasets, are scalable but may introduce higher uncertainty due to generalization. Conversely, local ML models, calibrated with site-specific data, capture fine-scale variability, and deliver more precise estimates for targeted areas (Heuvelink et al., 2021).

To enhance SOC stock predictions and account for uncertainty, advanced ML techniques like Quantile Regression Forest (QRF) models are increasingly employed. QRF offers probabilistic predictions with prediction intervals and assigns weights to observations, allowing for a robust assessment of uncertainty in SOC stock estimates. Addressing uncertainty is crucial, as errors in PSS-derived predictions and their associated errors need to be propagated into SOC sequestration estimates, affecting final C removal units' uncertainty at project level. ML techniques do not assume specific data distributions and can efficiently handle multi-source covariate data, focusing on improving prediction accuracy. They outperform conventional methods by minimizing prediction error, especially when mapping accuracy is prioritized (Wadoux et al., 2020). However, challenges with interpretability and the need for large, resource-intensive datasets remain, particularly when detecting small changes in SOC stocks (Bradford et al., 2023; Stanley et al., 2023). Efficient sampling designs, such as those based on covariate space, can improve ML model calibration by optimizing sample locations using methods like Conditioned Latin Hypercube Sampling (cLHS) and the Kennard-Stone algorithm (Biswas and Zhang, 2018; Wadoux et al., 2019). Local calibration is essential for transferring models to new areas and enhancing prediction accuracy. Despite ongoing advances in local calibration methods, challenges persist in selecting effective covariates and strategies to ensure model transferability (Viscarra Rossel et al., 2024; Xia et al., 2024). Balancing the need for large datasets with minimizing sampling costs remains a central challenge in ML-driven SOC quantification.

In this study, the Emilia Romagna region was selected as a test bed to evaluate the capability of PSS to detect field-scale differences in SOC stocks across a number of fields with varying soil types and textural classes. The primary aim is to develop and validate a robust, accurate, and cost-

effective PSS-based SOC stock accounting approaches to support any hybrid sampling and modelling MRV protocol to ensure the scalability of CF programs in northern Italy. Additionally, the region provides high-quality, publicly available data on SOC, BD, soil texture, and textural classes at a 100 m resolution, making it an ideal setting for PSS- and ML-based prediction model development. The PSS used in this study is the Veris iScan, an on-the-go PSS device from Veris Technologies (Salina, Kansas, USA). Mounted on vehicles, this sensor collects rapid, high-resolution soil measurements as it moves across the field, providing insights into soil properties and within-field variability. For example, Kweon (2012) demonstrated the SOM estimates using the sensor readings in a simple regression model have an R^2 value of 0.80, compared to laboratory analyses. However, more robust calibration is necessary to adapt the model prediction to the specific soil conditions of northern Italy.

More specifically the objectives of this work are as follow:

- i) to calibrate and validate a series of QRF models using a global dataset from the PSS, RS, and auxiliary data to predict key properties for SOC stock accounting: SOC concentration, soil texture and soil BD. These predictions will be used to estimate SOC stock at a 10-meter resolution at the field scale across a series of fields representative of the main soil conditions and types in Emilia-Romagna region.
- ii) To propose and validate a stepwise procedure for selecting robust and accurate local prediction models, supported by a precision soil sampling approach, aimed at minimizing the number of soil samples required for a PSS-based, but field-specific, SOC stock accounting approach.
- iii) To quantify model uncertainty, using both global and local datasets, associated with the SOC concentration and SOC stock predicted values. The study will also evaluate the cost-effectiveness and accuracy of the PSS-based SOC stock accounting approach compared to conventional soil sampling methods at the field level.

The main hypothesis that this study aim to address are:

- 1) On-the-go vis-NIR and electrical conductivity (EC) readings are valuable predictors of soil properties, such as SOC concentration and soil texture, needed for calculating SOC stock.
- 2) Combining auxiliary covariates, such accurate regional soil maps, with RS and PSS enhances the accuracy of QRF global models for predicting soil properties essential for SOC stock accounting at field scale.

- 3) Smart sampling improves site-specific calibration in the development of local dataset models.
- 4) SOC stock can be accurately predicted with a reasonable cost-accuracy tradeoff when the local model is calibrated and validated for main soil type of a region.

5.2 Materials and Methods

5.2.1 Workflow of model predictions

To develop the soil property prediction models, the workflow outlined in Fig. 5.1 was followed, comprising three main steps: 1) PSS data, along with available RS and auxiliary data, were integrated with ground truth soil measurement collected at field scale; 2) modeling step involved processing the data to develop QRF ML models to predict SOC, soil texture, and BD. The models' accuracy and associated uncertainties were assessed, and the resulting predictions were used to calculate SOC stock at a 10-meter resolution at the field scale; 3) for the out-of-sample prediction the robustness and reliability of the developed QRF models were tested using an independent validation dataset to ensure their generalizability and predictive performance.

The database created has two purposes: i) building a dataset for the QRF model and ii) creating raster covariate layers at a 10 m resolution at field scale, representing the covariates used in the QRF model. A Raster Look-Up Table links the raster covariates with the corresponding covariates names in the QRF model, enabling spatial mapping by applying the QRF model developed using the ModelMap package in R (Freeman and Frescino, 2023).

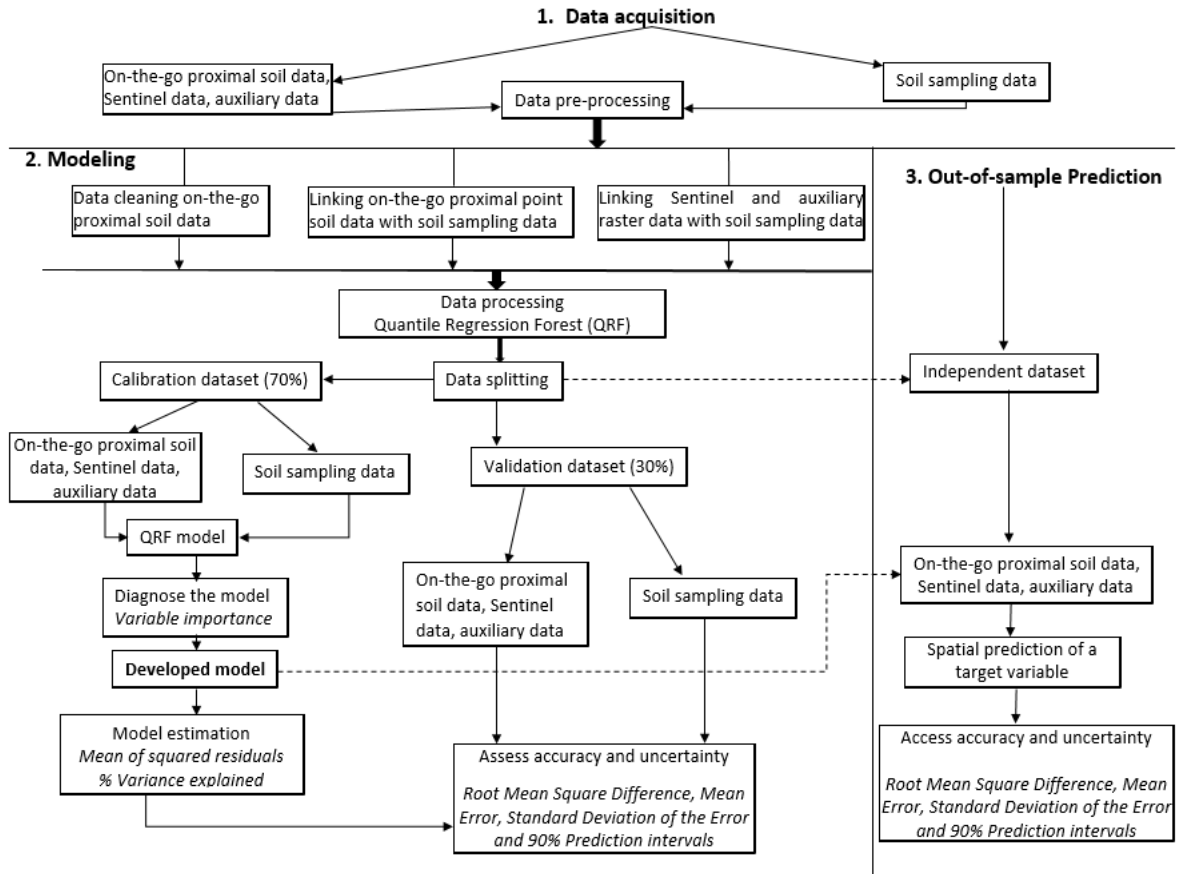


Figure 5.1 Flowchart illustrating the workflow: from integrating multisource data to develop the QRF model, through assessing model prediction accuracy, to evaluating associated uncertainties.

5.2.2 Dataset description and data acquisition strategy

Thirty-five fields were mapped using the iScan PRO sensor in the Emilia Romagna region, while fifteen fields were located in the neighboring regions of Piemonte, Lombardy, and Veneto, as shown in Figure 5.2. The field areas, dates of soil mapping and sample collection, and the number of soil samples taken per field are summarized in Table 5.1. In total, 578, 374, and 187 data points were collected for SOC, soil texture (percentage of sand, silt, and clay), and BD, respectively, with the frequency distributions shown in Figure 5.3.

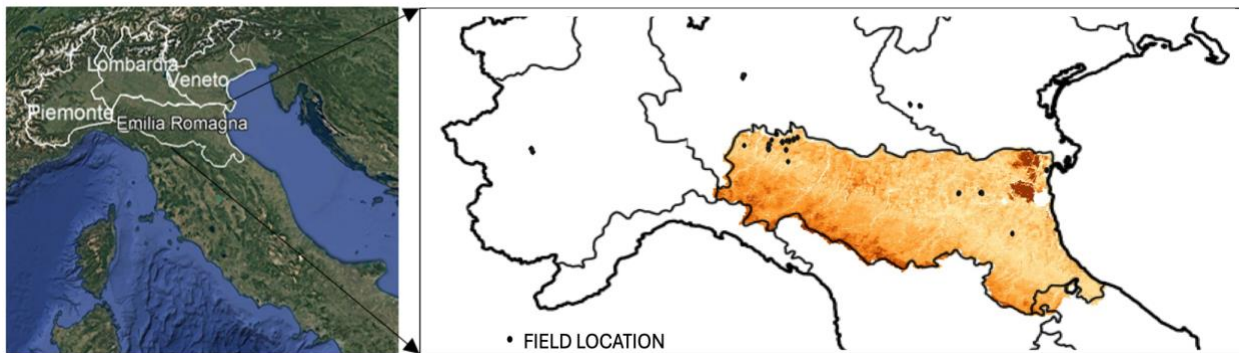


Figure 5.2 Fields' location used in this work. Most of the fields are in the Emilia Romagna region (35), with the remaining fields located in the Piemonte region (2), the Lombardy region (10), and the Veneto region (3).

Table 5.1 Summary of fields information, including soil sampling details such as the sampling date, the number of soil samples collected per field, and the laboratory analyses conducted for SOC, soil texture, and soil BD.

Field Name	Longitude (degree)	Latitude (degree)	Area (ha)	Mapping date	Sampling date	Sample number
Field1	9.730295	45.037593	2.5	Dec-21	Dec-21	4
Field2	9.703730	45.003442	1.0	Dec-21	Dec-21	4
Field3	9.701474	44.970270	3.0	15-Apr-22	22-Apr-22	48
Field4	9.826117	45.026575	3.4	29-Apr-22	3-May-22	8
Field5	9.826330	45.022760	4.3	29-Apr-22	3-May-22	8
Field6	9.828369	45.022160	7.0	29-Apr-22	3-May-22	10
Field7	9.857238	44.968912	1.5	4-May-22	12-May-22	4
Field8	9.859762	44.964977	3.0	4-May-22	12-May-22	4
Field 9	9.860436	44.966567	1.7	4-May-22	12-May-22	4
Field10	9.878744	44.878947	3.4	13-May-22	16-May-22	9
Field11	9.775484	45.080201	9.5	21-May-22	23-May-22	4
Field12	9.767784	45.086198	2.6	21-May-22	23-May-22	3
Field13	9.771441	45.088790	3.5	21-May-22	23-May-22	2
Field14	9.707046	45.003344	9.7	17-Aug-22	17-Aug-22	70
Field15	9.703683	45.003410	1.0	17-Aug-22	17-Aug-22	4
Field16	9.704338	45.007565	4.0	16-Aug-22	17-Aug-22	4
Field17	9.700940	44.983464	3.2	11-Sep-22	12-Sep-22	9
Field18	7.552481	44.974454	6.5	12-Sep-22	13-Sep-22	44
Field19	7.565337	44.951581	4.6	12-Sep-22	13-Sep-22	26
Field20a	9.888744	45.031197	1.4	14-Sep-22	22-Sep-22	4
Field20b	9.888048	45.031931	2.6	14-Sep-22	22-Sep-22	4
Field21a	9.927939	45.043965	4.2	14-Sep-22	12-Sep-22	10
Field21b	9.927230	45.044256	1.4	14-Sep-22	12-Sep-22	3
Field22a	9.961487	45.058650	2.5	14-Sep-22	22-Sep-22	5
Field22b	9.962407	45.058321	3.5	14-Sep-22	22-Sep-22	5
Field23	9.872833	45.033719	4.5	20-Sep-22	22-Sep-22	4
Field24	9.702141	45.007062	6.9	23-Mar-23	22-Sep-22	4
Field25	9.701942	45.003822	5.1	23-Mar-23	22-Sep-22	4
Field26	9.479843	44.997368	4.7	12-Oct-22	12-Oct-22	6
Field27	9.866458	45.033565	6.8	14-Oct-22	15-Oct-22	7
Field28	11.07118	45.291334	14.0	21-Jan-22	28-Jan-22	5
Field29	10.98503	45.298375	2.6	6-Apr-22	11-Apr-22	4
Field30	10.98047	45.303646	2.6	6-Apr-22	11-Apr-22	4
Field31	9.465670	45.502115	7.2	10-Mar-22	15-Apr-22	4
Field32	9.467513	45.495442	8.7	9-Mar-22	15-Apr-22	4
Field33	9.474631	45.508529	3.2	9-Mar-22	15-Apr-22	4
Field34	9.469255	45.516922	6.0	17-Dec-21	15-Apr-22	4
Field35	9.483941	45.517417	4.0	17-Dec-21	15-Apr-22	4
Field36	9.468072	45.499400	12.9	10-Mar-22	15-Apr-22	5
Field37	11.422841	44.646044	50.0	17-Mar-22	19-Oct-22	7
Field38	11.631319	44.650313	39.7	17-May-22	19-Oct-22	6
Field39	11.623751	44.648926	59.5	17-May-22	19-Oct-22	9
Field40	12.218634	44.817543	4.6	17-Nov-23	29-Nov-23	15
Field41	11.909658	44.349801	0.9	7-Dec-23	7-Dec-23	10
Field42a	9.888935	45.031218	1.4	21-Mar-23	22-Mar-23	15
Field42b	9.887704	45.031868	2.6	21-Mar-23	22-Mar-23	15
Field43a	9.961473	45.058546	2.5	21-Mar-23	21-Mar-23	18

Field43b	9.962610	45.058387	3.5	21-Mar-23	21-Mar-23	22
Field44a	9.825125	45.022698	4.4	21-Mar-23	23-Mar-23	15
Field44b	9.823871	45.023540	5.3	21-Mar-23	23-Mar-23	15
Field45a	9.701010	44.983436	3.4	22-Mar-23	22-Mar-23	26
Field46b	9.699530	44.983588	3.3	22-Mar-23	22-Mar-23	19
Field47a	9.927218	45.044131	4.2	21-Mar-23	21-Mar-23	30
Field47b	9.927913	45.044455	1.4	21-Mar-23	21-Mar-23	10
Field48	9.891072	45.002683	3.3	23-Nov-23	29-Nov-23	42
Field49	9.997268	45.045875	5.9	17-Aug-23	18-Aug-23	24
Field50	10.788820	45.252277	12.8	4-Aug-23	3-Oct-23	24
Field51	10.786483	45.250159	7.2	23-Aug-23	3-Oct-23	6
Field52	10.792559	45.249349	3.2	4-Aug-23	3-Oct-23	14
Field53	10.794713	45.247811	7.0	23-Aug-23	3-Oct-23	8

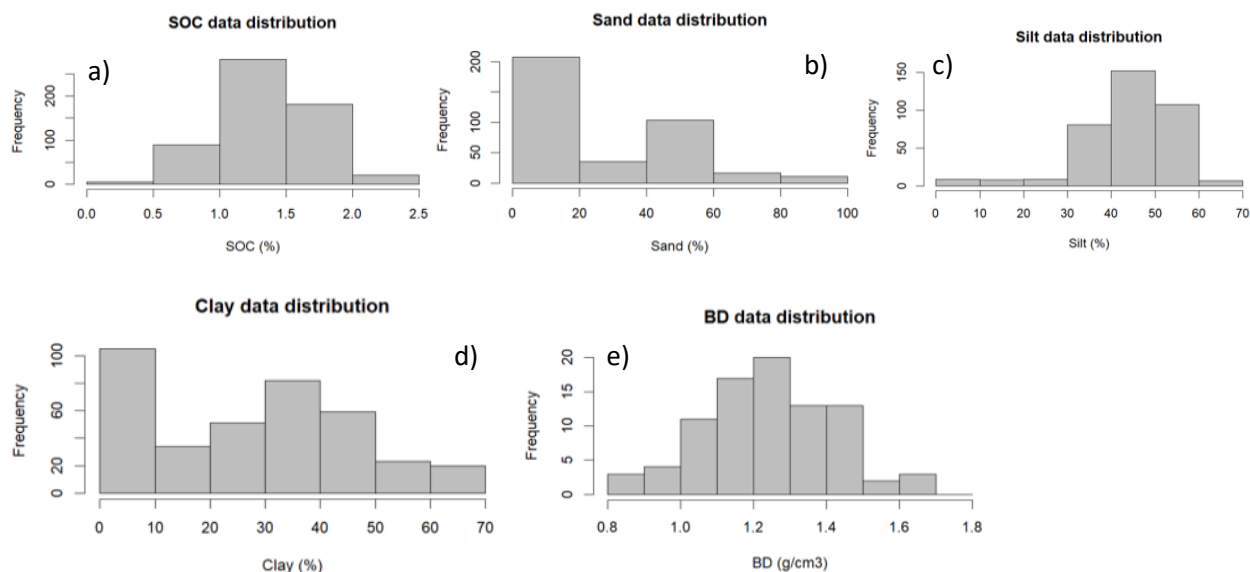


Figure 5.3 Frequency distribution of the measured data for: SOC with 578 observations (a), soil texture with 374 observations, that include sand (b), silt (c), and clay (d), and BD with 187 observations (e)

5.2.2.1 On-the-go proximal soil sensing data

To acquire on-the-go PSS measurements, the Veris iScan PRO sensor, equipped with a GPS receiver, was used (Veris Technologies, Salina, Kansas, USA). The iScan PRO sensor module contains red and infrared (IR) light sensors, as shown in Figure 5.4a. This dual-wavelength optical sensor measures the spectral reflectance in the red and IR bands, with wavelengths of 660 nm and 940 nm, respectively, which are used to estimate SOM content. Additionally, soil electrical conductivity (EC) readings (in mS m^{-1}) collected with the iScan PRO sensor help identify soil texture variability across the field (Corwin and Lesch, 2005). Red, IR and EC readings were taken along transects spaced 6 m to 16 m apart, depending on the field size, with measurements taken every 2 to 3 meters along each pass, determined by the data collection rate and field travel speed. On-the-go PSS measurements were performed when the agricultural field was bare, either before seeding or after harvesting, provided no wheel tracks were present. After the mapping process, raw measurements were visually inspected, and any points with negative readings were removed. The cleaned proximal data

(red, IR, and EC) were then uploaded on the FieldFusion cloud service (<https://fieldfusion.veristech.com/>) for further analysis. Soil measurements of IR, the IR/red ratio, and EC were converted into raster data at a 10 x 10 m resolution (aligned with Sentinel-2 pixel size) using the median function in the raster package in R, as depicted in Figure 5.4b.

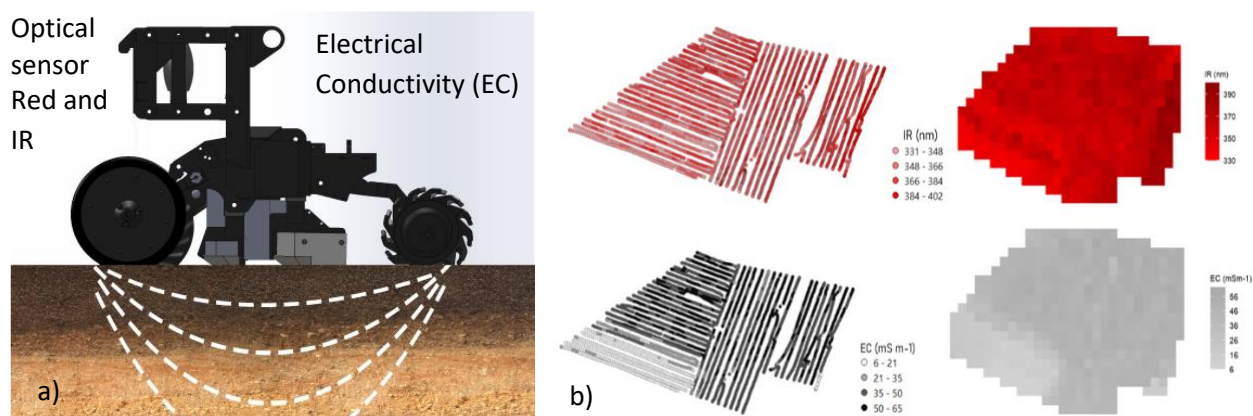


Figure 5.4 On the left (a), a schematic representation of the iScan PRO setup is shown, highlighting the sensor positions: electrical conductivity (EC) is measured by the two mechanical wheels of the sensor, among which humidity sensor (on the left) and optical sensor (for red and IR on the right) are present. On the right (b), an example of PSS soil point readings for IR (above) and EC (below) is displayed, along with the corresponding IR and EC raster data at 10 m resolution.

5.2.2.2 Soil sampling, laboratory analysis, and soil organic carbon stock calculation

Soil sampling was performed prior to mapping using two strategies: 50% of the fields were sampled using a systematic grid sampling (40 x 40 m), while the remaining 50% followed a systematic random sampling approach, with a minimum of 3 and a maximum of 24 samples per field. Further details on precision sampling after mapping are provided in Section 5.2.5.1.

For sampling, a 2 cm wide soil core auger was used to collect three soil sub-samples from a depth of 30 cm around each sampling point (approximately 3 m apart), which were then composited into a single sample. The soil samples were air-dried and ground to 2 mm for textural analysis, or ground to < 53 µm for elemental analysis. Soil texture was measured following the method of Moebius-Clune et al. (2016), while SOC concentration was determined using the Dumas method with an elemental analyzer (Skalar PRIMACS™ SNC-100). Soil BD was measured using the volumetric ring method, with two sub-samples collected per sampling point. The BD of the fine soil mass (stone/gravel-free) was calculated by dividing the oven-dried (105°C) soil mass by the known volume of the sample core (575 cm³). The amount of gravel/stones was also calculated on a dry weight basis. SOC stock (Mg C ha⁻¹) was then calculated by considering SOC concentration, BD, and gravel content at a depth of 0–30 cm, using the formula (IPCC, 1997):

$$\text{SOC stock (Mg C ha}^{-1}\text{)} = \text{SOC (\%)} \times \text{BD (g cm}^{-3}\text{)} \times \text{Depth (m)} \quad (1)$$

5.2.2.3 Sentinel-2 data of field mapped with proximal sensor.

The Synthetic Bare Soil Image (SBSI) was calculated for each field using field boundaries detected from mapping. The SBSI was derived from three indices: the Normalized Difference Vegetation Index (NDVI), the Normalized Burn Ratio 2 (NBR2), and the Bare Soil Index (BSI) (Castaldi et al., 2019). To extract the maximum bare soil pixels from an image, pixels with NDVI values greater than 0.35, NBR2 values greater than 0.125 (Castaldi et al., 2019), and BSI values less than 0.021 (Diek et al., 2017) were excluded. Sentinel-2 imagery covering the agricultural fields was selected using the Google Earth Engine (GEE) platform. For each field, the median of the bare soil pixels filtered from April to September over the period 2020–2024 was calculated to produce the SBSI. The SBSI was derived from Sentinel-2's 13 spectral bands, covering wavelengths from 443–2190 nm. These include visible and near-infrared bands with a 10 m pixel size, red-edge and shortwave infrared bands with a 20 m pixel size, and atmospheric correction bands with a 60 m pixel size. In this study, the SBSI for the visible red band (B4) and the shortwave infrared band (B12) were selected as covariates for SOC concentration prediction, while the near-infrared band (B8) and shortwave infrared bands (B11, B12) were used for clay content prediction.

5.2.2.4 Auxiliary variables dataset

5.2.2.4.1 Regional dataset used for the global model

The soil maps used as covariates were raster maps with a 100 m resolution, produced by soil survey staff of Emilia Romagna region (<https://ambiente.regione.emilia-romagna.it>). The following soil maps were incorporated into the prediction models: texture soil maps (percentage of sand, silt, and clay), soil textural classes based on USDA classification, SOC stock maps, and BD maps. Using a Scorpan Kriging approach, raster maps for SOC, sand, silt, and clay percentages, and USDA textural class at 100 m resolution were generated for topsoil (0–30 cm), based on a database of 18,969 sampling points (Ungaro et al., 2010). These maps are accompanied by accuracy maps indicating prediction reliability, ranging from high to low. Considering the accuracy of the available maps is crucial, as low accuracy is associated with high errors in the estimated soil properties used as covariates in the global ML model. To link soil sampling locations with the nearest high-accuracy SOC, sand, silt, clay and BD estimation points, a nearest-neighbor search was performed based on their projected coordinates (UTM zone 32N) using the "*ngeo*" package in R. The resulting SOC concentration, sand, silt, clay, and BD values were incorporated in the ML global dataset. In some fields, the textural class maps had low accuracy. To address this, a probability frequency distribution

was calculated, and the most frequent textural class was assigned to the entire field. The regional SOC, sand, silt, clay, and BD raster data were resampled using a bilinear interpolation method to achieve a 10 m resolution at the field scale. For textural class (a categorical variable), the nearest-neighbor resampling technique was applied to derive a 10 m resolution covariate at the field scale.

Additionally, another soil map used as a covariate is the soil type map of the Emilia-Romagna region, which describes soils and their geographical distribution across the alluvial plain and parts of the Apennines at a 1:50,000 scale. The identified polygons are classified according to the World Reference Base for Soil Resources (WRB) classification (Tarocco and Aprea, 2021). For agricultural fields located outside the Emilia-Romagna region, 1:250,000-scale soil maps were used as described in section 5.2.2.4.2. These polygon-based soil maps were rasterized at a 10 m resolution to align with the other soil covariate maps.

5.2.2.4.2 LUCAS dataset used for the global model

For the agricultural fields located in the Lombardy, Veneto, and Piemonte regions, SOC, BD, sand, silt, clay, and textural classes were obtained from the LUCAS database. Using the LUCAS topsoil database, the percentages of sand, silt and clay were predicted using the Multivariate Adaptive Regression Splines (MARS) approach (Ballabio et al., 2016). Additionally, the USDA-based textural class classification is available in the database. SOC was predicted using the LUCAS topsoil database by de Brogniez et al. (2015) through an implemented generalized additive model (GAM). This approach revealed large SOC uncertainties in northern latitudes or wetland areas, while smaller uncertainties were observed in croplands. The LUCAS soil BD prediction map was generated using the Cubist rule-based regression ML model (Panagos et al., 2024). The raster maps produced at the European scale generally have a pixel size of 500 m, except the BD map, which has a pixel size of 100 m.

5.2.3 Building global dataset for ML model

To relate soil sampling point measurements with on-the-go PSS data, a circular buffer with a 2 meters radius was created around each soil sampling location, following the LUCAS sampling procedure as shown in Figure 5.5 (Fernández-Ugalde et al., 2018). If multiple on-the-go PSS data points were found within the 2-meter buffer of a soil sampling location, their average value was used as the final dataset value. For soil sampling points located more than 2 meters away from the on-the-go PSS data, the shortest distance between the sampling location and the nearest PSS reading was considered.

We used the procedure outlined in Section 5.2.2.4.1 to link soil sampling locations with the SBSI Sentinel-2 point data by performing a nearest-neighbor search based on their projected coordinates (UTM zone 32N) using the "*ngeo*" package in R. This multisource dataset was used to develop a QRF (Quantile Regression Forest) model for predicting SOC, clay, silt, sand, and BD at a 10 m resolution at the field scale. The SOC dataset contains 578 observations, the soil texture dataset contains 374 observations, and the soil BD dataset contains 187 observations.

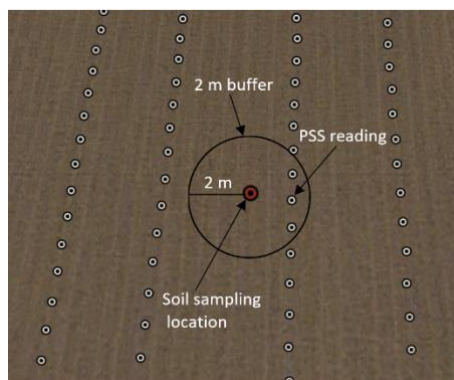


Figure 5.5 Example of linking on-the-go proximal soil sensing (PSS) point readings (empty circle) with a soil sampling location (red circle) in an agricultural field using a 2-meter buffer.

5.2.4 Quantile Regression Forest model

In the QRF model, weighted observations were used to estimate the full conditional distribution (Meinshausen, 2006). The QRF predicts the median and individual quantiles within the terminal nodes. Using the predicted median rather than the mean avoids biased predictions often associated with the mean (Anderson et al., 2014). Key parameters influencing the QRF model's performance include the number of predictors used to determine the splits (`mtry`) and the number of trees (`ntree`) (Breiman, 2001). The `mtry` parameter is automatically optimized using the `tuneRF` function from the `randomForest` package, which typically sets it as the number of covariates divided by three. The `ntree` parameter is selected based on the exponential relationship between model error and the number of trees. As the number of trees increases, model error decreases until stabilization. For this study, the error stabilized at 1000 trees, which was chosen as the final `ntree` value.

For model calibration and validation, the SOC and soil texture global datasets were randomly divided into 70% for calibration and 30% for validation. Due to the relatively small size of BD dataset, an 80%-20% split was applied instead. The QRF model was implemented using the `quantregForest` package in R (Meinshausen and Schiesser, 2015).

The QRF dataset uses multisource covariates derived from PSS, RS, and auxiliary data to predict SOC and soil texture (sand, silt, and clay). To evaluate the most influential variables and their impact

on the model performance, a backward selection approach was used. Several QRF models were tested with different combinations of data sources as covariates. Model performance was assessed based on root mean square error (RMSE) and Spearman correlation (results provided in supplementary materials). The findings revealed that incorporating all data sources (PSS, RS, and auxiliary) significantly improved prediction accuracy compared to models using covariates from a single source. To address multicollinearity among covariates, variable importance was calculated to understand which predictors contributed most to model performance. Two measures of variable importance were used: the percentage increase in Mean Standard Error (MSE), which indicates the model's reliance on a predictor by measuring the performance drop when the predictor values are permuted; and the increase in Node Purity, which evaluates how effectively a variable split the data into homogenous groups with respect to the target variable (Breiman, 2001). Covariate selection prioritized predictors with high values for both metrics. The selected covariates for each target variable were as follows:

- SOC: EC, IR/Red ratio, SBSI (B4 and B12), soil type, and estimated SOC.
- Soil texture (sand, silt, clay): EC, IR, textural class, and estimated sand, silt, or clay, respectively. Additionally, clay prediction included SBSI (B8, B11, and B12).
- BD: Observed SOC, sand, silt, and clay.

This systematic approach ensured that the QRF models achieved high predictive accuracy at a 10 m resolution for SOC, soil texture, and BD at the field scale.

5.2.5 *Building QRF local model dataset*

A local dataset was created for each mapped field by selecting samples from the global dataset using the Kennard–Stone (KS) algorithm. This algorithm ensures a uniform distribution of samples across the covariate space (Kennard and Stone, 1969). The KS algorithm begins by selecting the pair of points that are the farthest apart. Subsequently, the distance (d) between each unassigned point (i_0) and the selected points (i) is calculated as:

$$d = \max_{i_0} (\min_i (d_{i, i_0})) \quad (2)$$

This method ensures that the next selected point (i_0) is the one farthest from its nearest neighbor (i) within the global dataset. The KS algorithm employs the Mahalanobis distance to select the points, based on the principal component analysis (PCA) of the covariates' correlation matrix. While the global dataset contains both continuous and categorical covariates, only continuous covariates were used for sample selection. For the SOC local model, 50 points were selected from the global

dataset, whereas 30 points were chosen for sand, silt, clay, and BD. The KS algorithm was executed using the *prospectr* package in R (Stevens and Ramirez-Lopez, 2022).

5.2.5.1 Precision soil sampling

In addition to the local dataset created using the procedure described above (section 5.2.5), a field-specific set of $n=3$ samples per field was added to the local dataset. After mapping, the sampling locations within each field were selected using the cLHS algorithms, with PSS and SBSI data at a field scale used as covariates. In the cLHS sampling design, marginal strata are derived for each covariate space. Given the sample size (n) and the number of covariates (C) the total number of marginal strata is C^n . To create evenly spaced marginal strata, quantiles of cumulative probabilities are used as bounds (Minasny and McBratney, 2006). The simulated annealing algorithm, configured with default arguments and 50,000 iterations, was applied to optimize the sampling design, and minimize the criterion function (Brus, 2019). The soil sample size was set to three for each field, and the cLHS sampling was implemented using the *clhs* package in R (Roudier, 2021). These three precision-selected soil samples were subsequently added to the local dataset described in Section 5.2.5.

5.2.6 Metrics for prediction models performances evaluation

The accuracy of the fitted model was evaluated using the following metrics:

1. Root Mean Square Difference (RMSD,) which measures the inaccuracy of the model predictions:

$$\text{RMSD} = \sqrt{\frac{1}{n} \sum_{i=1}^n (y_i - x_i)^2} \quad (3)$$

where y_i is the predicted value for the i -th observation, x_i is the actual observed value for the i -th observation, and n is the number of observations.

2. Mean Error (ME), which measures the model bias:

$$\text{ME} = \frac{1}{n} \sum_{i=1}^n (y_i - x_i) \quad (4)$$

3. Standard Deviation of the Error (SDE), which measures the imprecision of the model:

$$\text{SDE} = \sqrt{\frac{1}{n-2} \sum_{i=1}^n (y_i - x_i)^2} \quad (5)$$

4. Spearman Correlation (ρ), which provides a coefficient of correlation between two variables based on ranks of observed and predicted values (Spearman, 1904). For each pair of variables, the difference (d_i) between the ranks of X_i (observed) and Y_i (predicted) is calculated:

$$d_i = R(X_i) - R(Y_i) \quad (6)$$

where $R(X_i)$ and $R(Y_i)$ represent the ranks X_i and Y_i respectively. The Spearman correlation is then derived as:

$$\rho = 1 - \frac{6 \sum d_i^2}{n(n^2 - 1)} \quad (7)$$

where d_i is the rank difference and n is the number of observations.

The Spearman correlation ranges from -1 to 1:

- $\rho=1$ indicates a perfect monotonic increasing relationship,
- $\rho=-1$ indicates a perfect monotonic decreasing relationship,
- $\rho=0$ indicates no monotonic relationship between the variables.

These metrics were calculated for both the global and local QRF models.

5.2.6.1 SOC stock error propagation

Overall and field-specific errors in SOC stock estimates estimated were calculated through analytical error propagation, following the formula proposed for input data in soil models by Verra VM0042 standards (Verra, 2024). The error propagation was carried out on a validation dataset, where the variance of ground truth observations and model predictions were used to estimate SOC stock uncertainties.

First, the variance of model's predictions, which reflects the spread of the predictions and how much they deviate from the ground truth, accounting for any linear relationship between the errors and observations, was calculated.

$$s_{\text{model}}^2 = s^2(\text{obs} - \text{pred}) = 2[s^2_{\text{model}} - \text{cov}(\text{pred}, \text{obs})] \quad (8)$$

where s_{model}^2 is the variance of the model's predictions, s^2 is the variance of the residuals, and cov is the covariance between predictions and ground truth observations.

Second, the correlation coefficient between pairs of residuals was calculated:

$$\rho = \frac{\text{cov}(\text{pred}, \text{obs})}{\sqrt{(s_{\text{model}}^2) * (s_{\text{observed}}^2)}} \quad (9)$$

Where the s_{observed}^2 is the variance of the ground truth observations.

Finally, the variance of the differences, based on the variance of predictions, the variance of the ground truth, and their correlation, was calculated:

$$s_{\text{model}}^2 = 2 s_{\text{model}}^2 (1 - \rho) \quad (10)$$

5.2.7 Uncertainty analysis

The QRF model uses the information from terminal nodes to generate uncertainty estimates. This allows for the mapping of lower and upper bounds for spatial predictions, such as a 90% prediction interval (PI), identified by the 5th and 95th percentiles (Arrouays et al., 2014; Heuvelink, 2014). The PI represents the range of values within which the true value is expected to fall 90% of the time. The width of the PI is calculated by subtracting the 5th percentile from the 95th percentile, which defines the absolute uncertainty (Poggio et al., 2021). Narrow intervals indicate more reliable predictions, while wider intervals suggest greater uncertainty (Kasraei et al., 2021). To evaluate the quality of the prediction intervals, the Prediction Interval Coverage Probability (PICP) was calculated (Lagacherie et al., 2019). The PICP represent the percentage of the validation dataset in which the target variable fall within the PI.

$$PICP = \frac{1}{n} \sum_i^n \delta(li \leq obs_i \leq ui) \quad (11)$$

where δ is an indicator function with a Boolean argument, in which 1 indicates TRUE and 0 otherwise. l_i and u_i are the lower and upper bounds of the respective observation (obs_i) in the validation dataset.

For a 90% PI, a PICP of 0.9 suggests an accurate uncertainty assessment. If the PICP is higher than 0.9, it suggests overestimation of uncertainty, while a value lower than 0.9 indicates underestimation.

5.2.8 Independent validation of single soil properties and SOC stock estimates

The purpose of independent validation is to test the model's robustness and reliability in conditions where the data may differ significantly from the calibration (e.g. Garsia et al. 2023). Thus, independent validation is performed to evaluate the QRF model on a completely new dataset that was not used during the model development process (calibration, validation, or hyperparameter tuning). The SOC concentration, sand, silt, clay, and BD have been independently validated. The independent validation includes SOC concentration, sand, silt, clay, and BD. This external dataset comes from two agricultural fields in Emilia Romagna, Field 48, and Field 49 (see Table 5.2). Soil sampling was conducted using a systemic grid approach for Field 48 and random sampling for Field 49. Soil laboratory analysis, soil mapping, and linking soil sample locations with multisource data were carried out as described in Sections 5.2.2.1, 5.2.2.2, 5.2.2.3, and 5.2.3, respectively. Once the QRF model has been calibrated and validated internally, it is tested on the independent dataset.

Performance metrics and uncertainties are calculated and compared with those obtained during internal validation, as detailed in Sections 5.2.6 and 5.2.7.

5.2.9 *Model accuracy and cost effectiveness.*

To compare the cost-accuracy of the proposed PSS-based C accounting schemes using global versus local models, a common physical soil sampling method, referred to here as the "compositing scheme," was used as a benchmark. The compositing scheme involves stratification (with a maximum of three strata per field) based on RS using SBSI S2 bands. The number of samples across strata was optimized using the Neyman allocation method, with a maximum of 5 per field smaller than 5 ha. Stratified simple random sampling was then used to collect soil aliquots, which were bulked into composite samples, each composite consisting of one aliquot per stratum (compositing across strata). The mean of each composite sample provided an unbiased estimate of the spatial mean of the field. At each sampling point, two BD sub-samples were also taken. Samples measurement and SOC stocks calculation was done as reported in Section 5.2.2.1. Averaging the composite sample means resulted in the final estimate of the spatial mean.

The total costs of sampling, mapping, and laboratory analyses (C_T) was modeled for a subset of 22 fields (total area of 90.5 hectares) where sampling points were used only for model validation. SOC stocks were calculated for both C accounting approaches. The costs were calculated using the equation proposed by Viscarra Rossel and Brus (2018) as follows:

$$C_T = C_F + C_L \quad (12)$$

where C_F represents the cost of fieldwork, and C_L is the cost of laboratory analyses (Table 5.2). Fieldwork costs comprise fixed costs (C_{Fx}) and variable costs (C_{Fv}). C_{Fx} include all preparations necessary for a soil sampling or mapping campaign, such as organizing, packing, maintaining equipment, loading sampling points onto GPS, reconnaissance field visits, and identifying sampling sites. The C_{Fv} for the compositing scheme were calculated as the cost per sampling site multiplied by the total number of sampling locations, while the C_{Lv} is the cost of analyzing a composite sample, multiplied by the number of composites. C_{Lv} includes costs of sample preparation (drying, grinding, and sieving) and analysis.

Total costs for the PSS method were modelled similarly for both the "global" (mapping and prediction model using global dataset) and "local" (mapping, precision sampling, laboratory analysis of texture and SOC concentration, and prediction model using a local dataset) C accounting approach. The C_F was modelled as C_{Fx} . However, variable costs consisted of two components: (a)

the variable cost of sensing and model prediction, which is calculated per hectare (for both global and local models), and (b) the variable costs of sampling and analysis, which include the costs per sampling site plus the cost of sensing (local model).

The C_L for sensing were modelled as the cost per calibration samples sent to the laboratory (C_{Lcal}) times the number of calibration samples.

The cost-efficiency of the different PSS-based C accounting methods was assessed by comparing prediction models using local and global datasets for SOC stock estimation and comparing these models against the compositing approach.

To evaluate the relative cost-efficiency (ϵ) of a C accounting approach, we calculate the ratio of C_T and associated uncertainty of SOC stock estimation:

$$\epsilon = \frac{C_{Ta} * RMSE_a}{C_{Tb} * RMSE_b} \quad (13)$$

where C_{Ta} and $RMSE_a$ represent the C_T and inaccuracy involved in SOC stock estimation using a C accounting scheme, and C_{Tb} and $RMSE_b$ represent C_T costs and inaccuracy involved in the alternative approach. The compositing scheme was used as the reference C accounting scheme, while the alternative designs were the sensing schemes using global or local models. When the relative cost-efficiency (ϵ) was larger than one ($\epsilon > 1$), the alternative sampling scheme was more cost-efficient than the reference scheme. For the inaccuracy of the PPS-based SOC stock accounting approach, the average value was used, and area-weighted RMSE (across the 22 fields) of the estimated mean SOC stocks at the field level from the validation dataset. For the compositing scheme, the inaccuracy was estimated by computing the RMSE for each field based on paired differences between the composite mean and the average of the aliquots from that composite.

Table 5.2 Breakdown of unitary (a) and total (b) costs in euro for soil sampling/sensing and laboratory analyses for the compositing and the proximal sensing SOC stock accounting schemes (global model with mapping only and local model with mapping + precision sampling).

a) Unitary costs				b) Total costs			
Item	Composites	Sensing global	Sensing local	Item	Composites	Sensing global	Sensing local
Fixed costs, C_{fx} (per field)	100	100	100	Fixed costs, C_{fx}	2,200	2,200	2,200
Field mobile sensor survey, C_{fv} (eur/ha)		60	60	Field mobile sensor survey, C_{fv}		7,240	7,240
				Subtotal, C_f	2,200	9,440	9,440
Field cost (average per field)				Field cost			
Soil coring	63		27	Soil coring	1380		990
Bulking of composites	8		6	Bulking of composites	184		81
BD sampling	63			BD sampling	920		
Transport	30	30	30	Transport	660	660	660
Subtotal, C_{fv}	163	30	63	Subtotal, C_{fv}	3,144	660	1,731
Proximal soil sensor (per field)				Proximal soil sensor			
fee cost for data cloud cleaning		16	16	fee cost for data cloud cleaning		352	352
Subtotal, C_{vs} (per field)		16	16	Subtotal, C_{vs}		352	352
Laboratory (per sample)				Laboratory			
Sample preparation	30		30	Sample preparation	2,760		1,980
Dry weight (for bulk density)	5		5	Dry weight (for bulk density)	460		
Gravel	5		5	Gravel	460		660
Elementar analyzer	45		45	Elementar analyzer	4,140		2,970
Texture	35		35				
Subtotal, C_{lv}	93		93	Subtotal, C_{lv}	3,220		2,310
				Total cost	13,164	10,452	16,473
				Total (per ha)	145	115	182
				Total (per field)	598	475	749

5.3 Results

5.3.1 Overall performance of QRF models using global and local datasets of single soil properties

The QRF models were run using combinations of PSS, RS, and auxiliary data as covariates for SOC and texture (sand, silt, and clay) predictions, and the model prediction accuracy, based on RMSE, was investigated. The model accuracy improved when all data sources (PSS, RS, and auxiliary data) were used as covariates compared to models using covariates from a single data source (Tab. S4.1, S4.2). When RS data were used as a covariate for SOC and clay predictions, improvements in RMSE of 9.52% and 7.95% were observed for SOC and clay, respectively. However, RS data did not affect the predictions of sand and silt. Regarding auxiliary data, soil type and textural class were identified as most important covariates for SOC and soil texture prediction, respectively. Additional QRF model tests were conducted by adding estimated clay from auxiliary data (regional and LUCAS) as a covariate for SOC prediction. Running the QRF model with and without clay as a covariate for SOC

prediction did not change the RMSE, which was 0.19% and 0.23% for regional and LUCAS datasets, respectively. Likewise, using the estimated BD from the regional and LUCAS datasets as a covariate did not improve the BD prediction. Instead, the observed SOC, sand, silt, and clay were used to build the QRF BD model prediction.

Based on the percentage of variance explained, prediction models using the global dataset were highly effective in explaining the variability of observed data for soil texture components (clay, sand, and silt). The best goodness-of-fit was observed for clay (96.12%), followed by sand (94.93%), silt (80.81%), SOC (68.66%), and BD (41.31%). In the global dataset used for validation, the RMSE of 3.18 % represented approximately 4.80% of the range of clay values (2.93 % to 69.57%), the RMSE of 5.75 % corresponded to approximately 6.60% of the range of sand values (2.36% to 90 %), and the RMSE of 4.39 % related to approximately 7.50% of the range of silt values (4 % to 62.9 %). The relative RMSEs indicate that the clay, sand, and silt models using the global dataset had a low error compared to the variability in the observed clay, sand, and silt values, suggesting good model predictive performance. In the validation dataset, the RMSE of 0.17 % represented about 9.70% of the range of SOC values (0.50 % to 2.22 %), indicating that the model using the global dataset performed well in predicting SOC values within the given range, with relatively small prediction errors in the context of dataset variation. The RMSE of 0.11 g cm⁻³ represented approximately 20.6% of the range of BD values (1.00 g cm⁻³ to 1.54 g cm⁻³) in global validation dataset, suggesting that the model had moderate predictive performance for BD.

SOC concentration and BD maps derived from models using global and local datasets are required to calculate SOC stocks at 10 m resolution at a field scale. Table 5.3 summarizes the metrics for calibration, validation and uncertainties of QRF models using global and local datasets for SOC, soil texture, and BD predictions. The SOC concentration model using the global dataset showed better calibration (explained variance of 68.66 %) and validation (Spearman's correlation of 0.83) performance than the BD model using the global dataset, which had an explained variance of 41.31 % and a Spearman's correlation of 0.62. While model predictions using the global dataset were consistent across fields, predictions using the local dataset differ depending on the field. The metrics of the QRF model predictions (SOC, soil texture and BD) using the local dataset represent the average values of all the fields used in this work (Table 5.3). The RMSE for SOC concentration model calibrations using the local dataset ranged from 0.18 % to 0.30 %, with RMSE for the local validation dataset ranging from 0.10 % to 0.30 %. The explained variance for the SOC model using the local dataset ranged from 6.01 % to 49.48 %, with Spearman's correlation ranging from 0.09 to 0.90 in

model validation. The RMSE for clay calibration using the local dataset ranged from 6.12 % to 8.97 %, with RMSE for model validation varying from 4.25 % to 15.97 %. The explained variance of clay prediction using the local dataset ranged from 78.25 % to 91.59 %, and Spearman's correlation ranged from 0.57 to 1. The RMSE for silt calibration using the local dataset ranged from 6.43 % to 9.38 %, and for silt local validation dataset, RMSE ranged from 5.33 % to 12.27 %. The explained variance for silt ranged from 31.26 % to 81.22 %, with Spearman's correlation ranging from 0.29 to 1. The RMSE for sand calibration using the local dataset ranged from 6.64 % to 13.57 %, with the RMSE for model validation ranging from 2.51 % to 24.94 %. The explained variance for the sand model using the local dataset ranged from 62.48 % to 91 %, and Spearman's correlation of the validation model ranges from 0.40 to 1. Lastly, the RMSE for BD calibration using the local validation dataset ranged from 0.08 g cm⁻³ to 0.11 g cm⁻³, with RMSE for the local validation dataset ranging from 0.15 g cm⁻³ to 0.20 g cm⁻³. The explained variance for the BD model using the local dataset ranged from 10.36 % to 34.99 %, with Spearman's correlation for local validation ranging from 0.14 to 0.63. The SDE of all predictors was higher for models using local dataset compared to the global dataset.

The results indicated a significant improvement in model prediction performance when using the local dataset. For 15 of the 45 fields in which the model using the local dataset was beneficial, the SOC model using the local dataset improved the ME by 52.51% on average compared to the global dataset model. The sand (26 out of 45 fields), silt (21 out of 45 fields), and clay (29 out of 45 fields) models using the local dataset indicated an average ME improvement of 43.13%, 40.87% and 46.54%, respectively, compared to the global model dataset. The BD model (8 of 22 fields) using the local dataset improved the ME by 57.13% on average compared to the global model dataset. It is worth mentioning that a direct comparison of error improvement between target variables was not made, as the number of observations used in model predictions with the local dataset differed. The uncertainties for models using the local dataset, presented in Table 5.3, represent the average value for all fields used in this work. The sand, silt, clay and BD models using the local dataset exhibited higher uncertainty than models using the global dataset. Only the SOC model using the local dataset improved the uncertainty (reducing the PIW) by 8.02 % on average compared to the model using the global dataset. The PIW of the SOC model using the local dataset ranged from 0.38 % to 0.62 %, while the PICP ranged from 0.50 to 1. The clay model using the local dataset had a PIW ranging from 17.50 % to 32.87 % with a PICP ranging from 0.60 to 1. The PIW of the silt model using the local dataset ranged from 14.85 % to 23.26 %, and the PIW for the sand model ranged from 18.46 % to

38.91 %. The PICP for silt and sand models using the local dataset ranged from 0.5 to 1. The PIW for clay, silt, sand, and BD predictions using the local datasets was higher than the global models. SOC, clay, and BD models using the global datasets showed higher PICP than those using the local dataset. However, for silt and sand models, opposite was observed. For a 90% PI, the PICP of 0.9 indicated accurate uncertainty assessment. The models (using both global or local datasets) tend to overestimate the uncertainty of their predictions (PICP below 0.9). For example, the PICP for the global clay prediction is 0.89 for a 90% prediction interval, meaning that 1% of the true values are expected to fall outside the interval.

Table 5.3 Summary of the QRF model performance metrics for SOC, clay, silt, sand, and BD predictions using global and local dataset. Note: RMSE= Root Mean Square Error, ME= Mean Error, SDE=Standard Deviation of the Error, PIW= Prediction Interval Width, PICP= Prediction Interval Coverage Probability

	Calibration		ME	Validation		Spearman's Corr	Uncertainty	
	RMSE	% variance explained		RMSE	SDE		PIW	PICP
SOC concentration global (%)	0.2	68.66	0.03	0.17	0.16	0.83	0.53	0.83
SOC concentration local (%)	0.22	24.76	0.01	0.21	0.21	0.43	0.52	0.82
Clay global (%)	3.52	96.12	-0.03	3.18	2.97	0.95	10.34	0.89
Clay local (%)	7.24	85.15	-1.64	8.02	6.19	0.93	24.31	0.82
Silt global (%)	4.93	80.81	0.05	4.39	4.15	0.89	10.50	0.75
Silt local (%)	8.02	60.92	-0.82	9.64	7.77	0.76	20.18	0.78
Sand global (%)	5.16	94.93	0.23	5.75	5.25	0.93	12.09	0.76
Sand local (%)	10.6	81.21	3.44	14.01	11.08	0.90	30.40	0.82
BD global (g cm ⁻³)	0.08	41.31	-0.02	0.11	0.11	0.62	0.16	0.70
BD local (g cm ⁻³)	0.09	22.68	-0.07	0.18	0.13	0.39	0.19	0.60

5.3.1.1 SOC concentration prediction using global and local dataset

The prediction of SOC using the local dataset showed better performance for SOC concentration than the global dataset without precision sampling. The validation global dataset for the SOC model consisted of 174 observations (30 % random data split from a total of 578 observations, Figure 5.6a, b). On the other hand, the validation local dataset for the SOC model consisted of 10 observations (20 % randomly split from a total of 53 observations used to build the local model, Figure 5.6 c, d). It is worth noting that the SOC model using local dataset is represented by a field with similar metrics to those in Table 5.3. The ME value of 0.03 suggests minimal bias in the SOC global model, indicating good accuracy in capturing the central tendency of the SOC values. An RMSE of 0.17 % indicates that the model has relatively small deviations from the observed values on average, suggesting a reasonable fit and good predictive accuracy. The high Spearman correlation (0.83) indicates a strong monotonic relationship between the observed and predicted SOC values. The SOC local model metrics shown minimal bias (ME of 0.01 %), meaning the model is unbiased. Prediction errors are

relatively moderate (RMSE of 0.21 %), suggesting moderate accuracy. For the SOC model using the local dataset, the Spearman correlation averages 0.43 considering the 45 fields used. In Field18 (Piemonte region), the predicted SOC was the worst (Spearman correlation of 0.09), while the best prediction was observed for Field46 (Spearman correlation of 0.90). For model prediction using the global dataset, approximately 16.2% of observations fell outside the predicted range. In contrast, for model prediction using local dataset, 10% of observations fell outside the interval, which is consistent with the interval's intended coverage.

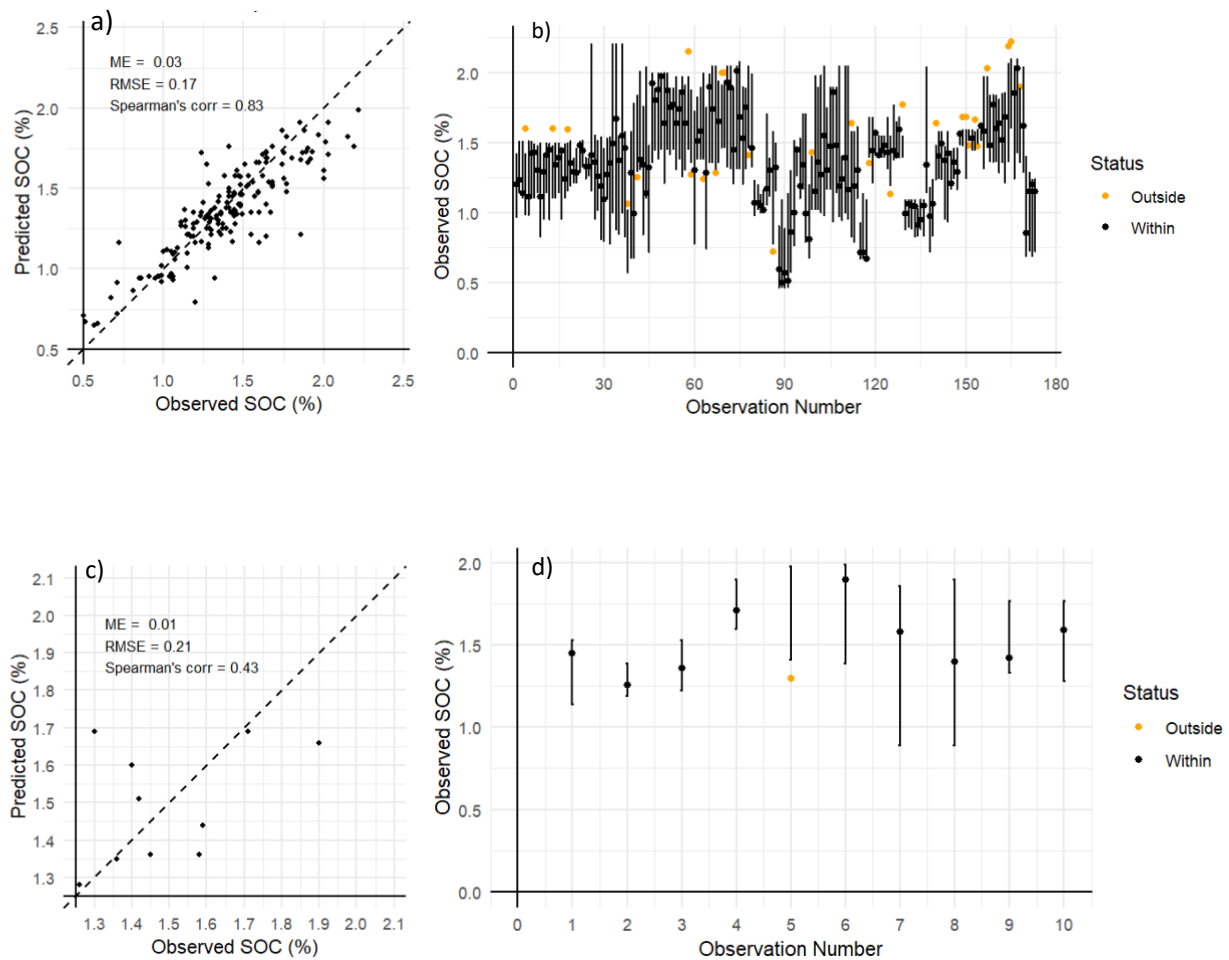


Figure 5.6 SOC QRF model predictions using global and local datasets. Figures 6a and 6c show the observed vs. predicted values for the global (a) and local (c) validation dataset, including the root mean square error (RMSE), model mean error (ME), and Spearman's correlation. Figures 6b and 6d present the observed SOC values with a 90% prediction interval (PI) for the global (b) and local (d) validation dataset; observed values within the 90% PI are shown in black, while those outside the PI are highlighted in orange.

5.3.1.2 Bulk density prediction using global and local dataset

The prediction of BD using the local dataset demonstrated worse performance compared to predictions using the global dataset without precision sampling. Among the fields considered, only two (Field13 and Field18) showed a positive percentage of explained variance, while the remaining

fields exhibited negative percentages. Since a negative percentage of explained variance reflects a poorly performing model for bulk density predictions, the performance metrics of the BD prediction using the local dataset are represented by the average of Field13 and Field18 (Table 5.3). The validation global dataset for the BD model comprised 37 observations (20 % randomly split from a total of 187 observations, Figure 5.7 a and b). In contrast, the local validation dataset of the BD model consisted of 6 observations (20 % randomly split from a total of 32 observations used to build the local model, Figure 5.7 c and d). For the global dataset, the RMSE of 0.11 g cm^{-3} represents a moderate error relative to the mean BD value (1.27 g cm^{-3}). The model also showed a moderate positive Spearman correlation (0.62) between the predicted and observed BD values. The global model's mean error (ME) was -0.02 g cm^{-3} , indicating relatively low bias. In contrast, BD model using local dataset exhibited higher bias, with an ME of -0.07 g cm^{-3} , and an RMSE of 0.18 g cm^{-3} , indicating greater deviations from the observed mean BD value (1.26 g cm^{-3}). The Spearman correlation for the local model was lower, at 0.39, reflecting weaker predictive performance. Furthermore, approximately 29.7% of observations fell outside the predicted range for the global dataset, whereas 66.67% of observations fell outside the predicted range for the local dataset. This indicates that the global model provides a more reliable prediction of BD compared to the local model.

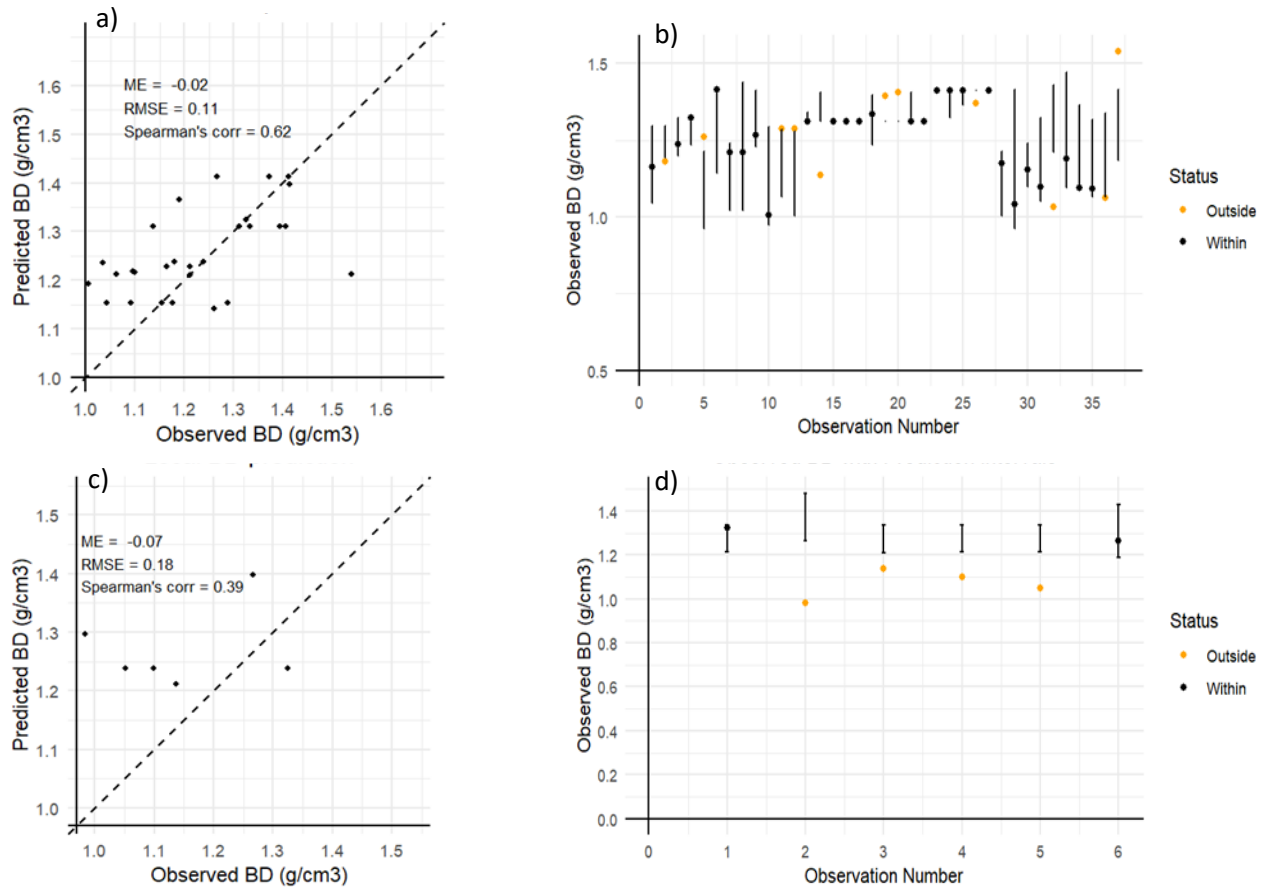


Figure 1 BD QRF model predictions using the global and local datasets. Figures 7a and 7c present the observed vs. predicted values for the global (a) and local (c) validation dataset, including the root mean square error (RMSE), model mean error (ME), and Spearman's correlation. Figures 7b and 7d present the observed BD values with a 90% prediction interval (PI) for the global (b) and local (d) validation dataset; observed values within the 90% PI are shown in black, while those outside the PI are highlighted in orange.

5.3.2 Field-level estimates results of single soil properties.

A summary of the results of the field-level estimates of SOC concentration, soil texture (sand, silt, and clay), and BD using global and local datasets are presented in Tables 5.4, 5.5 and 5.6, respectively. The use of local dataset generally produced narrower underestimations for maximum SOC content at field level and slightly overestimated the average SOC, whereas with the global dataset the SOC field average was underestimated. Overall, the local predictions performed better in capturing the SOC range compared to the global model. The SOC field level estimates using the global dataset ranged from 0.64% to 1.75%, with an average of 1.26%, while the observed SOC ranged from 0.54% to 2.00%, with an average of 1.27% (Tab. 5.4). The application of the global dataset overestimated the minimum SOC by 0.10%, underestimated the maximum SOC by 0.25%, and underestimated the average SOC by 0.01%. In contrast, SOC field level estimates using the local dataset ranged from 0.77% to 1.82%, with an average of 1.30%. The minimum SOC prediction using the local dataset was overestimated by 0.23%, like the global dataset. The maximum SOC prediction

was also underestimated, but with a smaller magnitude (0.18%) compared to the global dataset. Unlike the global dataset, the average SOC prediction using the local dataset was overestimated by 0.03%.

BD field level estimates using both datasets showed overestimation for the minimum BD values, with the local dataset exhibiting a larger bias. Maximum BD predictions were underestimated in both cases, and average BD predictions were slightly overestimated, with nearly identical magnitudes. Measured BD values ranged from 0.95 g cm^{-3} to 1.44 g cm^{-3} , with an average of 1.21 g cm^{-3} (Tab. 5.5). Field-level estimates using the global dataset ranged from 1.15 g cm^{-3} to 1.42 g cm^{-3} , with an average of 1.25 g cm^{-3} . The minimum and average BD predictions were overestimated by 0.21 g cm^{-3} and 0.05 g cm^{-3} , respectively, while the maximum prediction was underestimated by 0.02 g cm^{-3} . Field-level estimates using the local dataset showed similar trends, with the minimum BD overestimated by 0.25 g cm^{-3} , a higher bias than the global dataset. The maximum BD prediction was underestimated by 0.02 g cm^{-3} , while the average BD prediction of 1.26 g cm^{-3} showed an overestimation of 0.051 g cm^{-3} , like the global dataset.

Sand estimates at field level using global and local datasets were underestimated for the maximum sand content, with the local dataset showing a larger bias. The use of the global dataset overestimated the average sand content, while with the local dataset it was underestimated. Minimum sand predictions were consistent between the two datasets. Observed sand values ranged from 3.25% to 88%, with an average of 23.45% (Tab 5.6). Field level estimates using the global dataset ranged from 2.75% to 87.82%, with an average of 24.17%. While the minimum and maximum predictions were underestimated by 0.50% and 0.28%, respectively, the average was overestimated by 0.71%. Field level estimates using the local dataset showed a minimum sand value consistent with the global dataset (2.75%). However, the maximum sand prediction (85.85%) was underestimated by 2.15%, a higher bias than the global dataset. The average sand prediction using the local dataset (22.51%) was underestimated by 0.94%.

Silt estimates at field level using both datasets overestimated the minimum and average silt values, with higher biases observed in the local dataset. Maximum silt values were underestimated in both cases, with the local dataset showing greater bias. Observed silt values ranged from 5.20% to 60.93%, with an average of 43.61% (Tab. 5.6). Field level estimates using the global dataset were overestimated for the minimum and average silt values by 0.40% and 0.12%, respectively, and underestimated for the maximum by 1.56%. Field level estimates using the local dataset were overestimated for the minimum and average silt values by 0.80% and 0.50%, respectively, which

were larger than the global dataset. The maximum silt prediction (57.56%) was underestimated by 3.37%, a larger bias compared to the global dataset.

Clay estimates at field level using both datasets overestimated the minimum clay content and underestimated the maximum and average values. The local dataset exhibited higher biases for minimum and average clay predictions but a lower bias for the maximum clay prediction compared to the global dataset. Observed clay values ranged from 3.30% to 62.55%, with an average of 32.94% (Tab. 5.6). Field level estimates using the global dataset overestimated the minimum clay by 3.11% and underestimated the maximum and average values by 2.96% and 1.18%, respectively. Field level estimates using the local dataset overestimated the minimum clay by 3.86% and underestimated the maximum and average clay values by 2.57% and 1.85%, respectively. While the minimum and average biases were higher for the local dataset, the maximum clay prediction bias was lower than that of the global dataset.

Table 5.4 Results of QRF model predictions of the SOC concentration (%) using global and local datasets for each field. Next to the SOC predicted value is the mean error (ME).

Field	SOC (%) measured	SOC (%) predicted			
		Global dataset	ME	Local dataset	ME
Field1	1.23	1.18	0.04	1.18	0.04
Field2	1.34	1.42	-0.09	1.39	-0.05
Field3	1.31	1.31	0.01	1.48	-0.16
Field4	1.32	1.33	-0.002	1.31	0.01
Field5	1.28	1.29	-0.01	1.34	-0.06
Field6	1.44	1.32	0.12	1.32	0.12
Field7	1.30	1.34	-0.05	1.33	-0.03
Field8	1.09	1.14	-0.05	1.16	-0.06
Field9	1.34	1.25	0.09	1.33	0.01
Field11	1.07	0.95	0.12	1.10	-0.03
Field13	1.39	1.39	0.00	1.40	-0.02
Field14	1.72	1.73	-0.01	1.76	-0.04
Field15	1.33	1.31	0.02	1.38	-0.04
Field16	1.92	1.72	0.20	1.82	0.10
Field17	1.14	1.27	-0.13	1.24	-0.10
Field18	1.76	1.72	0.04	1.49	0.27
Field20a	1.07	0.99	0.07	1.14	-0.08
Field20b	0.84	0.89	-0.05	1.01	-0.17
Field21a	0.71	0.93	-0.22	0.92	-0.22
Field21b	0.54	0.64	-0.10	0.77	-0.23
Field22a	1.13	1.28	-0.14	1.28	-0.14
Field22b	1.23	1.29	-0.06	1.30	-0.07
Field23	0.82	1.09	-0.27	1.05	-0.23
Field24	1.42	1.65	-0.23	1.46	-0.04
Field25	1.37	1.49	-0.12	1.37	0.00
Field26	1.30	1.28	0.02	1.30	0.00
Field30	0.82	0.88	-0.06	0.88	-0.06
Field32	1.50	1.40	0.10	1.46	0.04
Field33	1.31	1.22	0.09	1.35	-0.05
Field34	1.55	1.35	0.20	1.48	0.07

Field36	1.23	1.22	0.01	1.21	0.02
Field37	1.33	1.23	0.10	1.23	0.10
Field38	0.83	0.83	0.00	0.85	-0.02
Field39	0.73	0.84	-0.10	0.89	-0.16
Field40	0.95	0.88	0.07	1.04	-0.09
Field42a	1.41	1.22	0.19	1.37	0.04
Field42b	1.33	1.29	0.04	1.38	-0.05
Field43a	1.40	1.42	-0.02	1.49	-0.09
Field43b	1.47	1.45	0.02	1.50	-0.03
Field44a	1.57	1.53	0.04	1.54	0.03
Field44b	1.54	1.54	0.00	1.53	0.01
Field45a	1.70	1.66	0.04	1.63	0.07
Field46b	2.00	1.75	0.25	1.71	0.29
Field47a	0.97	0.90	0.07	1.01	-0.04
Field47b	1.08	1.04	0.04	1.12	-0.04

Table 5.5 Results of QRF model predictions of the BD (g cm^{-3}) using global and local datasets for each field. Next to the BD predicted value is the mean error (ME).

Field	BD (g cm^{-3}) measured	BD predicted (g cm^{-3})			
		Global dataset	ME	Local dataset	ME
Field1	-	1.15	-	1.24	-
Field2	-	1.14	-	1.28	-
Field3	-	1.21	-	1.28	-
Field4	1.32	1.24	0.09	1.24	0.08
Field5	1.20	1.24	-0.04	1.27	-0.07
Field6	1.24	1.24	0.00	1.27	-0.03
Field7	1.23	1.23	0.00	1.25	-0.02
Field8	1.26	1.23	0.03	1.25	0.01
Field9	1.41	1.23	0.18	1.29	0.13
Field11	1.40	1.41	-0.02	1.30	0.10
Field13	1.27	1.34	-0.08	1.35	-0.08
Field14	1.19	1.19	0.00	1.19	0.00
Field15	1.29	1.15	0.13	1.26	0.03
Field16	1.29	1.19	0.10	1.28	0.01
Field17	1.15	1.16	-0.01	1.23	-0.08
Field18	1.31	1.27	0.04	1.29	0.02
Field20a	0.96	1.23	-0.27	1.23	-0.27
Field20b	1.07	1.21	-0.15	1.21	-0.15
Field21a	1.09	1.41	-0.31	1.23	-0.14
Field21b	1.22	1.41	-0.19	1.23	-0.02
Field22a	1.10	1.22	-0.12	1.24	-0.14
Field22b	1.10	1.20	-0.10	1.21	-0.11
Field23	-	1.23	-	1.23	-
Field24	1.10	1.19	-0.09	1.25	-0.15
Field25	0.95	1.17	-0.22	1.25	-0.30
Field26	-	1.19	-	1.24	-
Field30	-	1.41	-	1.27	-
Field32	-	1.39	-	1.30	-
Field33	-	1.31	-	1.28	-
Field34	-	1.34	-	1.29	-
Field36	-	1.41	-	1.28	-
Field37	-	1.22	-	1.22	-
Field38	-	1.35	-	1.27	-
Field39	-	1.34	-	1.34	-

Field40	1.44	1.42	0.02	1.42	0.02
Field42a	-	1.21	-	1.28	-
Field42b	-	1.21	-	1.28	-
Field43a	-	1.22	-	1.31	-
Field43b	-	1.22	-	1.29	-
Field44a	-	1.24	-	1.24	-
Field44b	-	1.24	-	1.24	-
Field45a	-	1.20	-	1.26	-
Field46b	-	1.17	-	1.29	-
Field47a	-	1.39	-	1.39	-
Field47b	-	1.39	-	1.39	-

Table 5.6 Results of QRF model predictions of the sand (%), silt (%) and clay (%) using global and local datasets for each field. Next to the sand, silt and clay predicted value is the mean error (ME).

Field	Sand (%) measured	Sand predicted (%)				Silt (%) measured	Silt predicted (%)				Clay (%) measured	Clay predicted (%)			
		Global dataset	ME	Local dataset	ME		Global dataset	ME	Local dataset	ME		Global dataset	ME	Local dataset	ME
Field1	17.00	13.91	3.09	14.21	2.79	60.93	53.64	7.28	55.29	5.64	22.08	34.96	-5.87	27.95	-12.89
Field2	19.13	11.59	7.54	16.43	2.70	59.08	53.71	5.36	53.57	5.50	22.03	34.63	-8.87	30.90	-12.60
Field3	13.34	16.91	-3.57	12.37	0.97	54.38	57.85	-3.48	57.56	-3.18	32.28	26.06	7.05	25.23	6.22
Field4	3.40	2.75	0.65	2.75	0.65	34.05	43.87	-9.82	44.29	-10.24	62.55	53.74	3.31	59.24	8.81
Field5	3.25	2.83	0.42	2.95	0.30	36.70	43.46	-6.75	38.71	-2.01	60.05	53.71	1.71	58.34	6.34
Field6	3.40	3.33	0.07	3.00	0.40	42.71	44.31	-1.59	45.89	-3.18	53.89	51.77	2.91	50.98	2.12
Field7	25.14	20.16	4.98	12.95	12.20	42.76	37.91	4.85	45.21	-2.45	32.10	35.00	-1.98	34.07	-2.91
Field8	14.83	22.36	-7.52	10.27	4.57	50.81	42.14	8.67	45.70	5.11	34.36	31.79	1.64	32.71	2.57
Field9	7.17	17.11	-9.94	8.89	-1.73	55.14	40.79	14.36	47.14	8.00	37.69	36.86	1.74	35.95	0.83
Field11	41.21	48.50	-7.29	38.23	2.99	42.71	41.36	1.36	45.70	-2.98	16.07	16.50	1.45	14.62	-0.43
Field13	61.61	74.71	-13.10	65.81	-4.20	25.82	14.79	11.04	25.74	0.08	12.57	9.50	0.00	12.57	3.07
Field14	8.86	12.92	-4.06	11.69	-2.83	47.43	51.50	-4.07	53.55	-6.12	43.71	37.58	9.41	34.29	6.13
Field15	15.71	11.91	3.80	11.57	4.14	38.93	52.63	-13.70	51.79	-12.86	45.36	35.95	14.64	30.71	9.41
Field16	13.04	11.89	1.14	12.50	0.54	46.79	52.27	-5.48	49.52	-2.73	40.18	37.29	2.86	37.32	2.89
Field17	10.71	12.10	-1.39	13.53	-2.83	52.34	53.62	-1.28	52.38	-0.04	36.96	36.26	0.04	36.91	0.69
Field18	52.58	20.93	31.64	19.17	33.40	39.88	53.10	-13.22	53.29	-13.41	7.54	9.77	0.38	7.16	-2.23
Field20a	14.77	31.21	-16.45	17.50	-2.73	55.27	41.65	13.62	44.79	10.48	29.96	26.93	1.82	28.14	3.04
Field20b	12.73	15.29	-2.55	17.89	-5.16	53.82	56.16	-2.34	53.43	0.39	33.45	26.96	11.75	21.70	6.49
Field21a	31.15	45.58	-14.43	38.74	-7.59	49.76	41.80	7.96	43.61	6.15	19.08	14.67	2.53	16.54	4.41
Field21b	32.60	46.84	-14.24	34.21	-1.62	51.36	41.68	9.68	41.83	9.52	16.05	17.61	-2.72	18.77	-1.56
Field22a	12.50	4.99	7.51	7.09	5.41	41.63	34.09	7.54	33.89	7.74	45.87	56.89	-6.33	52.20	-11.01
Field22b	10.20	7.18	3.02	8.43	1.77	44.71	35.25	9.46	35.09	9.62	45.12	57.27	-8.92	54.04	-12.15
Field23	4.64	21.05	-16.41	15.76	-11.12	55.54	39.80	15.73	46.18	9.36	39.82	34.89	-0.27	40.09	4.93
Field24	9.64	12.14	-2.50	10.27	-0.62	49.11	51.18	-2.07	53.57	-4.46	41.25	37.11	6.73	34.52	4.14
Field25	18.60	12.73	5.86	13.71	4.88	46.31	51.36	-5.05	52.61	-6.30	35.10	36.39	0.40	34.70	-1.30
Field26	7.39	7.45	-0.06	5.44	1.95	42.95	43.64	-0.69	43.76	-0.81	49.65	49.23	0.70	48.95	0.43
Field30	60.98	48.50	12.48	54.17	6.80	33.05	36.65	-3.60	38.02	-4.97	5.98	11.06	-1.22	7.20	-5.08
Field32	46.20	50.60	-4.40	49.30	-3.10	50.50	39.65	10.85	40.70	9.80	3.30	9.26	-5.60	8.90	-5.96
Field33	46.40	33.99	12.41	39.37	7.03	44.93	59.36	-14.44	54.93	-10.00	8.68	9.28	-0.41	9.08	-0.60
Field34	53.53	52.30	1.23	52.30	1.23	37.88	38.57	-0.70	38.50	-0.63	8.53	8.90	-1.46	9.98	-0.37
Field36	49.94	51.60	-1.66	51.60	-1.66	41.50	39.62	1.88	40.70	0.80	8.58	9.19	-0.74	9.32	-0.61
Field37	8.49	4.78	3.70	8.14	0.34	51.57	50.10	1.47	47.14	4.43	39.94	44.70	-5.89	45.84	-4.76
Field38	48.77	42.62	6.15	55.42	-6.65	32.85	36.91	-4.06	20.42	12.43	18.38	19.49	2.92	15.47	-1.10
Field39	48.23	40.53	7.71	34.64	13.59	30.94	38.86	-7.92	41.87	-10.93	20.82	22.03	-1.09	21.91	-1.21
Field40	88.00	87.72	0.28	85.85	2.15	5.20	5.60	-0.40	6.00	-0.80	6.80	6.41	-0.36	7.16	0.39
Field42a	10.73	16.34	-5.61	16.00	-5.27	46.21	52.61	-6.40	53.42	-7.20	43.06	26.43	25.66	17.40	16.63
Field42b	9.57	16.28	-6.71	11.91	-2.34	47.34	55.81	-8.47	53.57	-6.23	43.09	27.01	24.31	18.78	16.07
Field43a	8.21	5.60	2.62	5.35	2.86	35.06	34.01	1.05	33.63	1.42	56.73	58.36	-1.58	58.31	-1.63

Field43b	6.50	7.03	-0.53	5.76	0.74	34.07	34.04	0.04	35.05	-0.98	59.43	59.59	-0.55	59.98	-0.16
Field44a	3.69	4.19	-0.50	4.43	-0.74	33.83	42.77	-8.94	47.03	-13.20	62.49	53.86	3.63	58.86	8.63
Field44b	6.57	3.98	2.59	4.37	2.20	33.41	44.62	-11.21	45.58	-12.16	60.01	53.25	2.80	57.22	6.76
Field45a	10.76	11.75	-1.00	10.81	-0.06	46.10	53.80	-7.70	48.89	-2.79	43.14	37.63	3.60	39.54	5.51
Field46b	14.59	13.33	1.26	13.27	1.32	52.37	51.84	0.53	53.63	-1.26	33.04	34.82	-1.04	34.08	-1.78
Field47a	39.13	44.51	-5.38	43.74	-4.61	39.74	39.59	0.15	35.27	4.47	21.13	19.25	0.68	20.45	1.88
Field47b	30.54	43.47	-12.93	41.29	-10.74	44.93	39.70	5.22	36.63	8.30	24.53	19.56	3.68	20.84	4.97

5.3.3 Field level SOC stock estimates using QRF applied to global and local dataset

SOC stocks were calculated using SOC concentration and BD predictions derived from the QRF model. These stocks were computed at a 30 cm soil depth at the field scale with a resolution of 10 m. SOC stock values estimated using predicted values from the global dataset across 22 fields ranged from 28.75 t ha⁻¹ to 65.36 t ha⁻¹, with an average of 46.82 t ha⁻¹. Similarly, SOC stocks calculated using the local dataset ranged from 28.66 t ha⁻¹ to 70.10 t ha⁻¹, with an average of 48.55 t ha⁻¹. The minimum SOC stock estimates derived from the global and local datasets differed by only 0.09 t ha⁻¹, whereas the maximum SOC stock estimated by the local dataset was 4.74 t ha⁻¹ higher than that of the global dataset.

SOC stock estimates using the global dataset had a 90% prediction interval (PI) ranging from 29.37 t ha⁻¹ (lower bound) to 72.31 t ha⁻¹ (upper bound), which captured the observed average SOC stock value of 45.33 t ha⁻¹. However, the wide prediction interval width (PIW) of 42.94 t ha⁻¹ indicated high uncertainty in the SOC stock estimates. The prediction interval coverage probability (PICP) of 1 suggested that the uncertainty was slightly overestimated.

SOC stock estimates using the local dataset for the 22 fields had a 90% PI ranging from 33.53 t ha⁻¹ to 75.06 t ha⁻¹, representing the range in which SOC stocks were expected to fall with 90% confidence. The observed average SOC stock of 45.33 t ha⁻¹ fell within this range, but the average PIW of 41.53 t ha⁻¹ still indicate high uncertainty. A PICP of 0.95 suggested that the local model slightly underestimated the uncertainty in SOC stock predictions. Figure 5.8 illustrates the SOC stock estimates derived from the global and local datasets compared to the observed values.

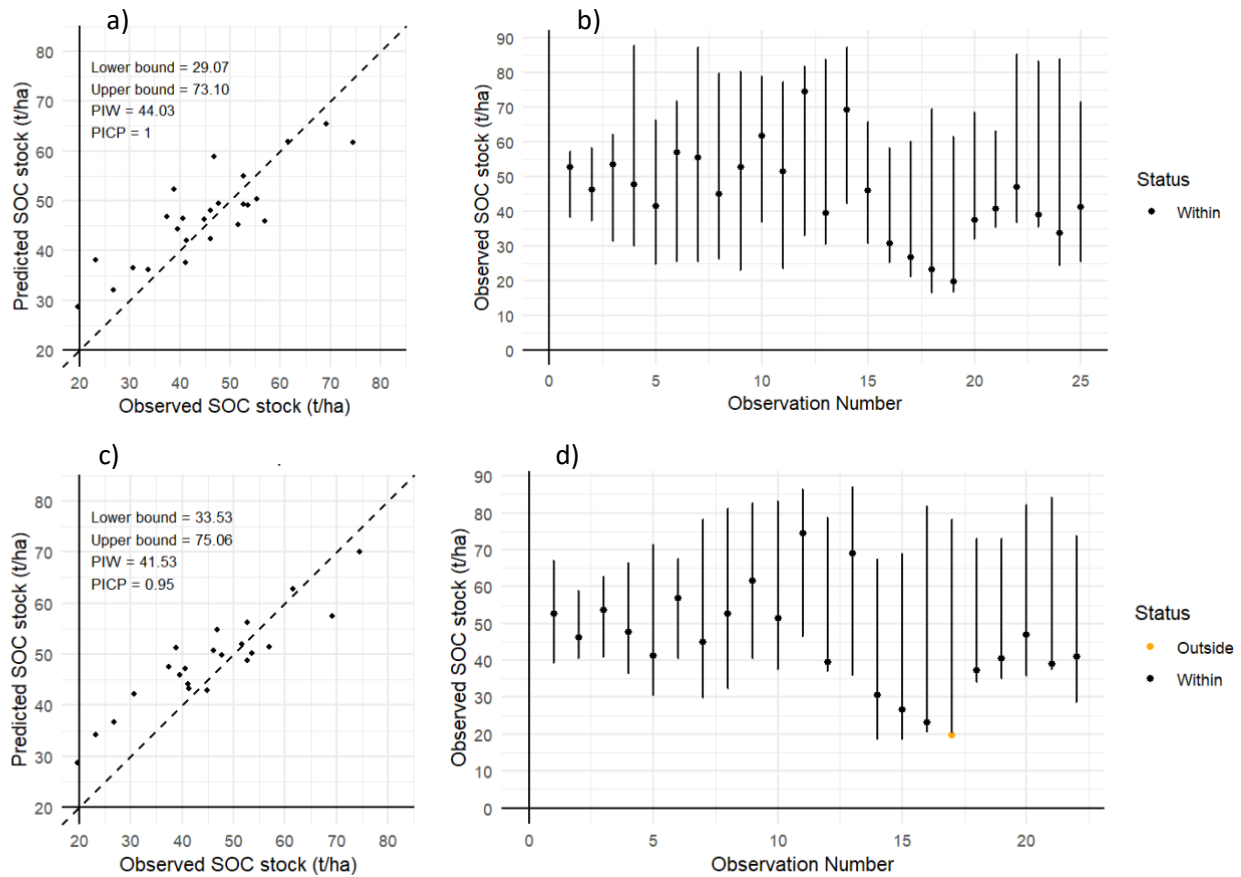


Figure 5.8 Scatter plots of observed versus predicted SOC stocks using the global (8a) and local (8c) model dataset. The lower and upper bounds represent uncertainties quantified by the QRF model using the 5th and 95th quantiles. PIW refers to the prediction interval width, and PICP represent the prediction interval coverage probability. Fig 8b and 8d illustrate observed SOC stock values with a 90% prediction interval (PI) for the global (b) and local (d) model dataset, where values within the 90% PI are shown in black, and those outside the PI are shown in orange.

5.3.3.1 SOC stock error propagation

The uncertainty in SOC stock estimation was measured using the standard deviation (SD). Table 5.7 presents the field-level average SOC stock and the corresponding SD for 22 fields, calculated using both global and local datasets. Error propagation (SD) for the SOC stock estimate was determined by considering the SD of SOC concentration and BD, from which SOC stock was derived. The average SOC stock estimated using the global dataset was 46.82 t ha^{-1} with an SD of 10.26 t ha^{-1} , indicating a relative error of 22.90% within the model predictions. Similarly, the SOC stock estimated using the local dataset had an average of 48.55 t ha^{-1} with an SD of 8.64 t ha^{-1} , corresponding to a relative error of 19.25% within the model predictions.

Table 5.7 Results of QRF model predictions of the SOC concentration (%) and BD (g cm^{-3}) using global and local datasets for 22 fields. Next to the SOC and BD predicted value is the standard deviation (SD). On the right site, the SOC stock estimated derived from SOC concentration and BD using global and local datasets are presented with their corresponding SD.

Field	SOC (%)	SOC (%) predicted				BD (g cm^{-3})	BD predicted (g cm^{-3})				SOC Stock	SOC stock (t ha^{-1}) estimated			
	measured					measured					Measured (t ha^{-1})				
		Global	SD	Local	SD		Global	SD	Local	SD		Global	SD	Local	SD
Field4	1.35	1.33	0.05	1.30	0.11	1.32	1.24	0.06	1.24	0.08	53.49	49.22	3.07	48.17	4.91
Field5	1.2	1.23	0.09	1.33	0.08	1.20	1.24	0.07	1.25	0.08	43.17	45.72	4.34	45.82	3.90
Field6	1.33	1.32	0.17	1.31	0.10	1.24	1.24	0.07	1.28	0.05	51.51	49.22	7.03	50.22	4.39
Field7	1.35	1.34	0.32	1.35	0.15	1.23	1.23	0.11	1.28	0.08	50.14	49.77	12.66	51.83	6.75
Feld8	1.25	1.14	0.20	1.25	0.12	1.26	1.23	0.11	1.26	0.07	47.26	46.08	9.06	47.26	5.46
Field9	1.23	1.25	0.31	1.35	0.10	1.41	1.23	0.08	1.28	0.09	57.26	45.35	11.64	51.83	5.35
Field11	1.11	1.09	0.36	1.15	0.23	1.40	1.42	0.07	1.32	0.08	46.15	46.37	15.63	45.86	9.64
Field13	0.99	1.02	0.43	1.03	0.35	1.27	1.42	0.10	1.40	0.10	37.61	43.39	18.51	43.17	15.01
Field14	1.72	1.73	0.21	1.77	0.16	1.19	1.20	0.09	1.19	0.10	61.43	62.23	8.93	63.27	7.93
Field15	1.41	1.27	0.32	1.35	0.21	1.29	1.15	0.10	1.26	0.09	53.80	43.98	11.82	50.95	8.81
Field16	1.98	1.55	0.31	1.82	0.22	1.29	1.19	0.10	1.28	0.06	76.70	55.47	12.08	70.10	9.05
Field17	1.12	1.26	0.34	1.24	0.24	1.15	1.16	0.10	1.23	0.08	38.52	43.85	12.47	46.52	9.33
Field19	1.83	1.77	0.20	1.51	0.24	1.31	1.26	0.08	1.29	0.09	71.95	66.28	8.79	58.27	10.29
Field20a	1.13	1.03	0.19	1.12	0.21	0.96	1.23	0.09	1.23	0.12	31.59	38.10	7.65	41.41	8.61
Field20b	0.88	0.9	0.27	0.98	0.30	1.07	1.21	0.09	1.21	0.10	27.97	32.69	10.25	35.62	11.40
Field21a	0.64	0.59	0.28	0.99	0.30	1.22	1.41	0.09	1.24	0.08	23.39	39.40	18.68	37.00	11.36
Field21b	0.65	0.95	0.31	0.67	0.32	1.09	1.40	0.10	1.21	0.08	21.35	28.39	9.44	24.37	11.88
Field22a	1.06	1.33	0.20	1.31	0.20	1.10	1.23	0.05	1.20	0.10	35.45	48.90	7.76	47.13	8.13
Field22b	1.35	1.37	0.13	1.38	0.20	1.13	1.20	0.04	1.18	0.09	46.00	49.30	5.09	48.62	7.86
Field24	1.42	1.65	0.23	1.46	0.20	1.10	1.19	0.09	1.25	0.12	46.87	58.95	9.29	54.72	9.10
Field25	1.37	1.49	0.29	1.37	0.25	0.95	1.17	0.10	1.25	0.10	38.97	52.30	10.92	51.27	10.29
Field40	0.78	0.91	0.24	1.12	0.24	1.44	1.42	0.08	1.42	0.10	33.38	38.69	10.61	47.56	10.67

5.3.4 Independent field-level validation of estimates for single properties

An independent validation of the estimates for SOC concentration, sand, silt, clay, and BD predictions was conducted in two new fields (Field48 and Field49) to evaluate the performance of the QRF models under previously unseen conditions using global and local datasets. Figures 5.9 and 10 illustrate the SOC predictions for these fields.

For Field48, the observed SOC field average was 1.21%. The SOC prediction based on the global dataset had a field average of 1.30% with an RMSE of 0.30%, while the local dataset yielded a field average of 1.22% with an RMSE of 0.29%. The 90% confidence interval for SOC predictions in Field48, based on the global dataset, ranged on average from 0.95% to 1.98% (Figures 5.9a and 5.9c). In

contrast, the local dataset produced narrower lower and upper bounds, averaging from 0.44% to 0.61% (Figures 5.9a and 5.9c).

For Field49, the observed SOC field average was 1.34%. The global dataset predicted an average SOC of 1.28% with an RMSE of 0.10%, while the local dataset provided a slightly higher average SOC prediction of 1.32% with an RMSE of 0.08%. The global dataset's 90% confidence interval for SOC predictions spanned from 0.91% to 1.96% on average (Figures 5.9e and 5.9g), whereas the local dataset's predictions fell within a narrower range, averaging from 1.08% to 1.71% (Figures 5.9e and 5.9g).

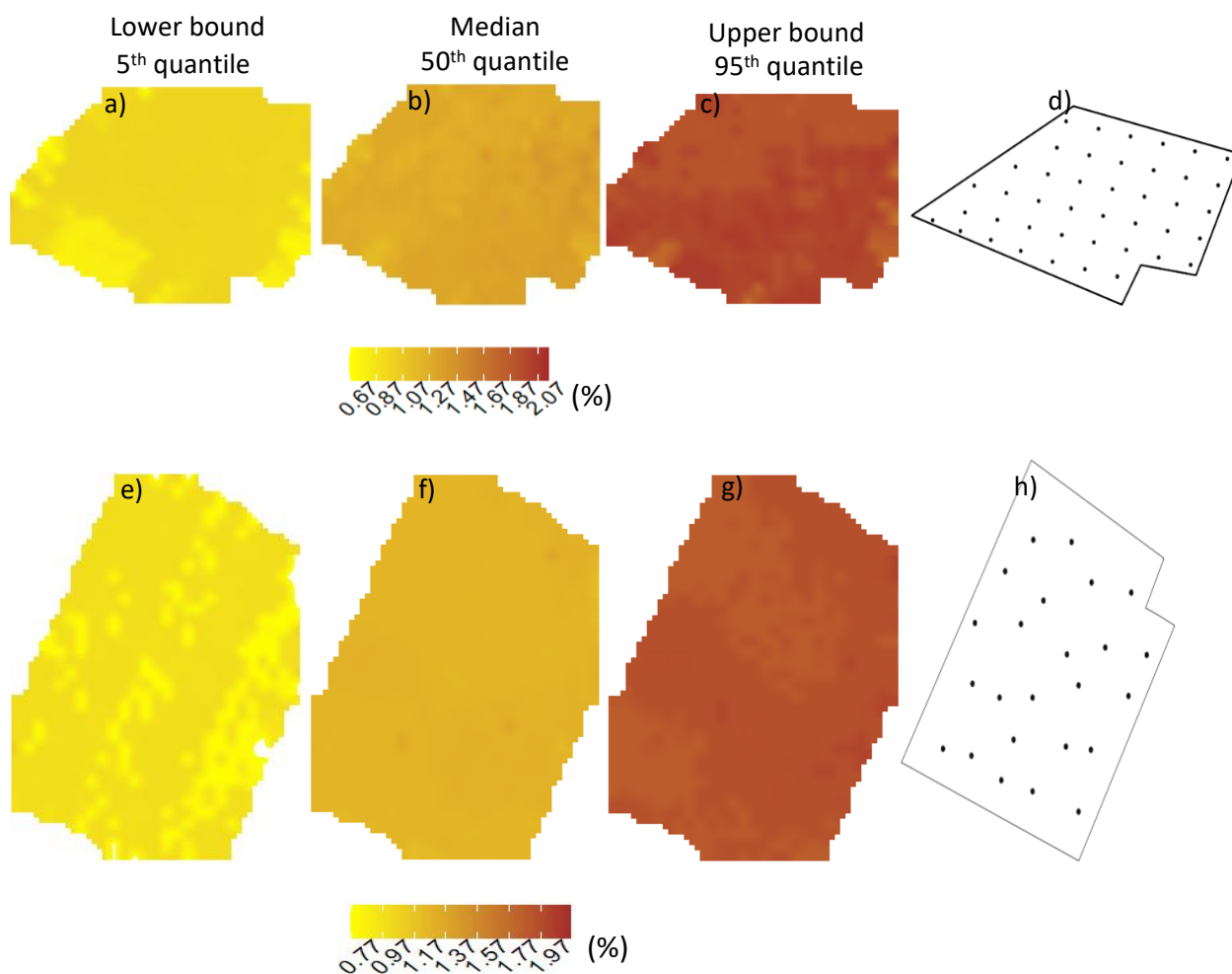


Figure 5.9 Independent SOC concentration (5) predictions using the global dataset at 10 m resolution. The lower bound (5th quantile) is shown in panels (a) and (e), the median (50th quantile) in panels (b) and (f), and the upper bound (95th quantile) in panels (c) and (g) for Field48 and Field49, respectively. The regular sampling grid used for the independent validation of estimates is displayed in panels (d) and (h).

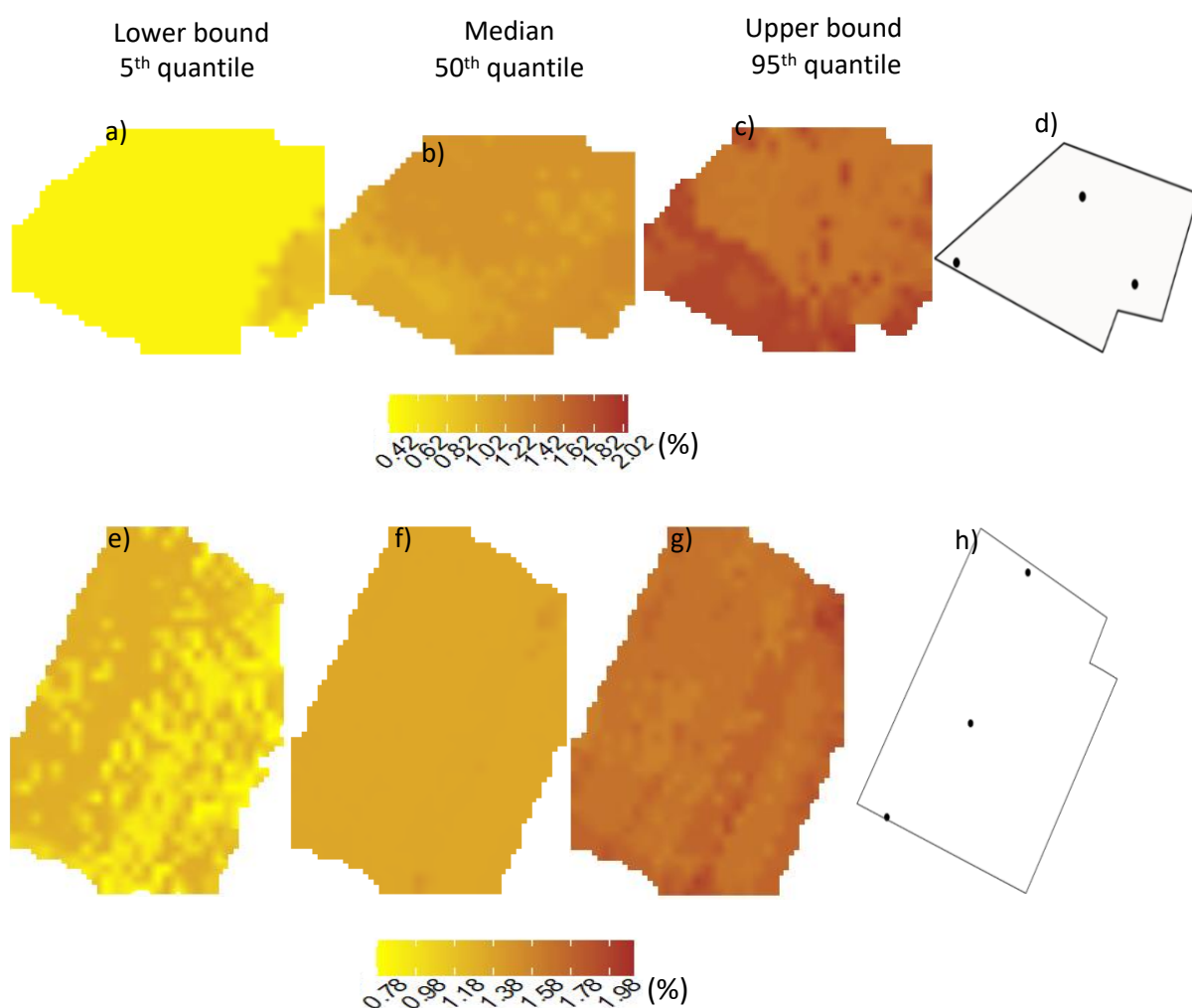


Figure 5.10 Independent SOC concentration (%) predictions using the local dataset at 10 m resolution. The lower bound (5th quantile) is shown in panels (a) and (d), the median (50th quantile) in panels (b) and (e), and the upper bound (95th quantile) in panels (c) and (f) for Field48 and Field49, respectively. Panels (d) and (h) display the 3-point sample locations derived from precision sampling for Field48 and Field49.

Figures 5.11 and 5.12 show the BD predictions for the two fields (Field48 and Field49) based on the global and local datasets. For both fields, observed BD data were unavailable. The average BD prediction for Field48 is estimated at 1.19 g cm^{-3} using the global dataset and 1.22 g cm^{-3} using the local dataset. In Figures 5.11a and 5.11c, the lower and upper bounds of BD prediction for Field48 using the global dataset range from 1.01 g cm^{-3} to 1.36 g cm^{-3} for a 90% confidence interval. For the same field, the lower and upper bounds of BD prediction using the local dataset range from 1.10 g cm^{-3} to 1.35 g cm^{-3} (Figures 5.12a and 5.12c).

For Field49, the average BD predictions using the global and local datasets are 1.22 g cm^{-3} and 1.32 g cm^{-3} , respectively. The lower and upper bounds for BD prediction using the global dataset (Figures 11d and 11f) range from 1.09 g cm^{-3} to 1.37 g cm^{-3} , while the local dataset estimates the bounds from 1.10 g cm^{-3} to 1.40 g cm^{-3} (Figures 5.12d and 5.12f).

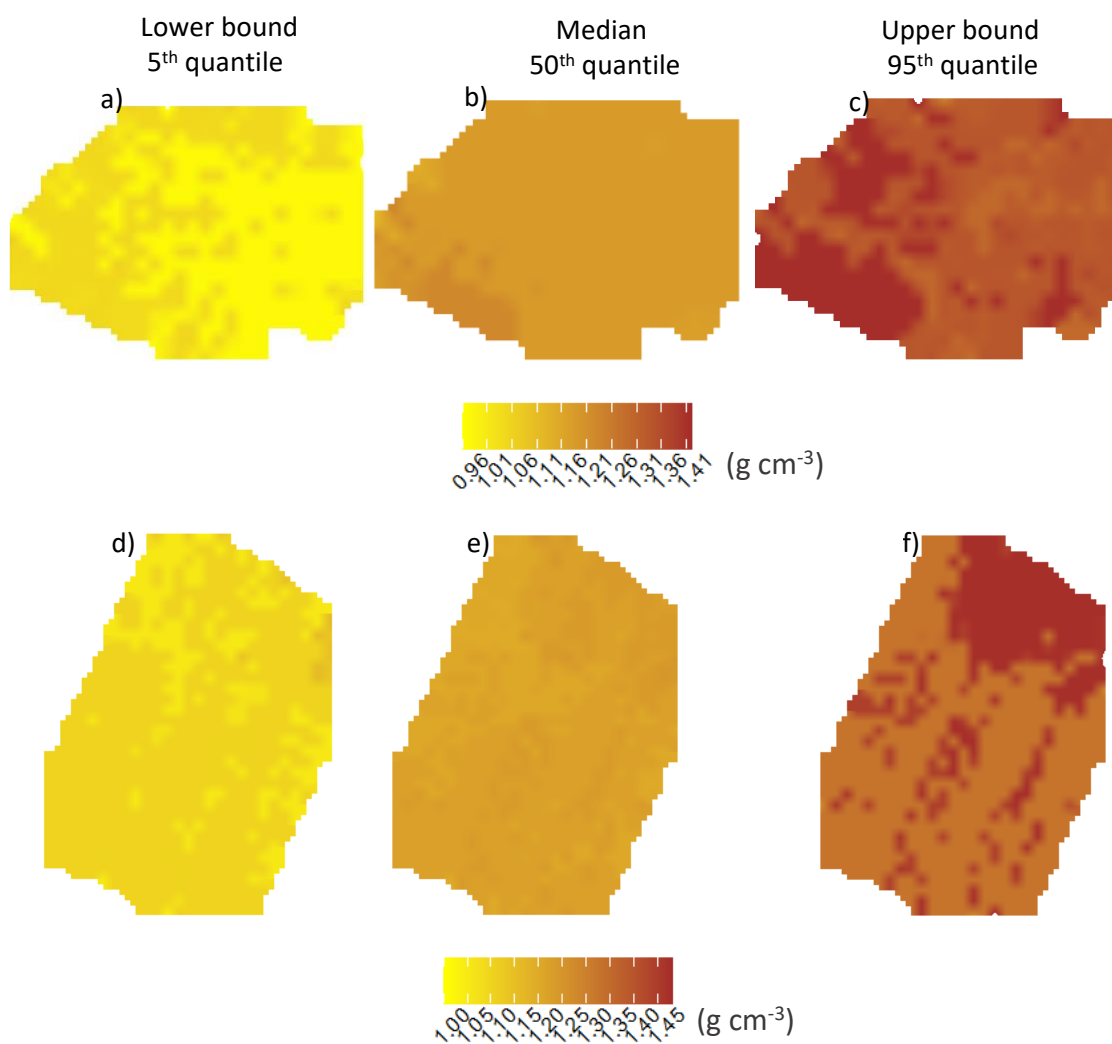


Figure 5.11 Independent BD global predictions (g cm^{-3}) at 10 m resolution, showing the lower bound as 5th quantile (a, d), median as 50th quantile (b, e) and upper bound as 95th quantile (c, f) for Field48 and Field49.

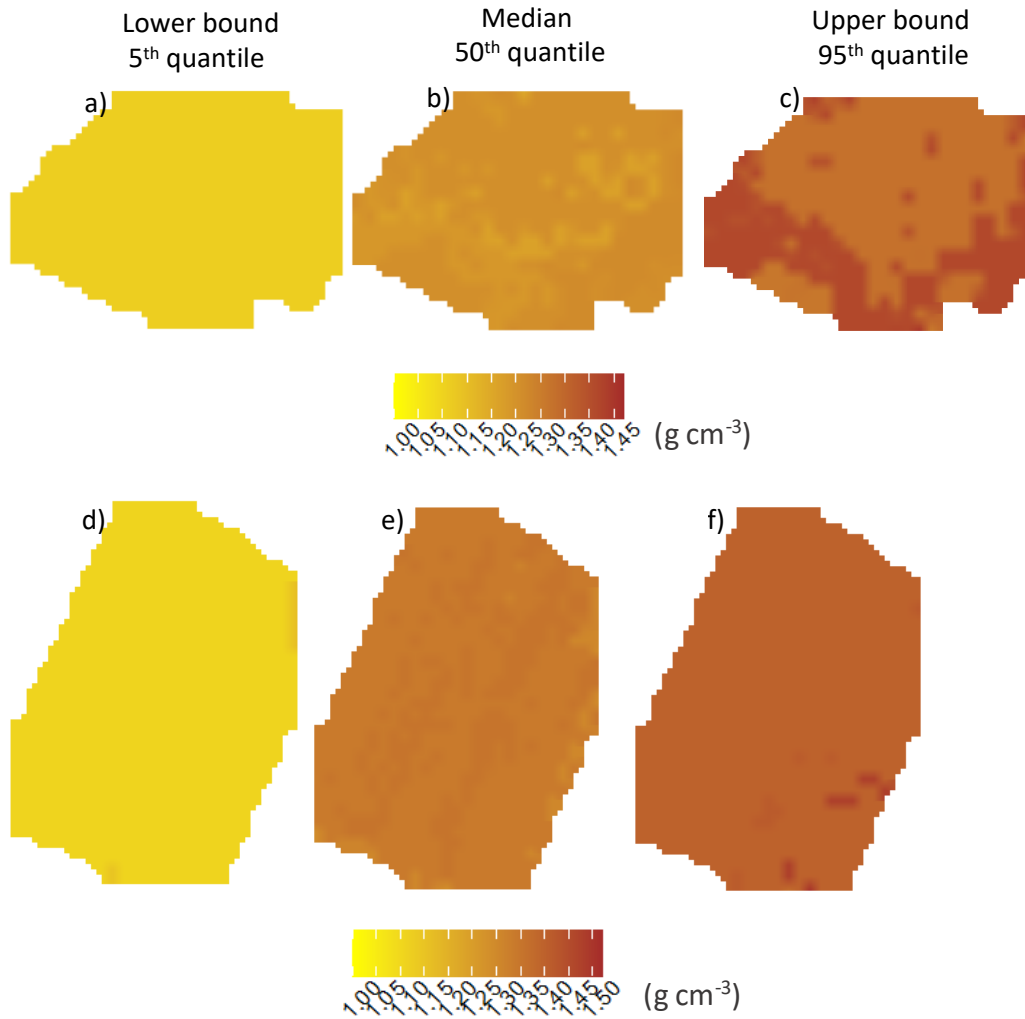


Figure 5.12 Independent BD local prediction (g cm^{-3}) at 10 m resolution, showing the lower bound as 5th quantile (a, d), median as 50th quantile (b, e) and upper bound as 95th quantile (c, f) for Field48 and Field49.

5.3.4.1 SOC stock independent validation

The SOC stock was calculated using the SOC and BD predictions from the global and local datasets at the field scale, with a 10 m resolution, for independent fields. Figures 5.13 and 5.14 show the SOC stock field distribution calculated by using the global and local model dataset respectively.

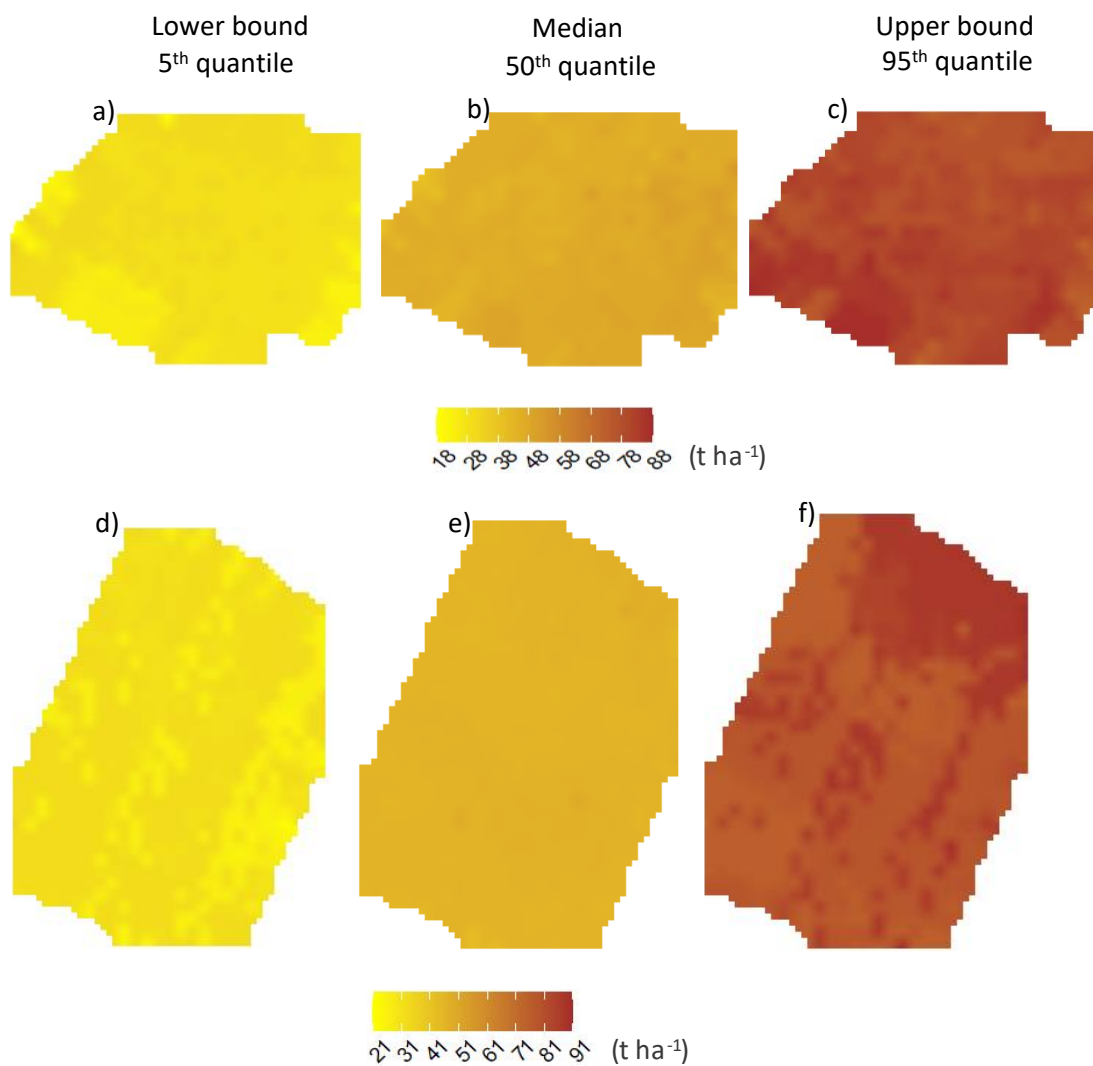


Figure 5.13 SOC stock calculations (t ha^{-1}) at a 30 cm soil depth and 10 m resolution using the global SOC and BD prediction, showing the lower bound as 5th quantile (a, d), median as 50th quantile (b, e) and upper bound as 95th quantile (c, f) for Field 48 and Field 49.

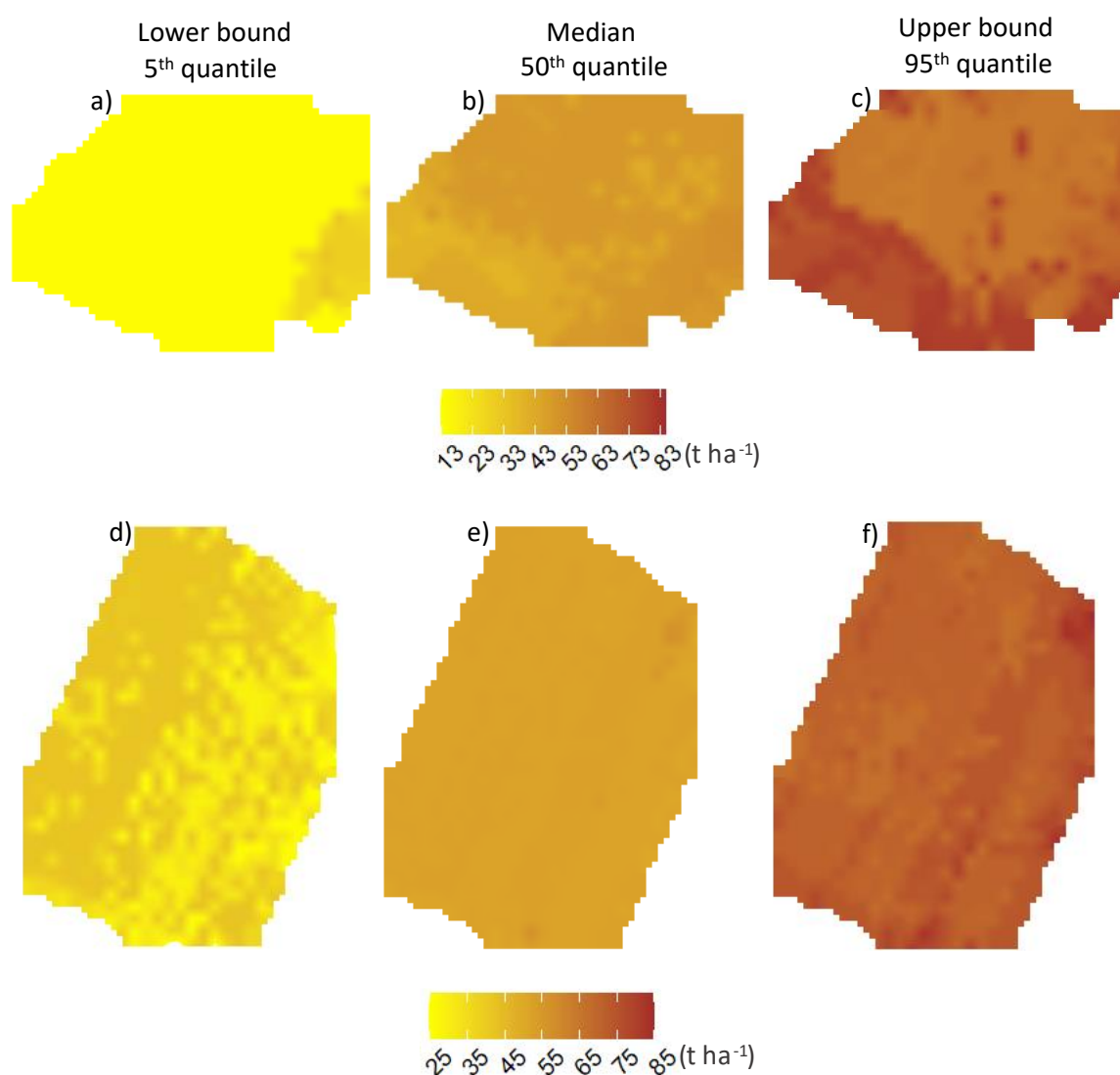


Figure 5.14 SOC stock calculations (t ha⁻¹ at 30 cm soil depth at 10 m resolution using the local SOC and BD prediction, showing the lower bound as 5th quantile (a, d), median as 50th quantile (b, e) and upper bound as 95th quantile (c, f) for Field48 and Field49.

5.3.5 Cost- accuracy effectiveness

Table 5.8 presents the relative cost-efficiency (ϵ) values of 1.03 for the SOC stock accounting approach using the PSS-based global dataset and 1.46 for the local dataset. These values, based on the overall cost for the 22 fields, indicate that both PSS schemes are more cost-efficient than the compositing sampling scheme (the reference design), as the effectiveness is greater than 1. When comparing the two PSS schemes, the local dataset proves to be more cost-efficient than the global dataset. It is important to note that the RMSE values used in the calculations are area-weighted for the 22 fields.

Table 5.8. Relative cost-efficiency (ϵ) of the proximal sensing SOC stock accounting approach using the compositing sampling scheme as the reference designs for the 22 fields used for validation (total area of 90 hectares)

SOC stock accounting approach	Total costs (euros)	Area weighted	Effectiveness
		RMSE (Mg SOC ha ⁻¹)	(ϵ)
Composite	13,164 €	7.8	-
PSS-based global dataset	10,452 €	9.48	1.03
PSS-based local dataset	16,473 €	4.28	1.46

5.4 Discussions

5.4.1 Using global and local QRF models for SOC stock estimation

Accurate SOC stock estimation enables better-informed decisions on soil management and CF initiatives. This study calibrated and validated QRF models, using multisource data (PSS, RS, and auxiliary data) as covariates to predict SOC concentration, soil texture, and BD using both global and local datasets.

Global models were calibrated on large datasets representing diverse soil conditions across Northern Italy (Emilia Romagna, Piemonte, Lombardy and Veneto). The integration of PSS, RS, and auxiliary data significantly enhanced prediction accuracy compared to using covariates from a single data source, as detailed in the Supplementary Material. Using PSS data alone, SOC concentration was predicted with an RMSE of 0.24%. Incorporating estimated SOC concentration and soil type auxiliary data (from regional databases or from LUCAS) alongside PSS data, RMSE was reduced to 0.21%. Further, combining PSS, auxiliary data, and RS data (Sentinel-2 bands like SBSI B4, B12) improved prediction accuracy, achieving an RMSE of 0.19%. For clay content prediction, PSS data alone resulted in an RMSE of 8.47%. Adding estimated clay and textural class auxiliary data to PSS data reduced the RMSE to 3.52%. Integrating PSS, auxiliary, and RS data further improved clay prediction accuracy, achieving an RMSE of 3.24%. These results demonstrate that RS data enhances model performance and prediction accuracy. The model's performance metrics showed strong accuracy for SOC concentration predictions, with an R^2 of 0.75 and an RMSE of 0.17%. Predictions for soil texture were even more robust, achieving excellent performance with R^2 values of 0.97 for clay (RMSE = 3.18%), 0.95 for sand (RMSE = 5.75%), and 0.83 for silt (RMSE = 4.39%). However, the model struggled to predict BD, which exhibited poor performance with an R^2 of 0.36 and RMSE of 0.11 g cm⁻³. PSS techniques, especially Vis-NIR spectroscopy and EC measurements, were critical for assessing soil properties that influenced SOC stocks. Reyes and Ließ (2023) underscored the critical

role of PSS data in achieving high spatial and temporal resolution monitoring of SOC at the field scale. Moreover, coupling PSS with RS and ML algorithms provided a cost-effective alternative for large-scale SOC mapping (Majumdar et al., 2022), while increasing model accuracy (Kok et al., 2024). For example, Kok et al. (2024) combined proximal NIR spectroscopy, Sentinel-2 RS data, and deep learning techniques to predict soil texture and SOC content with $R^2 > 0.80$. Similarly, Li et al. (2023) achieved $R^2 = 0.83$ for SOC concentration prediction in Australian soils by integrating MIR spectroscopy, RS, and ML approaches. At larger scale, Van der Voort et al. (2023) demonstrated the potential of the SoilCASTOR method to estimate SOC stocks in U.S. arable fields at a 10-m spatial resolution, reporting SOC stock values ranging from 19 to 55 t C ha⁻¹. These findings collectively highlight the potential of integrating PSS and RS data for accurate and efficient SOC stock estimation. However, some limitations remain. For instance, in Gholizadeh et al. (2018) and Van der Voort et al. (2023), while Sentinel-2 RS data effectively mapped SOC and clay variability, it performed less accurately for silt and sand content, underscoring the need for further refinement in integrating PSS and RS for soil property assessments.

Global models, once calibrated, offer an efficient approach for large-scale SOC mapping, as they can be applied to new locations without additional calibration. However, their ability to capture local variations in soil properties is limited, particularly in areas underrepresented in the calibration dataset (Poggio et al., 2021). In contrast, local models tailored to specific fields using precision sampling strategies better account for soil variability, leading to more accurate predictions. In this work, the model performance was significantly improved when the local dataset was used for QRF model SOC concentration prediction, yielding an $R^2 = 0.60$ and RMSE 0.09% for Field9 (1.7 ha) and an $R^2 = 0.51$ and RMSE 0.08% for Field25 (5.1 ha) using 3 measurements derived from precision sampling. Moreover, Yuzugullu et al. (2024) applied a precision sampling approach, that combined spectral-temporal soil heterogeneity detected via Sentinel-1 and Sentinel-2 imagery to evaluate SOC prediction models across croplands at 10 m resolution. Their findings revealed that model performance varied with field size and sampling density. For example, a 4.2-ha field in Switzerland with 33 measurements yielded an R^2 of 0.76 and a mean absolute prediction error (MAPE) of 2.9% and a field near Paris, France (7.5 ha) with 32 measurements achieved R^2 values of 0.95 with corresponding MAPE values of 7.4% emphasizing the role of dense, targeted ground measurements in improving accuracy. Although SOC concentration predictions obtained with the QRF model had a lower model accuracy than Yuzugullu et al. (2024) it is important to point out that our approach used few measurements to build the QRF local model, which makes it more cost-efficient.

However, building local models can be time-consuming, requiring separate calibration and validation for each field. They also risk overfitting due to smaller dataset sizes, as reflected in this study in higher RMSE values for soil texture and BD predictions in local validation dataset compared to local calibration dataset. For instance, RMSE values for clay, silt, sand, and BD were respectively of 8.02%, 9.64%, 14.01%, and 0.18 g cm⁻³ in the validation dataset compared to 7.24%, 8.02%, 10.6%, and 0.09 g cm⁻³ in calibration. Notably, SOC concentration predictions were an exception, with slightly lower RMSE values in validation than calibration datasets, indicating that dataset size and representativeness are critical to model performance.

For instance, Lucà et al. (2017) found that reducing the calibration dataset size from 216 to subsets of 14–144 samples led to performance declines, with different regression techniques requiring between 29 and 115 samples to match full dataset results. Similarly, Ramirez-Lopez et al. (2014) showed that smaller calibration datasets increased prediction errors for clay content, underscoring the importance of sufficient dataset size to capture soil variability. Adeniyi and Maerker (2024) demonstrated that a 10-m resolution offered the best balance between detail and performance for soil type classification in Lombardy, Italy, highlighting the significance of selecting the optimal spatial resolution for covariates to ensure accurate model predictions. These findings emphasize the trade-offs between the broad applicability of global models and the precision of local models. High-resolution SOC stock estimates derived from ML models are essential for driving process-based models like RothC (Coleman and Jenkinson, 1996), which require accurate initial SOC stock inputs to simulate SOC stock changes under different management scenarios. The integration of these high-resolution estimates with RothC facilitates field-scale SOC stock monitoring and management, enabling more precise assessments of the impact of agricultural practices (Sulman et al., 2018). While the development of local models can be resource-intensive, their ability to capture fine-scale spatial variability makes them invaluable for site-specific SOC stock predictions. In contrast, global models, though less precise, remain effective for large-scale SOC stock estimations, providing a broader perspective that can complement the detailed insights offered by local models. Together, these approaches form a complementary framework, balancing the need for precision at the field scale with efficiency at regional or global scales, and supporting improved decision-making for soil management and CF.

5.4.2 Importance of building robust local dataset and equipping the SOC stock accounting with precision sampling

In soil science, sub-setting and spiking are common localization methods. Local calibration datasets often result in models with poor generalization, highlighting the importance of selecting a restricted number of samples (Brown et al., 2005). Spiking involves re-calibrating a model using a global dataset, augmented with weighted local samples (Gogé et al., 2014.; Guy et al., 2015). This study uses the subsetting method with the KS approach to select a sample subset from a global dataset based on proximal (EC, IR, IR/Red) and RS (SBSI from Sentinel-2 bands) covariates at the field scale. While this approach identifies highly dissimilar samples, it may limit the model's exposure to broader data, reducing its robustness (Tessarolo et al., 2014). Consequently, models based on local datasets may not represent all field conditions effectively, leading to variable model performance across different fields. Nevertheless, our results indicate a 14.57% improvement in RMSE of the SOC stock predicted using the local dataset compared to the model using the global dataset. Recent advances in transfer learning and domain adaptation, which transfer knowledge from global datasets to local settings (Viscarra Rossel et al., 2024), suggest the potential for leveraging these methods for precision soil sampling.

Precision soil sampling targets areas with significant spatial variation, enhancing the efficiency and accuracy of data collection. In this study, expert knowledge determined three soil samples per field, and the cLHS design was used to select samples that represent the multidimensional feature space defined by proximal soil sensing (EC, IR, IR/Red) and RS data (SBSI from Sentinel-2 bands 4, 8, 11, 12) (Wadoux et al., 2019; Xia et al., 2024). The cLHS method ensures well-distributed sampling, minimizing bias and avoiding over-sampling areas with similar soil properties (Brungard and Boettinger, 2010; Minasny and McBratney, 2006). Combining local datasets and field-specific soil data for model calibration improved model performance across various fields. For example, the model for SOC concentration, sand, silt, clay, and BD showed a decrease in ME of 36.36%, 63.63%, 43.18%, 52.27%, and 30.77%, respectively. This improvement may be influenced by the SBSI input data, and the thresholds used to distinguish bare soil from other areas, which are crucial for precision soil sampling. Additionally, multi-temporal composite images with adequate lengths are essential to avoid vegetation cover during satellite passes (Castaldi, 2021; Zhou et al., 2021). For fields where subsetting and precision sampling were beneficial, the SOC model using a local dataset showed a 52.51% improvement in error compared to the global dataset model. Similarly, improvements of 43.13%, 40.87%, and 46.54% were observed for sand, silt, and clay models,

respectively, and BD showed a 57.13% improvement when local dataset were compared to global dataset. Although the number of observations and fields differed across models, the results highlight the significant impact of precision sampling on model performance. Precision agriculture can also benefit from high-resolution (10 m) local dataset models, enabling the creation of soil maps for variable-rate fertilization strategies. Accurate SOC and soil texture maps generated using local models enhanced with PSS could improve the accuracy of nitrogen analytical approaches for determining the optimal fertilizer application rates at the field level. This precision in nitrogen management can help farmers enhance nitrogen use efficiency, leading to a reduction in fertilizer-induced N₂O emissions from soils (Wu and Ma, 2015). Furthermore, the cost-efficiency of the PSS-based local dataset ($\epsilon = 1.46$) for SOC stock accounting is superior to the compositing sampling scheme and PSS-based global dataset ($\epsilon = 1.03$), making it an important tool for precision fertilization.

5.4.3 Uncertainty and cost-accuracy of SOC stock estimates using the PSS-based approach.

QRF models offer the significant advantage of quantifying prediction uncertainty, making them particularly valuable for SOC stock estimations, which inherently exhibit high spatial and temporal variability. By using QRF, 90% prediction intervals can be obtained rather than single-point estimates, which is advantageous when dealing with the heterogeneous nature of soils (Poggio et al., 2021; Rudiyanto et al., 2016; Vaysse and Lagacherie, 2017). The PIW for clay, silt, sand, and BD in models using global datasets were narrower than those in models using local datasets, except for SOC. Narrow intervals represent lower uncertainty in model predictions, while wider intervals indicate greater uncertainty (Kasraei et al., 2021). For a 90% PI, a PICP of 0.9 suggested accurate uncertainty assessment. Except for the SOC model using local dataset (which has narrower PI than the global dataset model), models using local datasets for texture (clay, silt, sand fractions) and BD show wider PIs than global models. Wider prediction intervals suggest that models using local datasets were less confident in their estimates compared to global ones. This may arise from limited data, a narrower range of input variables, the quality of covariates, or the local model's inability to capture complex relationships. Indeed, uncertainty predictions tend to be higher when fewer observations were used in model prediction (Heuvelink et al., 2021). Only clay prediction intervals obtained with the global QRF ML model provided a realistic representation of prediction uncertainty (PICP = 0.89). The SOC and BD models, whether using global or local datasets, overestimate prediction intervals. All target variables had values with large spread considering the PICP of 0.9. A

large PICP spread inconsistent uncertainty estimation. Moreover, uncertainties were not uniformly distributed across predictions, meaning the model performs better in some fields and worse in others.

The accuracy of models using global or local datasets highlights the potential of predicted clay content as an input variable for soil carbon models like RothC. While clay predictions are not perfectly accurate, the associated errors (RMSE of 3.18% for global datasets and 8.02% for local datasets) have minimal impact on overall SOC sequestration estimates, provided that other input variables remain constant. Precision sampling effectively reduces uncertainty by capturing local variability, leading to more accurate model predictions. Models based on global datasets offer scalability at a lower cost but tend to propagate uncertainty, making them less effective for precise local predictions. A balance between uncertainty reduction and scalability can be achieved by integrating approaches: using global datasets for broader applicability while applying precision sampling at the field scale to fine-tune model accuracy.

This finding has notable cost implications, suggesting that precision sampling efforts could prioritize SOC and clay content measurements over BD sampling. Given that errors in clay content predictions remain within an acceptable range, this approach optimizes resource allocation while maintaining reliable input data for soil carbon models. To optimize resource allocation in CF programs, scenario analyses should be conducted to identify the most effective approaches for clay and SOC stock accounting, considering factors such as field size (in hectares) and spatial soil variability (Bradford et al., 2023). SOC stock estimation, calculated as the product of SOC concentration and BD at 30 cm depth, was found to be sensitive to BD variability. BD predictions from global and local datasets had RMSE values of 0.11 g cm^{-3} and 0.18 g cm^{-3} , respectively, introducing considerable uncertainty in SOC stock estimates. For 22 fields, intra-field variability in SOC stock was 22.31 t ha^{-1} with global models and 14.81 t ha^{-1} with local models, demonstrating that BD is a key source of variability. Our results show that using the global and local datasets, 48.85 % and 37.09% of the overall SOC stock estimate uncertainty is attributed to BD error. Prior studies (De Vos et al., 2005; Goidts et al., 2009) reported similar challenges, with indirect BD estimates resulting in errors of up to 36% in SOC stock calculations, and random measurement errors contributing up to 40% uncertainty (Zhou et al., 2019). Improving BD predictions is therefore critical for enhancing the reliability and cost-effectiveness of SOC stock estimations. Strategies to improve BD prediction accuracy include incorporating additional covariates, such as soil moisture and land management practices, or employing advanced PSS technologies like gamma-ray spectroscopy or Vis-NIR spectroscopy for

indirect estimation. If BD prediction accuracy improves sufficiently to achieve an RMSE of 2 for SOC stock (that would represent an 5 % of uncertainty over the 70 Mg SOC ha⁻¹ measured value in our 90 hectares mapped), the cost-to-accuracy ratio of local SOC stock accounting approaches could increase significantly (e.g. from 1.5 to 3.1), making them far more viable for large-scale implementation in CF and soil monitoring programs.

Uncertainty in SOC stock predictions were accounted for using the 5th and 95th quantiles generated by the SOC and BD QRF models (Figure 5.6). Both global and local datasets used for SOC stock estimation yielded wide prediction intervals, with the local dataset model showing slightly narrower PI (section 5.3.3). Other studies have also pointed out the issue of high prediction uncertainty in SOC stock estimation, which prohibits the detection of SOC stock changes (Heuvelink et al., 2021; Szatmári et al., 2019). In this work, wide prediction intervals in SOC stock estimation using the QRF model were directly influenced by uncertainties in SOC concentration and BD estimations. In addition, the PICP of models using global and local datasets suggested an underestimation of uncertainty. A wide prediction interval reflects low confidence in identifying the actual value, indicating significant uncertainty in SOC stock estimates. These results align with other studies, where over-optimistic probabilistic predictions occurred when the QRF model underestimated uncertainty (Kasraei et al., 2021; Szatmári and Pásztor, 2019). Practically, wide intervals and underestimated uncertainty reduce the usability of SOC stock estimates, as they provide less precise information, which is critical for C accounting. The local model's consistency and low error propagation variability provided reliable average predictions at the field scale. For two independent validation fields in the Emilia Romagna region, results of this study indicated an average intra-field variability of estimated values of 9.83 t ha⁻¹ and 11.18 t ha⁻¹, respectively for models using global and local dataset. These differences suggested that local datasets provide a more nuanced understanding of SOC stocks within fields, while global datasets are more generalized. Although the intra-field variability observed in these validation fields is better than that estimated by the model used to calibrate the data, further testing in other unknown soil conditions with larger intra-field SOC stock variability is needed to assess the scalability of the proposed method. The SOC stock estimate depends on the margin of error, the scale of SOC stock verification, and the actual C sequestration value of a practice. The average area-weighted error (RMSE) of 4.28 Mg SOC ha⁻¹ for the model using the local dataset suggests that current measurements or re-measurements using PSS are not reliable for assessing C sequestration in most eligible CF practices in croplands. Given that the average SOC stock sequestration rate, when cover crops and minimum tillage are adopted

(the most widely adopted practice in CF programs), is around $0.5 \text{ t C ha}^{-1} \text{ year}^{-1}$, in five-year CF programs (the minimum duration under CRCF EU regulation) would result in 2.5 tons sequestered that is much lower than our area-weighted error (RMSE) of 4.28 Mg SOC. If we scale up this CR removal potential to the average farm size of Emilia Romagna (50 hectares) this would mean being able to measure and verify an amount of C sequestered at the farm level of 200 tons C. The RMSE of 4.28 Mg SOC makes it challenging to detect small-scale changes in SOC stock, such as the 2.5 tons of C sequestered over 5 years at the farm level raising the issue of scaling up the approach. When re-measuring to track changes over time in SOC stock and the RMSE is large relative to the amount of C expected to be sequestered, detecting changes will require frequent and precise measurements to reduce the error. However, propagating uncertainty and calculating C sequestration at a finer resolution may help reduce the margin of error by increasing sample point estimates. If cost-effective, PSS could be used to monitor C removals at a larger scale, though cost-effectiveness needs to be evaluated across multiple fields and hectares.

ML models are powerful predictive tools, but achieving high accuracy often incurs higher costs. Balancing accuracy with associated costs is crucial (Smith et al., 2020). The cost-accuracy tradeoff reflects the relationship between the data required to develop an ML model and the level of predictive performance. In general, larger datasets yield more accurate predictions but also incur higher costs, as seen with global models. Instead, local models provide acceptable accuracy at reduced costs. In the context of SOC stock accounting using PSS-based global and local datasets, both schemes are more cost-efficient than compositing sampling. However, the PSS-based local dataset is more cost-efficient than the global dataset. The cost and accuracy of spectral sensing technologies for lab analysis could further reduce costs, and improvements in data quality for QRF model calibration and better sampling strategies (e.g., more field maps and stratified sampling) could reduce costs for local SOC stock accounting, enhancing cost-accuracy ratios for CF programs and improving scalability for C quantification methodologies.

The uncertainty analysis suggests that QRF model predictions require improvement. Increasing sample density and improving covariate data quality can narrow prediction intervals and yield more reliable field-scale predictions (Heuvelink et al., 2021; Szatmári et al., 2019). Incorporating additional relevant covariates, such as land management history, can reduce unexplained variability in SOC and BD, leading to more precise predictions. For instance, Abramoff et al. (2022) emphasized the need to include land management as a covariate to capture SOC dynamics in croplands. Other approaches, such as Bayesian (Li et al., 2022) or Monte Carlo (Padarian et al., 2022; Verra, 2024)

could also be considered for SOC stock uncertainty estimates. Monte Carlo uses a probabilistic approach where each input variable is assigned a probability distribution based on observed data. It handles multiple input uncertainties simultaneously, allowing for an assessment of total uncertainty in SOC stock variability. Kakhani et al. (2024) demonstrated how conformal prediction can correct prediction intervals from ML regression models. Although these techniques focus on uncertainty estimation, integrating soil process information into ML models is essential for improving their reliability and achieving higher accuracy (Minasny et al., 2024).

A higher spatially dependent accuracy of the proposed PSS-based SOC stock accounting approach, combined with an integrated smart sampling strategy to minimize the number of samples required for local calibration (e.g., clustering fields under similar pedo-climatic and management conditions, such as crop rotation or tillage practices), could significantly enhance the overall precision and cost-effectiveness of the hybrid sampling and modeling approach for crop residue estimates. Reducing uncertainty in model inputs at finer spatial scales is particularly beneficial, as it minimizes errors when crop residue estimates are performed across larger scales.

Future research should focus on conducting scenario analyses to evaluate the cost-effectiveness and error propagation associated with implementing CF programs of varying sizes and under diverse initial soil conditions. Such studies would be instrumental in refining SOC estimation methods to ensure scalability and reliability in real-world applications.

5.5 Conclusions

Advances in soil sensing technologies, machine learning algorithms, and precision sampling strategies have significantly improved the ability to predict SOC stocks. On-the-go PSS technologies, such as VIS-NIR spectroscopy and EC sensors, enable the collection of high-resolution data, which supports the prediction of soil properties required for SOC stock estimation. Integrating PSS data with RS information and auxiliary data as covariates in QRF global models has proven particularly effective for predicting key soil properties.

In this study, the QRF model using global datasets demonstrated good to excellent performance in predicting SOC concentration and soil texture (clay, silt, and sand). However, when applied to estimate BD based on measured soil properties such as SOC concentration and soil texture, the model showed poor performance, highlighting BD as a critical challenge in SOC stock accounting.

The novelty of this work lies in developing and testing a cost-effective C accounting framework to estimate SOC stocks at a 10 m resolution at the field scale. This framework utilizes the QRF model

with multisource covariates and minimal ground measurements, defined through precision sampling. By emphasizing site-specific calibration, the QRF model with a local dataset achieved accurate SOC stock predictions (RMSE of 4.28 Mg SOC ha⁻¹) with a relative error of 19.3%, while maintaining cost-efficiency. Our findings demonstrate that the SOC stock accounting approach using the local dataset is more cost-effective than using both a compositing sampling scheme (reference design) and the global dataset.

Nevertheless, the uncertainty in SOC stock estimation using the local dataset exceeded 10%, making the *measure or re-measure* approach using PSS unreliable for accurately assessing SOC sequestration under typical CF practices in croplands. Despite this limitation, the QRF model using local data effectively predicted soil variables with a high spatial resolution (10 m), potentially supporting applications such as precision fertilization. These high-resolution predictions facilitate the design of smart fertilization strategies for crops in rotation, potentially reducing N₂O emissions. The QRF model's accurate predictions of clay content and SOC stock show promise as input variables for soil C models like Roth-C, further advancing C accounting and precision agriculture strategies.

AUTHORS CONTRIBUTION

Irena Ymeti, Marta Bertola, Andrea Ferrarini: manuscript editing. **Marta Bertola, Lorenzo Cremonesi, Andrea Ferrarini:** field data collection. **Irena Ymeti, Marta Bertola, Lorenzo Cremonesi:** Laboratory analysis. **Irena Ymeti, Michele Croci, Andrea Ferrarini:** data analysis. **Irena Ymeti, Andrea Ferrarini:** conceptualization and study design. **Andrea Ferrarini, Stefano Amaducci:** funding acquisition and supervision.

ACKNOWLEDGEMENTS

The authors acknowledged UCSC field crop group collaborators for helping in field campaign and laboratory analysis, in particular Edoardo Volpato, Levyn Eric Perez Alvarado, and Luis Brayan Lopez Lazo.

FUNDING INFORMATION

This study is part of the project Farms4Climate, co-funded by the Partnership for Research and Innovation in the Mediterranean Area (PRIMA) and the European Union.

Supplementary Material

Table S5.1 Summary of various SOC prediction models using proximal sensing (PS) data, auxiliary data such as soil type (ST), and soil organic carbon estimates (SOC_EST). The SOC_EST values were derived using geographic matching, which relates point SOC observations to raster SOC estimated values. For SOC observations classified under accuracy levels 2 (moderate) or 3 (low), the nearest SOC estimated value with accuracy 1 (high) was used as the final SOC_EST value. Sentinel-2 SBSI (Synthetic Bare Soil Image) bands utilized include B2, B3, B4, B8, B11, and B12.

Model Name	RMSE	Pearson's Corr	Spearman's Corr
PS	0.24	0.73	0.76
PS+ST+ SOC_EST geographic matching	0.2	0.81	0.82
PS+ST + SOC_EST field average	0.19	0.84	0.86
PS+ST + SOC_EST accuracy 1	0.21	0.81	0.83
PS+ST+ SOC_EST geographic matching + Sentinel 6 bands	0.19	0.83	0.84
PS+ST+ SOC geographic matching + Sentinel 3 bands	0.2	0.82	0.84
PS+ST+ SOC_EST field average + Sentinel 6 bands	0.19	0.84	0.85
PS+ST+ SOC_EST field average + Sentinel 3 bands	0.19	0.83	0.84
PS+ST+SOC_EST accuracy 1 + Sentinel 6 bands	0.19	0.84	0.85
PS+ST+ SOC_EST accuracy 1 + Sentinel 3 bands	0.19	0.83	0.85
Sentinel 6 bands	0.25	0.72	0.68
Sentinel 3 bands	0.26	0.67	0.66
ST+ SOC_EST geographic matching + Sentinel 6 bands	0.23	0.76	0.77
ST +SOC_EST geographic matching + Sentinel 3 bands	0.24	0.73	0.74
ST+ SOC_EST field average + Sentinel 6 bands	0.21	0.8	0.8
ST +SOC_EST field average + Sentinel 3 bands	0.22	0.78	0.78
ST+ SOC_EST accuracy 1 + Sentinel 6 bands	0.23	0.77	0.77
ST +SOC accuracy 1 + Sentinel 3 bands	0.24	0.74	0.76
PS+ST+ Sentinel 6 bands	0.24	0.8	0.81

Table S5.2 Summary of various soil texture prediction models using proximal sensing (PS) data, auxiliary data such as the textural class (TC), Clay, Silt and Sand estimated, and Sentinel-2 SBSI.

Model Name	RMSE	Pearson's Corr	Spearman's Corr
PS	8.47	0.88	0.86
PS+TC+ CLAY_EST	3.52	0.98	0.97
PS+TC + CLAY_EST+SENTINEL	3.38	0.98	0.97
TC + CLAY_EST +SENTINEL	4.05	0.97	0.96
SENTINEL DATA ONLY	9.86	0.84	0.83
PS+TC+CLAY_EST+ B8, B11, B12	3.24	0.98	0.97
PS+TC+ B8, B11, B12	4.00	0.98	0.96
PS+TC+SAND_EST	4.52	0.97	0.94
PS+TC+SAND_EST+SENTINEL	4.75	0.97	0.95
TC+SAND_EST+SENTINEL	5.39	0.96	0.94
SENTINEL DATA ONLY	13.01	0.76	0.78
PS+TC+SAND_EST+ B8, B4, B11	4.78	0.97	0.95
PS+TC+ B8, B4, B11	5.44	0.96	0.94
PS+TC+SILT_EST	4.52	0.84	0.86
PS+TC+SILT_EST+SENTINEL	4.64	0.82	0.86
TC+SILT_EST+SENTINEL	4.75	0.81	0.84
SENTINEL DATA ONLY	7.64	0.41	0.50
PS+TC+SILT_EST+ B3, B2, B4	4.66	0.82	0.85
PS+TC+ B3, B2, B4	4.80	0.80	0.84

Chapter 6

General conclusions

Marta Bertola

This thesis addresses the urgent need for sustainable agricultural practices to make operational measurable and verifiable C removals as temporary soil C sequestration for climate change mitigation, to support soil quality improvement, and ultimately contribute to farm climate resilience. By integrating experimental, analytical, and modeling approaches across diverse cropping systems and scales, this research explores operational CF strategies and advances state-of-the-art knowledge on soil economy opportunities for farmers and the agro-food sector. Through a multi-faceted approach spanning systemic agronomic solutions and advanced MRV tools, the findings highlight the potential of tailored CF initiatives to achieve climate and agricultural sustainability goals. The outcomes provide actionable insights into the design, implementation, and scalability of CF practices, while addressing key challenges and opportunities in operationalizing CF strategies. The major conclusions are summarized as follows:

SOC sequestration and soil quality in perennial systems (Chapter 2)

The cultivation of novel *Miscanthus* hybrids on marginal lands offers a promising approach to transforming underutilized landscapes into productive systems that contribute to SOC sequestration and biomass production. Over four years, an average SOC sequestration rate of 0.43 Mg SOC ha⁻¹ y⁻¹ was observed, though this varied significantly with previous land use and site conditions, highlighting the role of soil stoichiometry in SOC dynamics. While *Miscanthus* cultivation provided a reliable biomass supply for bio-based industries, site-specific challenges, including nutrient imbalances—particularly K and N deficiencies—and negative impacts on soil physical quality were noted. However, targeted nutrient management could mitigate these issues, sustaining long-term productivity and soil health. This study underscores that longer cultivation periods are critical to fully realizing the soil quality improvements and C sink potential of perennial systems.

Measurable and verifiable net climate and agronomic benefits in industrial tomato supply chain through CarbonFarming-done-right (Chapter 3)

Carbon Farming (CF) practices co-developed for the industrial tomato supply chain—including diversified crop rotations, winter cover crop integration, reduced tillage, and integrated residue and organic nutrient management—demonstrated significant environmental and economic benefits. Key findings over a 2-year crop rotation span include: 1) increased SOC stocks on an equivalent soil mass basis of approximately +1.25 Mg SOC ha⁻¹ y⁻¹; 2) enhanced stable SOC fractions (MAOM-C) by +3 Mg ha⁻¹ over 2.5 years; 3) and improvements in soil health indicators, such as MBC, nutrient cycling, and aggregate stability.

Although GHG emission reductions were relatively modest ($-0.1\text{-ton CO}_2\text{e ha}^{-1}$) compared to the baseline—primarily due to residue incorporation and machinery use—optimized management strategies have the potential to further minimize trade-offs and amplify net climate benefits.

The integration of digital tools into a hybrid sampling and modelling quantification approach (aka MRV approach) of C removals, including hybrid remote sensing (RS)-based models and smart soil sampling strategies, minimized uncertainties in C removal estimates against real farm dynamic baseline and ensured compliance with certification standards like the EU CRCF and Verra protocols. These tools demonstrated high accuracy:

- Time series SOC stock estimation: RMSE = 3.19 Mg ha^{-1} , RE = 1%, ME = 0.95, and $R^2 = 0.95$.
- SOC stock change (ΔSOC) estimation: RMSE = 3.73 Mg ha^{-1} , RE = -153%, ME = 0.20, and $R^2 = 0.34$.
- Relative uncertainty of ΔSOC estimations was 4.74% across the entire project area.

Furthermore, high-resolution spatial data from proximal soil sensors could offer opportunities to enhance model initialization and validation through smart soil sampling strategies, improving accuracy and scalability.

The MRV tool enabled the development of a scalable framework for monitoring, reporting and verifying carbon credit generation at farm scale. Overall, per LCF 17.7 carbon certificates (measured as net temporary carbon removal certifiable units in $\text{ton CO}_2\text{e ha}^{-1}$) over a wheat-maize rotation were generated, passed the accuracy and uncertainty test and thus verified according to standards required by certification bodies. Preliminary economic assessments of this certificates confirmed the financial viability of the CF practices adopted. If issued and priced in VCM these units would correspond to a net farm income of approximately $\text{€}92\text{ ha}^{-1}\text{ year}^{-1}$, covering around 97% of implementation costs when co-benefits were included. However, the study emphasized the importance of addressing risks such as carbon leakage and the need to balance economic returns with operational costs to enhance the credibility and long-term sustainability of CF programs.

Overall, these results underscore the potential of CF practices to improve the sustainability of industrial tomato supply chains in northwestern Italy, while aligning with decarbonization goals of agri-food industries and the increasing confidence of their Scope 3 reporting.

Microbial Mechanisms Driving SOC Stabilization (Chapter 4)

The microbial processes underpinning SOC stabilization are pivotal for optimizing long-term C storage and sustaining soil fertility. This study demonstrated that incorporating crop residues with

stoichiometrically balanced nutrient supplementation significantly enhances microbial biomass, activity, and humification efficiency, leading to higher stabilization of SOC in mineral-associated fractions (+0.68 mg C g⁻¹ of MAOM-C over 3-month incubation). Microbial metabolic processes, such as the microbial-C-pump, play a critical role in ensuring long-term SOC storage, particularly in soils with lower initial fertility. In contrast, imbalanced nutrient availability or poor soil conditions can drive microbial mining of native SOM, reducing stabilization efficiency. Organic fertilizers proved especially effective in promoting microbial growth and SOC stabilization, offering a sustainable alternative to mineral fertilizers. Although targeted nutrient additions may lead to higher soil emissions (+70% compared to the control), the corresponding increase in SOC sequestration efficiency per unit of N input (+0.1 mg C g⁻¹ over the 3-month incubation) suggests that, if the overall CO₂-equivalent C balance results in a net climate benefit through C removals, this strategy holds significant potential to achieve the dual goals of Carbon Farming: climate mitigation and soil health improvement. These findings emphasize the importance of integrating the understanding of microbial processes and targeted nutrient management into CF frameworks to maximize SOC stabilization and enhance soil functionality to support crop yield.

Advances in SOC stock accounting with a proximal soil sensing-based regional and ML-based prediction model to support soil C modelling and precision agriculture.

This study calibrated and validated a cost-effective SOC stock accounting approach that integrates on-the-go proximal soil sensing (PSS), remote sensing technologies, precision sampling strategies, and machine learning models (QRF). The accounting approach leverages a QRF prediction model for key properties for SOC stock calculation based on a regional dataset or a local dataset supported by smart sampling. The accounting approach provided high-resolution (10 m) SOC stock maps, which serve as critical spatial input data for soil C modeling in order to reduce error propagation across scales. By achieving a favorable cost-accuracy ratio ($\epsilon = 1.46$) compared to traditional composite sampling approaches, this methodology demonstrates added value in carbon accounting applications when C removals need to be quantified and verified in large scale CF programs. Accurate clay content estimations (RMSE of 3.18%) for indirect BD estimates enhanced the reliability of SOC stock predictions (area-weighted RMSE of 4.28 Mg SOC ha⁻¹), highlighting the potential of these maps in reducing error propagation in soil C modeling and improving the efficiency of CF initiatives.

Despite uncertainties in SOC stock estimation (19.3% relative error) are exceeding 10% of the validation dataset due to actual limitations in bulk density (BD) predictions via PS, the proposed

accounting framework remains a robust tool for MRV applications in CF programs. It might support accurate and spatially explicit soil C model initialization and contemporary support the deployment of precision fertilization strategies that optimize nutrient management, thereby reducing fertilizers-related soil GHG emissions. This integrated approach aligns with the broader goals of carbon farming by enabling smarter fertilization and promoting sustainable agricultural practices.

Overarching Insights and Contributions

This research highlights the multifaceted nature of CF systems, where success depends on integrating agronomic practices, technological innovations, and policy alignment to achieve climate and agricultural sustainability goals. Diverse pathways to SOC sequestration were identified, with perennial systems like *Miscanthus* offering stable biomass feedstocks and contributing to SOC storage, while annual systems, such as industrial tomato production, present opportunities to incorporate CF practices into existing supply chains. Tailored, site-specific management approaches are essential, as SOC dynamics and soil quality outcomes are strongly influenced by soil properties, previous land use, and local constraints. Additionally, addressing microbial processes and nutrient cycling enhances SOC stabilization while mitigating environmental risks, such as GHG emissions and nutrient imbalances.

The adoption of advanced hybrid MRV approach —integrating remote sensing, proximal soil sensors, and biogeochemical models—improves the accuracy, reliability, and scalability of CF initiatives, facilitating their integration into voluntary carbon markets or Scope 3 reporting for agrifood-sector. Crucially, establishing robust specific baselines is essential to accurately quantify the impacts of CF practices to avoid any under crediting or over crediting situation across a supply chain if a biased standardized baseline is chosen. The data presented in this thesis are derived from field- and farm-scale monitoring and verification, often accompanied by reported estimation uncertainties, which reflect the real-world challenges of measuring and verifying C removals certificates. However, balancing trade-offs—such as field emissions from CF practices, nutrient depletion, and uncertainties in SOC accounting—remains critical to optimize outcomes and build confidence in CF initiatives. Economic feasibility remains a cornerstone for scaling CF practices, with carbon credits serving as a key incentive for adoption. Nonetheless, economic benefits must be carefully balanced against risks, certification costs, and potential trade-offs to ensure long-term viability. Future research should prioritize addressing gaps in BD prediction, refining long-term SOC sequestration assessments, and enhancing MRV systems. By aligning with certification standards

and fostering stakeholder collaboration, CF practices can be effectively scaled and integrated into climate finance mechanisms, driving progress in the soil carbon economy.

This thesis advances the broader vision of carbon farming as a cornerstone of sustainable agriculture and climate mitigation by delivering operational and scalable agronomic solutions aimed at decarbonizing the agrifood sector. It provides a pathway for the development and validation of locally relevant, cost-effective, and scalable MRV tools that ensure measurable and verifiable outcomes, unlocking economic and environmental opportunities for farmers and agrifood stakeholders. Importantly, the thesis underscores the need to fully assess the cost-effectiveness and uncertainties associated with MRV digital solutions and the carbon removal estimates they produce at both farm and project scales. Such assessments are essential for supporting the transition toward climate-neutral and climate-resilient farming systems. By addressing critical knowledge gaps—from the impacts of land-use transitions to the development of accurate MRV tools and microbial mechanisms of SOC stabilization—it provides actionable insights for scaling CF practices. Moving forward, research should emphasize long-term evaluations of CF impacts, leveraging CF platforms (e.g. <https://www.regrow.ag/platform> or <https://www.agricarbon.co.uk>) to integrate high-resolution soil carbon monitoring and scalable sampling technologies. Utilizing tools such as remote sensing, machine learning, and automated soil analysis, multi-scale data integration can improve monitoring accuracy and verification standards. Future strategies should also optimize trade-offs between agricultural productivity, carbon removals, and sustainability by combining SOC mapping, cost-effective CF practices, and emissions-reducing measures, ensuring a balanced approach to climate goals and efficient farm and crop management.

REFERENCES

- Abay, P., Gong, L., Luo, Y., Zhu, H., Ding, Z., 2024. Soil extracellular enzyme stoichiometry reveals the nutrient limitations in soil microbial metabolism under different carbon input manipulations. *Sci. Total Environ.* 913, 169793. <https://doi.org/10.1016/j.scitotenv.2023.169793>
- Abdulraheem, M.I., Zhang, W., Li, S., Moshayedi, A.J., Farooque, A.A., Hu, J., 2023. Advancement of Remote Sensing for Soil Measurements and Applications: A Comprehensive Review. *Sustainability* 15, 15444. <https://doi.org/10.3390/su152115444>
- Abid, M., Lal, R., 2009. Tillage and drainage impact on soil quality: II. Tensile strength of aggregates, moisture retention and water infiltration. *Contains Pap. Highl. 2006 Land Degrad. Soil Water Conserv. Trop. Highl. Mekelle Ethiop.* 21-25 Sept. 2006 103, 364–372. <https://doi.org/10.1016/j.still.2008.11.004>
- Abramoff, R.Z., Bertrand, G., Haicheng, Z., Katerina, G., Xiaofeng, X., Rossel, R.A.V., Wenping, Y., Philippe, C., 2022. Improved global-scale predictions of soil carbon stocks with Millennial Version 2. *Soil Biol. Biochem.* 164. <https://doi.org/10.1016/j.soilbio.2021.108466>
- Adeniyi, O.D., Maerker, M., 2024. Explorative analysis of varying spatial resolutions on a soil type classification model and it's transferability in an agricultural lowland area of Lombardy, Italy. *Geoderma Reg.* 37.
- Agostini, F., Gregory, A.S., Richter, G.M., 2015. Carbon Sequestration by Perennial Energy Crops: Is the Jury Still Out? *BioEnergy Res.* 8, 1057–1080. <https://doi.org/10.1007/s12155-014-9571-0>
- Aguilera, E., Lassaletta, L., Gattinger, A., Gimeno, B.S., 2013. Managing soil carbon for climate change mitigation and adaptation in Mediterranean cropping systems: A meta-analysis. *Agric. Ecosyst. Environ.* 168, 25–36. <https://doi.org/10.1016/j.agee.2013.02.003>
- Agumas, B., Blagodatsky, S., Balume, I., Musyoki, M.K., Marhan, S., Rasche, F., 2021. Microbial carbon use efficiency during plant residue decomposition: Integrating multi-enzyme stoichiometry and C balance approach. *Appl. Soil Ecol.* 159, 103820. <https://doi.org/10.1016/j.apsoil.2020.103820>
- Amelung, W., Bossio, D., Vries, W., Kögel-Knabner, I., Lehmann, J., Amundson, R., Bol, R., Collins, C., Lal, R., Leifeld, J., Minasny, B., Pan, G., Paustian, K., Rumpel, C., Sanderman, J., Groenigen, J.W., Mooney, S., Wesemael, B., Wander, M., Chabbi, A., 2020. Towards a global-scale soil climate mitigation strategy. *Nat. Commun.* 11, 5427. <https://doi.org/10.1038/s41467-020-18887-7>
- Amougou, N., Bertrand, I., Machet, J.-M., Recous, S., 2011. Quality and decomposition in soil of rhizome, root and senescent leaf from *Miscanthus x giganteus*, as affected by harvest date and N fertilization. *Plant Soil* 338, 83–97. <https://doi.org/10.1007/s11104-010-0443-x>
- Anderson, E., Arundale, R., Maughan, M., Oladeinde, A., Wycislo, A., Voigt, T., 2011. Growth and agronomy of *Miscanthus x giganteus* for biomass production. *Biofuels* 2, 71–87. <https://doi.org/10.4155/bfs.10.80>
- Andrade, R., Mancini, M., Teixeira, A.F.D.S., S.H.G., S., Weindorf, D.C., Chakraborty, S., Guilherme, L.R.G., Curi, N., 2022. Proximal sensor data fusion and auxiliary information for tropical soil property prediction: Soil texture. *Geoderma* v.422, 115936. <https://doi.org/10.1016/j.geoderma.2022.115936>
- Angers, D., Arrouays, D., Cardinael, R., Chenu, C., Corbeels, M., Demenois, J., Farrell, M., Martin, M., Minasny, B., Recous, S., Six, J., 2022. A well-established fact: Rapid mineralization of organic inputs is an important factor for soil carbon sequestration. *Eur. J. Soil Sci.* 73, e13242. <https://doi.org/10.1111/ejss.13242>
- Angers, D.A., Arrouays, D., Saby, N.P.A., Walter, C., 2011. Estimating and mapping the carbon saturation deficit of French agricultural topsoils. *Soil Use Manag.* 27, 448–452. <https://doi.org/10.1111/j.1475-2743.2011.00366.x>
- Ardenti, F., Abalos, D., Capra, F., Lommi, M., Maris, S.C., Perego, A., Bertora, C., Tabaglio, V., Fiorini, A., 2022. Matching crop row and dripline distance in subsurface drip irrigation increases yield and mitigates N₂O emissions. *Field Crops Res.* 289, 108732. <https://doi.org/10.1016/j.fcr.2022.108732>
- Arrouays, D., Grundy, M.G., Hartemink, A.E., Hempel, J.W., Heuvelink, G.B., Hong, S.Y., Lagacherie, P., Lelyk, G., McBratney, A.B., McKenzie, N.J., Lourdes MendoncaSantos, M., Minasny, B., Montanarella, L., Odeh, I.O., Sanchez, P.A., Thompson, J.A., Zhang, G.-L., 2014. GlobalSoilMap: Toward a Fine-Resolution Global Grid of Soil Properties, in: *Advances in Agronomy*. <https://doi.org/10.1016/B978-0-12-800137-0.00003-0>
- Awty-Carroll, D., Magenau, E., Al Hassan, M., Martani, E., Kontek, M., van der Pluijm, P., Ashman, C., de Maupeou, E., McCalmont, J., Petrie, G., Davey, C., van der Cruysen, K., Jurišić, V., Amaducci, S., Lamy, I., Shepherd, A., Kam, J., Hoogendam, A., Croci, M., Dolstra, O., Ferrarini, A., Lewandowski, I., Trindade, L.M., Kiesel, A., Clifton-Brown, J., 2022. Yield performance of fourteen novel inter- and intra-species *Miscanthus* hybrids across Europe. *GCB Bioenergy* gcb.13026. <https://doi.org/10.1111/gcb.13026>
- Baggs, E.M., Rees, R.M., Smith, K.A., Vinten, A.J.A., 2000. Nitrous oxide emission from soils after incorporating crop residues. *Soil Use Manag.* 16, 82–87. <https://doi.org/10.1111/j.1475-2743.2000.tb00179.x>
- Bai, Z., 2018. Effects of agricultural management practices on soil quality_ A review of long-term experiments for Europe and China 7.

- Bailey, V.L., Pries, C.H., Lajtha, K., 2019. What do we know about soil carbon destabilization? *Environ. Res. Lett.* 14, 083004. <https://doi.org/10.1088/1748-9326/ab2c11>
- Ballabio, C., Panagos, P., Montanarella, L., 2016. Mapping topsoil physical properties at European scale using the LUCAS database. *Geoderma* 261, 110–123.
- Barbato, C.T., Strong, A.L., 2023. Farmer perspectives on carbon markets incentivizing agricultural soil carbon sequestration. *Npj Clim. Action* 2, 26. <https://doi.org/10.1038/s44168-023-00055-4>
- Barton, K., 2023. MuMIn: multi-model inference. *Http-Forge R-Proj. Orgprojects* mumin.
- Bastida, F., García, C., Fierer, N., Eldridge, D.J., Bowker, M.A., Abades, S., Alfaro, F.D., Asefaw Berhe, A., Cutler, N.A., Gallardo, A., García-Velázquez, L., Hart, S.C., Hayes, P.E., Hernández, T., Hseu, Z.-Y., Jehmlich, N., Kirchmair, M., Lambers, H., Neuhauser, S., Peña-Ramírez, V.M., Pérez, C.A., Reed, S.C., Santos, F., Siebe, C., Sullivan, B.W., Trivedi, P., Vera, A., Williams, M.A., Luis Moreno, J., Delgado-Baquerizo, M., 2019. Global ecological predictors of the soil priming effect. *Nat. Commun.* 10, 3481. <https://doi.org/10.1038/s41467-019-11472-7>
- Bastida, F., Zsolnay, A., Hernández, T., García, C., 2008. Past, present and future of soil quality indices: A biological perspective. *Geoderma* 147, 159–171. <https://doi.org/10.1016/j.geoderma.2008.08.007>
- Batjes, N.H., Ceschia, E., Heuvelink, G.B.M., Demenois, J., Le Maire, G., Cardinael, R., Arias-Navarro, C., Van Egmond, F., 2024. Towards a modular, multi-ecosystem monitoring, reporting and verification (MRV) framework for soil organic carbon stock change assessment. *Carbon Manag.* 15, 2410812. <https://doi.org/10.1080/17583004.2024.2410812>
- Baveye, P.C., 2020. Bypass and hyperbole in soil research: Worrisome practices critically reviewed through examples 72, 1–20. <https://doi.org/10.1111/ejss.12941>
- Bell, L.W., Wade, L.J., Ewing, M.A., 2010. Perennial wheat: a review of environmental and agronomic prospects for development in Australia. *Crop Pasture Sci.* 61, 679. <https://doi.org/10.1071/CP10064>
- Berthelin, J., Laba, M., Lemaire, G., Powlson, D., Tessier, D., Wander, M., Baveye, P.C., 2022. Soil carbon sequestration for climate change mitigation: Mineralization kinetics of organic inputs as an overlooked limitation. *Eur. J. Soil Sci.* 73, e13221. <https://doi.org/10.1111/ejss.13221>
- Bertrand, I., Viaud, V., Daufresne, T., Pellerin, S., Recous, S., 2019. Stoichiometry constraints challenge the potential of agroecological practices for the soil C storage. A review. *Agron. Sustain. Dev.* 39, 54. <https://doi.org/10.1007/s13593-019-0599-6>
- Beuch, S., Boelcke, B., Belau, L., 2000. Effect of the Organic Residues of *Miscanthus x giganteus* on the Soil Organic Matter Level of Arable Soils. *J. Agron. Crop Sci.* 184, 111–120. <https://doi.org/10.1046/j.1439-037x.2000.00367.x>
- Bhattacharyya, S.S., Ros, G.H., Furtak, K., Iqbal, H.M.N., Parra-Saldívar, R., 2022. Soil carbon sequestration – An interplay between soil microbial community and soil organic matter dynamics. *Sci. Total Environ.* 815, 152928. <https://doi.org/10.1016/j.scitotenv.2022.152928>
- Biney, J.K.M., Vašát, R., Bell, S.M., Kebonye, N.M., Klement, A., Kingsley, J., Borůvka, L., 2022. Prediction of topsoil organic carbon content with Sentinel-2 imagery and spectroscopic measurements under different conditions using an ensemble model approach with multiple pre-treatment combinations. *Soil Tillage Res.* 220. <https://doi.org/10.1016/j.still.2022.105379>
- Biswas, A., Zhang, Y., 2018. Sampling designs for validating digital soil maps: A review. *Pedosphere* 28, 1–15. [https://doi.org/10.1016/S1002-0160\(18\)60001-3](https://doi.org/10.1016/S1002-0160(18)60001-3)
- Blanco-Canqui, H., Ruis, S.J., 2020. Cover crop impacts on soil physical properties: A review. *Soil Sci. Soc. Am. J.* 84, 1527–1576. <https://doi.org/10.1002/saj2.20129>
- Bolinder, M.A., Crotty, F., Elsen, A., Frac, M., Kismányoky, T., Lipiec, J., Tits, M., Tóth, Z., Kätterer, T., 2020. The effect of crop residues, cover crops, manures and nitrogen fertilization on soil organic carbon changes in agroecosystems: a synthesis of reviews. *Mitig. Adapt. Strateg. Glob. Change* 25, 929–952. <https://doi.org/10.1007/s11027-020-09916-3>
- Bolinder, M.A., Janzen, H.H., Gregorich, E.G., Angers, D.A., VandenBygaart, A.J., 2007. An approach for estimating net primary productivity and annual carbon inputs to soil for common agricultural crops in Canada. *Agric. Ecosyst. Environ.* 118, 29–42. <https://doi.org/10.1016/j.agee.2006.05.013>
- Bönecke, E., Meyer, S., Vogel, S., Schröter, I., Gebbers, R., Kling, C., Kramer, E., Lück, K., Nagel, A., Philipp, G., Gerlach, F., Palme, S., Scheibe, D., Zieger, K., Rühlmann, J., 2021. Guidelines for precise lime management based on high-resolution soil pH, texture and SOM maps generated from proximal soil sensing data. *Precis. Agric.* 22, 493–523. <https://doi.org/10.1007/s11119-020-09766-8>
- Bongiorno, G., Bünemann, E.K., Oguejiofor, C.U., Meier, J., Gort, G., Comans, R., Mäder, P., Brussaard, L., de Goede, R., 2019a. Sensitivity of labile carbon fractions to tillage and organic matter management and their potential as comprehensive soil quality indicators across pedoclimatic conditions in Europe. *Ecol. Indic.* 99, 38–50. <https://doi.org/10.1016/j.ecolind.2018.12.008>
- Bongiorno, G., Postma, J., Bünemann, E.K., Brussaard, L., de Goede, R.G.M., Mäder, P., Tamm, L., Thuerig, B., 2019b. Soil suppressiveness to *Pythium ultimum* in ten European long-term field experiments and its relation with soil parameters. *Soil Biol. Biochem.* 133, 174–187. <https://doi.org/10.1016/j.soilbio.2019.03.012>

- Bradford, M.A., Eash, L., Polussa, A., Jevon, F.V., Kuebbing, S.E., Hammac, W.A., Rosenzweig, S., Oldfield, E.E., 2023. Testing the feasibility of quantifying change in agricultural soil carbon stocks through empirical sampling. *Geoderma* 440, 116719. <https://doi.org/10.1016/j.geoderma.2023.116719>
- Bragato, G., Fornasier, F., Brus, D.J., 2016. Characterization of soil fertility and soil biodiversity with dsDNA as a covariate in a regression estimator for mean microbial biomass C: Soil dsDNA as a covariate for microbial biomass C. *Eur. J. Soil Sci.* 67, 827–834. <https://doi.org/10.1111/ejss.12387>
- Brami, C., Nathan Lowe, C., Menasseri, S., Jacquet, T., Pérès, G., 2020. Multi-parameter assessment of soil quality under *Miscanthus x giganteus* crop at marginal sites in Île-de-France. *Biomass Bioenergy* 142, 105793. <https://doi.org/10.1016/j.biombioe.2020.105793>
- Breiman, L., 2001. Random forests.
- Briones, M.J.I., Massey, A., Elias, D.M.O., McCalmont, J.P., Farrar, K., Donnison, I., McNamara, N.P., 2023. Species selection determines carbon allocation and turnover in *Miscanthus* crops: Implications for biomass production and C sequestration. *Sci. Total Environ.* 887, 164003. <https://doi.org/10.1016/j.scitotenv.2023.164003>
- Bronick, C.J., Lal, R., 2005. Soil structure and management: a review. *Geoderma* 124, 3–22. <https://doi.org/10.1016/j.geoderma.2004.03.005>
- Brown, D.J., Bricklemeyer Ross, S., Miller Perry, R., 2005. Validation requirements for diffuse reflectance soil characterization models with a case study of VNIR soil C prediction in Montana. *Geoderma* 129, 251–267. <https://doi.org/10.1016/j.geoderma.2005.01.001>
- Brummitt, C.D., Mathers, C.A., Keating, R.A., O'Leary, K., Easter, M., Friedl, M.A., DuBuisson, M., Campbell, E.E., Pape, R., Peters, S.J.W., Kumar, A.A., 2024. Solutions and insights for agricultural monitoring, reporting, and verification (MRV) from three consecutive issuances of soil carbon credits. *J. Environ. Manage.* 369, 122284. <https://doi.org/10.1016/j.jenvman.2024.122284>
- Brungard, C.W., Boettinger, J.L., 2010. Conditioned Latinhypercube sampling: Optimal sample size for digital soil mapping of arid rangelands in Utah, USA. P. 67–75 in *Digital soil mapping*. Springer, Dordrecht, The Netherlands.
- Bruni, E., Guenet, B., Huang, Y., Clivot, H., Virto, I., Farina, R., Kätterer, T., Ciais, P., Martin, M., Chenu, C., 2021. Additional carbon inputs to reach a 4 per 1000 objective in Europe: feasibility and projected impacts of climate change based on Century simulations of long-term arable experiments. *Biogeosciences* 18, 3981–4004. <https://doi.org/10.5194/bg-18-3981-2021>
- Brus, D.J., 2019. Sampling for digital soil mapping: A tutorial supported by R scripts. *Geoderma* 338, 464–480. <https://doi.org/10.1016/j.geoderma.2018.07.036>
- Bünemann, E.K., Bongiorno, G., Bai, Z., Creamer, R.E., De Deyn, G., de Goede, R., Fleskens, L., Geissen, V., Kuyper, T.W., Mäder, P., Pulleman, M., Sukkel, W., van Groenigen, J.W., Brussaard, L., 2018. Soil quality – A critical review. *Soil Biol. Biochem.* 120, 105–125. <https://doi.org/10.1016/j.soilbio.2018.01.030>
- Burney, J.A., Davis, S.J., Lobell, D.B., 2010. Greenhouse gas mitigation by agricultural intensification. *Proc. Natl. Acad. Sci.* 107, 12052–12057. <https://doi.org/10.1073/pnas.0914216107>
- Cadoux, S., Ferchaud, F., Demay, C., Boizard, H., Machet, J.-M., Fourdinier, E., Preudhomme, M., Chabbert, B., Gosse, G., Mary, B., 2014. Implications of productivity and nutrient requirements on greenhouse gas balance of annual and perennial bioenergy crops. *GCB Bioenergy* 6, 425–438. <https://doi.org/10.1111/gcbb.12065>
- Cadoux, S., Riche, A.B., Yates, N.E., Machet, J.-M., 2012. Nutrient requirements of *Miscanthus x giganteus*: Conclusions from a review of published studies. *Biomass Bioenergy* 38, 14–22. <https://doi.org/10.1016/j.biombioe.2011.01.015>
- Calvin, K., Dasgupta, D., Krinner, G., Mukherji, A., Thorne, P.W., Trisos, C., Romero, J., Aldunce, P., Barrett, K., Blanco, G., Cheung, W.W.L., Connors, S., Denton, F., Diongue-Niang, A., Dodman, D., Garschagen, M., Geden, O., Hayward, B., Jones, C., Jotzo, F., Krug, T., Lasco, R., Lee, Y.-Y., Masson-Delmotte, V., Meinshausen, M., Mintenbeck, K., Mokssit, A., Otto, F.E.L., Pathak, M., Pirani, A., Poloczanska, E., Pörtner, H.-O., Revi, A., Roberts, D.C., Roy, J., Ruane, A.C., Skea, J., Shukla, P.R., Slade, R., Slangen, A., Sokona, Y., Sörensson, A.A., Tignor, M., Van Vuuren, D., Wei, Y.-M., Winkler, H., Zhai, P., Zommers, Z., Hourcade, J.-C., Johnson, F.X., Pachauri, S., Simpson, N.P., Singh, C., Thomas, A., Totin, E., Arias, P., Bustamante, M., Elgizouli, I., Flato, G., Howden, M., Méndez-Vallejo, C., Pereira, J.J., Pichs-Madruga, R., Rose, S.K., Saheb, Y., Sánchez Rodríguez, R., Ürge-Vorsatz, D., Xiao, C., Yassaa, N., Alegría, A., Armour, K., Bednar-Friedl, B., Blok, K., Cissé, G., Dentener, F., Eriksen, S., Fischer, E., Garner, G., Guivarch, C., Haasnoot, M., Hansen, G., Hauser, M., Hawkins, E., Hermans, T., Kopp, R., Leprince-Ringuet, N., Lewis, J., Ley, D., Ludden, C., Niamir, L., Nicholls, Z., Some, S., Szopa, S., Trewin, B., Van Der Wijst, K.-I., Winter, G., Witting, M., Birt, A., Ha, M., Romero, J., Kim, J., Haïtes, E.F., Jung, Y., Stavins, R., Birt, A., Ha, M., Orendain, D.J.A., Ignon, L., Park, S., Park, Y., Reisinger, A., Cammaramo, D., Fischlin, A., Fuglestedt, J.S., Hansen, G., Ludden, C., Masson-Delmotte, V., Matthews, J.B.R., Mintenbeck, K., Pirani, A., Poloczanska, E., Leprince-Ringuet, N., Péan, C., 2023. IPCC, 2023: Sections. In: *Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland. Intergovernmental Panel on Climate Change (IPCC). <https://doi.org/10.59327/IPCC/AR6-9789291691647>

- Canal Filho, R., Molin, J.P., Wei, M.C.F., Silva, E.R.O.D.A., 2023. Soil Attributes Mapping with Online Near-Infrared Spectroscopy Requires Spatio-Temporal Local Calibrations. *AgriEngineering* v.5, 1163–1177,. <https://doi.org/10.3390/agriengineering5030074>.
- Castaldi, F., 2021. Sentinel-2 and Landsat-8 multi-temporal series to estimate topsoil properties on croplands. *Remote Sens.* 13, 3345.
- Castaldi, F., Chabrillat, S., Don, A., Wesemael, B., 2019. Soil organic carbon mapping using LUCAS topsoil database and Sentinel-2 data: An approach to reduce soil moisture and crop residue effects. *Remote Sens* 11, 2121.
- Chen, Y., Tian, W., Shao, Y., Li, Y.-J., Lin, L.-A., Zhang, Y.-J., Han, H., Chen, Z.-J., 2020. Miscanthus cultivation shapes rhizosphere microbial community structure and function as assessed by Illumina MiSeq sequencing combined with PICRUSt and FUNGUld analyses. *Arch. Microbiol.* 202, 1157–1171. <https://doi.org/10.1007/s00203-020-01830-1>
- Chimento, C., Almagro, M., Amaducci, S., 2016. Carbon sequestration potential in perennial bioenergy crops: the importance of organic matter inputs and its physical protection. *GCB Bioenergy* 8, 111–121. <https://doi.org/10.1111/gcbb.12232>
- Chimento, C., Amaducci, S., 2015. Characterization of fine root system and potential contribution to soil organic carbon of six perennial bioenergy crops. *Biomass Bioenergy* 83, 116–122. <http://dx.doi.org/10.1016/j.biombioe.2015.09.008>
- Christian, D., Poulton, P., Riche, A., Yates, N., Todd, A., 2006. The recovery over several seasons of ¹⁵N-labelled fertilizer applied to *Miscanthusxgiganteus* ranging from 1 to 3 years old. *Biomass Bioenergy* 30, 125–133. <https://doi.org/10.1016/j.biombioe.2005.11.002>
- Clarke, D.E., Stockdale, E., Hannam, J.A., Marchant, B.P., Hallett, S.H., 2024. Spatial-temporal variability in nitrogen use efficiency: Insights from a long-term experiment and crop simulation modeling to support site specific nitrogen management. *Eur. J. Agron.* 158. <https://doi.org/10.1016/j.eja.2024.127224>
- Clifton-Brown, J., Hastings, A., Mos, M., McCalmont, J.P., Ashman, C., Awty-Carroll, D., Cerazy, J., Chiang, Y., Cosentino, S., Cracroft-Eley, W., Scurlock, J., Donnison, I.S., Glover, C., Gołab, I., Greef, J.M., Gwyn, J., Harding, G., Hayes, C., Helios, W., Hsu, T., Huang, L.S., Jeżowski, S., Kim, D., Kiesel, A., Kotecki, A., Krzyzak, J., Lewandowski, I., Lim, S.H., Liu, J., Loosely, M., Meyer, H., Murphy-Bokern, D., Nelson, W., Pogrzeba, M., Robinson, G., Robson, P., Rogers, C., Scalici, G., Schuele, H., Shafiei, R., Shevchuk, O., Schwarz, K., Squance, M., Swaller, T., Thornton, J., Truckses, T., Botnari, V., Vizir, I., Wagner, M., Warren, R., Webster, R., Yamada, T., Youell, S., Xi, Q., Zong, J., Flavell, R., 2017. Progress in upscaling *Miscanthus* biomass production for the European bio-economy with seed-based hybrids. *GCB Bioenergy* 9, 6–17. <https://doi.org/10.1111/gcbb.12357>
- Clifton-Brown, J., Hastings, A., von Cossel, M., Murphy-Bokern, D., McCalmont, J., Whitaker, J., Alexopoulou, E., Amaducci, S., Andronic, L., Ashman, C., Awty-Carroll, D., Bhatia, R., Breuer, L., Cosentino, S., Cracroft-Eley, W., Donnison, I., Elbersen, B., Ferrarini, A., Ford, J., Greef, J., Ingram, J., Lewandowski, I., Magenau, E., Mos, M., Petrick, M., Pogrzeba, M., Robson, P., Rowe, R.L., Sandu, A., Schwarz, K.-U., Scordia, D., Scurlock, J., Shepherd, A., Thornton, J., Trindade, L.M., Vetter, S., Wagner, M., Wu, P.-C., Yamada, T., Kiesel, A., 2023. Perennial biomass cropping and use: Shaping the policy ecosystem in European countries. *GCB Bioenergy* 15, 538–558. <https://doi.org/10.1111/gcbb.13038>
- Clifton-Brown, J.C., Breuer, J., Jones, M.B., 2007. Carbon mitigation by the energy crop, *Miscanthus*. *Glob. Change Biol.* 13, 2296–2307. <https://doi.org/10.1111/j.1365-2486.2007.01438.x>
- Coleman, K., Jenkinson, D.S., 1996. RothC-26.3 - A Model for the turnover of carbon in soil, in: Powlson, D.S., Smith, P., Smith, J.U. (Eds.), *Evaluation of Soil Organic Matter Models*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 237–246. https://doi.org/10.1007/978-3-642-61094-3_17
- Coonan, E.C., Kirkby, C.A., Kirkegaard, J.A., Amidy, M.R., Strong, C.L., Richardson, A.E., 2020a. Microorganisms and nutrient stoichiometry as mediators of soil organic matter dynamics. *Nutr. Cycl. Agroecosystems* 117, 273–298. <https://doi.org/10.1007/s10705-020-10076-8>
- Coonan, E.C., Richardson, A.E., Kirkby, C.A., Kirkegaard, J.A., Amidy, M.R., Strong, C.L., 2020b. Soil fertility and nutrients mediate soil carbon dynamics following residue incorporation. *Nutr. Cycl. Agroecosystems* 116, 205–221. <https://doi.org/10.1007/s10705-019-10037-w>
- Corwin, D.L., Lesch, S.M., 2005. Characterizing soil spatial variability with apparent soil electrical conductivity; in: *Survey Protocols, Computers and Electronics in Agriculture*. pp. 1-3,2005, 103–133,. <https://doi.org/10.1016/j.compag.2004.11.002>.
- Cotrufo, M.F., Haddix, M.L., Kroeger, M.E., Stewart, C.E., 2022. The role of plant input physical-chemical properties, and microbial and soil chemical diversity on the formation of particulate and mineral-associated organic matter. *Soil Biol. Biochem.* 168, 108648. <https://doi.org/10.1016/j.soilbio.2022.108648>
- Cotrufo, M.F., Ranalli, M.G., Haddix, M.L., Six, J., Lugato, E., 2019. Soil carbon storage informed by particulate and mineral-associated organic matter. *Nat. Geosci.* 12, 989–994. <https://doi.org/10.1038/s41561-019-0484-6>
- COWI, Ecologic Institute, IEEP, 2021. Technical guidance handbook - Setting up and implementing result-based carbon farming mechanisms in the EU. Report to the European Commission, DG Climate Action, under Contract No.

CLIMA/C.3/ETU/2018/007. COWI, Kongens Lyngby.

Cowie, A.L., Lonergan, V.E., Rabbi, S.M.F., Fornasier, F., Macdonald, C., Harden, S., Kawasaki, A., Singh, B.K., 2013. Impact of carbon farming practices on soil carbon in northern New South Wales. *Soil Res.* 51, 707. <https://doi.org/10.1071/SR13043>

Cox, T.S., Glover, J.D., Van Tassel, D.L., Cox, C.M., DeHAAN, L.R., 2006. Prospects for Developing Perennial Grain Crops. *BioScience* 56, 649. [https://doi.org/10.1641/0006-3568\(2006\)56\[649:PFDPGC\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2006)56[649:PFDPGC]2.0.CO;2)

Croci, M., Impollonia, G., Marcone, A., Antonucci, G., Letterio, T., Colauzzi, M., Vignudelli, M., Ventura, F., Anconelli, S., Amaducci, S., 2022. RTM Inversion through Predictive Equations for Multi-Crop LAI Retrieval Using Sentinel-2 Images. *Agronomy* 12, 2835. <https://doi.org/10.3390/agronomy12112835>

Croft, G.K., Hoover, K., Ramseur, J.L., Stubbs, M., 2021. Agriculture and forestry offsets in carbon markets: background and selected issues.

Dămățircă, C., Moretti, B., Bertora, C., Ferrarini, A., Zavattaro, L., 2023. Residue incorporation and organic fertilisation improve carbon and nitrogen turnover and stabilisation in maize monocropping 15.

Das, A., Lal, R., Somireddy, U., Bonin, C., Verma, S., Rimal, B.K., 2016. Changes in soil quality and carbon storage under biofuel crops in central Ohio. *Soil Res.* 54, 371. <https://doi.org/10.1071/SR14353>

Davis, S.C., Parton, W.J., Dohleman, F.G., Smith, C.M., Grosso, S.D., Kent, A.D., DeLucia, E.H., 2010. Comparative Biogeochemical Cycles of Bioenergy Crops Reveal Nitrogen-Fixation and Low Greenhouse Gas Emissions in a *Miscanthus × giganteus* Agro-Ecosystem. *Ecosystems* 13, 144–156. <https://doi.org/10.1007/s10021-009-9306-9>

de Brogniez, D., Ballabio, C., Stevens, A., Jones, R.J.A., Montanarella, L., Wesemael, B., 2015. A map of the topsoil organic carbon content of Europe generated by a generalized additive model. *Eur. J. Soil Sci.* 66, 121–134. <https://doi.org/10.1111/ejss.12193>

De Vos, B., Meirvenne, M., Quataert, P., Deckers, J., Muys, B., 2005. Predictive quality of pedotransfer functions for estimating bulk density of forest soils. *Soil Sci. Soc. Am. J.* 69, 500–510.

Dechow, R., Franko, U., Kätterer, T., Kolbe, H., 2019. Evaluation of the RothC model as a prognostic tool for the prediction of SOC trends in response to management practices on arable land. *Geoderma* 337, 463–478. <https://doi.org/10.1016/j.geoderma.2018.10.001>

Delgado-Baquerizo, M., Reich, P.B., Khachane, A.N., Campbell, C.D., Thomas, N., Freitag, T.E., Abu Al-Soud, W., Sørensen, S., Bardgett, R.D., Singh, B.K., 2017. It is elemental: soil nutrient stoichiometry drives bacterial diversity. *Environ. Microbiol.* 19, 1176–1188. <https://doi.org/10.1111/1462-2920.13642>

De Rosa, D., Ballabio, C., Lugato, E., Fasiolo, M., Jones, A., Panagos, P., 2023. Soil organic carbon stocks in European croplands and grasslands: How much have we lost in the past decade? *Glob. Change Biol.* 30, e16992. <https://doi.org/10.1111/gcb.16992>

Diacono, M., Montemurro, F., 2011. Long-Term Effects of Organic Amendments on Soil Fertility, in: Lichtfouse, E., Hamelin, M., Navarrete, M., Debaeke, P. (Eds.), *Sustainable Agriculture Volume 2*. Springer Netherlands, Dordrecht, pp. 761–786. https://doi.org/10.1007/978-94-007-0394-0_34

Diek, S., Fornallaz, F., Schaepman, M.E., Jong, R., 2017. Barest Pixel Composite for Agricultural Areas Using Landsat Time Series. *Remote Sens* 9, 1245. <https://doi.org/10.3390/rs9121245>

Doane, T.A., Horwath, W.R., 2003. Spectrophotometric Determination of Nitrate with a Single Reagent. *Anal. Lett.* 36, 2713–2722. <https://doi.org/10.1081/AL-120024647>

Don, A., Osborne, B., Hastings, A., Skiba, U., Carter, M.S., Drewer, J., Flessa, H., Freibauer, A., Hyvönen, N., Jones, M.B., Lanigan, G.J., Mander, Ü., Monti, A., Djomo, S.N., Valentine, J., Walter, K., Zegada-Lizarazu, W., Zenone, T., 2012. Land-use change to bioenergy production in Europe: implications for the greenhouse gas balance and soil carbon. *GCB Bioenergy* 4, 372–391. <https://doi.org/10.1111/j.1757-1707.2011.01116.x>

Don, A., Seidel, F., Leifeld, J., Kätterer, T., Martin, M., Pellerin, S., Emde, D., Seitz, D., Chenu, C., 2023. Carbon sequestration in soils and climate change mitigation—Definitions and pitfalls. *Glob. Change Biol.* e16983. <https://doi.org/10.1111/gcb.16983>

Dondini, M., Hastings, A., Saiz, G., Jones, M.B., Smith, P., 2009. The potential of *Miscanthus* to sequester carbon in soils: comparing field measurements in Carlow, Ireland to model predictions. *GCB Bioenergy* 1, 413–425. <https://doi.org/10.1111/j.1757-1707.2010.01033.x>

Dorning, M., Nicholson, M., Almedia, I., 2021. The carbon market goldrush in American agriculture. *Bloom. News*.

Dvorakova, K., Heiden, U., Pepers, K., Staats, G., Os, G., Wesemael, B., 2023. Improving soil organic carbon predictions from a sentinel-2 soil composite by assessing surface conditions and uncertainties. *Geoderma* 429, 116128.

Dynarski, K.A., Bossio, D.A., Scow, K.M., 2020. Dynamic Stability of Soil Carbon: Reassessing the “Permanence” of Soil Carbon Sequestration. *Front. Environ. Sci.* 8, 514701. <https://doi.org/10.3389/fenvs.2020.514701>

Dzeletovic, Z., Glamoclija, D., 2015. Effect of nitrogen on the distribution of biomass and element composition of the root system of *Miscanthus × giganteus*. *Arch. Biol. Sci.* 67, 547–560. <https://doi.org/10.2298/ABS141010017D>

E.C., 2022. COMMISSION STAFF WORKING DOCUMENT EXECUTIVE SUMMARY OF THE IMPACT ASSESSMENT REPORT

- Accompanying the document - Proposal for a Regulation of the European Parliament and of the Council establishing a Union certification framework for carbon removals. <https://doi.org/10.5040/9781782258674>
- E.C. DG Environment, 2011. Soil :the hidden part of the climate cycle. Publications Office of the European Union, LU.
- Egnér, H., Riehm, H., Domingo, W., 1960. Studies on chemical soil analysis as a basis for assessing the nutrient status of the soil. II. Chem. Extr. Methods Determining Phosphorus Potassium 26, 199–215.
- Emmerling, C., Schmidt, A., Ruf, T., Francken-Welz, H., Thielen, S., 2017. Impact of newly introduced perennial bioenergy crops on soil quality parameters at three different locations in W-Germany. *J. Plant Nutr. Soil Sci.* 180, 759–767. <https://doi.org/10.1002/jpln.201700162>
- England, J.R., Viscarra-Rossel, R.A., 2018. Proximal sensing for soil carbon accounting. *The Soil* 4, 101–122. <https://doi.org/10.5194/soil-4-101-2018>
- EU Nature Restoration Regulation, 2024.
- European Commission, 2021. Sustainable carbon cycles. Communication from the Commission to the European Parliament and the Council.
- European Parliament and Council of the European Union., 2024. REGULATION (EU) 2024/3012 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 27 November 2024 establishing a Union certification framework for permanent carbon removals, carbon farming and carbon storage in products.
- Fang, Y., Singh, B.P., Collins, D., Armstrong, R., Van Zwieten, L., Tavakkoli, E., 2020. Nutrient stoichiometry and labile carbon content of organic amendments control microbial biomass and carbon-use efficiency in a poorly structured sodic-subsoil. *Biol. Fertil. Soils* 56, 219–233. <https://doi.org/10.1007/s00374-019-01413-3>
- Fang, Y., Singh, B.P., Cowie, A., Wang, W., Arachchi, M.H., Wang, H., Tavakkoli, E., 2019. Balancing nutrient stoichiometry facilitates the fate of wheat residue-carbon in physically defined soil organic matter fractions. *Geoderma* 354, 113883. <https://doi.org/10.1016/j.geoderma.2019.113883>
- FAO, 2020. A protocol for measurement, monitoring, reporting and verification of soil organic carbon in agricultural landscapes. FAO. <https://doi.org/10.4060/cb0509en>
- Farina, R., Coleman, K., Whitmore, A.P., 2013. Modification of the RothC model for simulations of soil organic C dynamics in dryland regions. *Geoderma* 200–201, 18–30. <https://doi.org/10.1016/j.geoderma.2013.01.021>
- Feng, X., Qin, S., Zhang, D., Chen, P., Hu, J., Wang, G., Liu, Y., Wei, B., Li, Q., Yang, Y., Chen, L., 2022. Nitrogen input enhances microbial carbon use efficiency by altering plant–microbe–mineral interactions. *Glob. Change Biol.* 28, 4845–4860. <https://doi.org/10.1111/gcb.16229>
- Fernández-Ugalde, O., Orgiazzi, A., Jones, A., Lugato, E., Panagos, P., 2018. LUCAS 2018 –SOIL COMPONENT: Sampling Instructions for Surveyors, in: EUR 28501 EN. <https://doi.org/10.2760/023673>
- Ferrarini, A., Fornasier, F., Serra, P., Ferrari, F., Trevisan, M., Amaducci, S., 2017. Impacts of willow and miscanthus bioenergy buffers on biogeochemical N removal processes along the soil–groundwater continuum. *Gcb Bioenergy* 9, 246–261.
- Ferrarini, A., Martani, E., Fornasier, F., Amaducci, S., 2021. High C input by perennial energy crops boosts belowground functioning and increases soil organic P content. *Agric. Ecosyst. Environ.* 308, 107247. <https://doi.org/10.1016/j.agee.2020.107247>
- Ferrarini, A., Martani, E., Mondini, C., Fornasier, F., Amaducci, S., 2022. Short-Term Mineralization of Belowground Biomass of Perennial Biomass Crops after Reversion to Arable Land. *Agronomy* 12, 485. <https://doi.org/10.3390/agronomy12020485>
- Fiorini, A., Maris, S.C., Abalos, D., Amaducci, S., Tabaglio, V., 2020. Combining no-till with rye (*Secale cereale* L.) cover crop mitigates nitrous oxide emissions without decreasing yield. *Soil Tillage Res.* 196, 104442. <https://doi.org/10.1016/j.still.2019.104442>
- Fornasier, F., Ascher, J., Ceccherini, M.T., Tomat, E., Pietramellara, G., 2014. A simplified rapid, low-cost and versatile DNA-based assessment of soil microbial biomass. *Ecol. Indic.* 45, 75–82. <https://doi.org/10.1016/j.ecolind.2014.03.028>
- Fowler, A.F., Basso, B., Millar, N., Brinton, W.F., 2023. A simple soil mass correction for a more accurate determination of soil carbon stock changes. *Sci. Rep.* 13, 2242. <https://doi.org/10.1038/s41598-023-29289-2>
- Freeman, E., Fresco, T., 2023. ModelMap: Modeling and Map Production using Random Forest and Related Stochastic Models.
- Gammoudi, N., Kovács, J., Gresina, F., Varga, G., 2024. Combined use of HYSPLIT model and MODIS aerosols optical depth to study the spatiotemporal circulation patterns of Saharan dust events over Central Europe. *Aeolian Res.* 67–69, 100899. <https://doi.org/10.1016/j.aeolia.2024.100899>
- Garsia, A., Moinet, A., Vazquez, C., Creamer, R.E., Moinet, G.Y.K., 2023. The challenge of selecting an appropriate soil organic carbon simulation model: A comprehensive global review and validation assessment. *Glob. Change Biol.* gcb.16896. <https://doi.org/10.1111/gcb.16896>
- Gaspar, M.L., Cabello, M.N., Pollero, R., Aon, M.A., 2001. Fluorescein Diacetate Hydrolysis as a Measure of Fungal Biomass in Soil. *Curr. Microbiol.* 42, 339–344. <https://doi.org/10.1007/s002840010226>

- Georgiou, K., Jackson, R.B., Vinduřková, O., Abramoff, R.Z., Ahlström, A., Feng, W., Harden, J.W., Pellegrini, A.F.A., Polley, H.W., Soong, J.L., Riley, W.J., Torn, M.S., 2022. Global stocks and capacity of mineral-associated soil organic carbon. *Nat. Commun.* 13, 3797. <https://doi.org/10.1038/s41467-022-31540-9>
- Gholizadeh, A., Viscarra Rossel Raphael, A., Saberioon, M., Borůvka, L., Kratina, J., Pavlů, L., 2021. National-scale spectroscopic assessment of soil organic carbon in forests of the Czech Republic. *Geoderma* 385, 0016-7061. <https://doi.org/10.1016/j.geoderma.2020.114832>.
- Gholizadeh, A., Žižala, D., Saberioon, M., Borůvka, L., 2018. Soil organic carbon and texture retrieving and mapping using proximal, airborne and Sentinel-2 spectral imaging(2018. *Remote Sens. Environ.* 218, 89-103,. <https://doi.org/10.1016/j.rse.2018.09.015>
- Glover, J.D., Cox, C.M., Reganold, J.P., 2007. Future Farming: A Return to Roots? *Sci. Am.* 297, 82–89. <https://doi.org/10.1038/scientificamerican0807-82>
- Gogé, F., Gomez, C., Jolivet, C., Joffre, R., 2014. Which strategy is best to predict soil properties of a local site from a national Vis–NIR database? *Geoderma* 213,2014,Pages, 1-9,. <https://doi.org/10.1016/j.geoderma.2013.07.016>.
- Goidts, E., Wesemael, b., Crucifix, M., 2009. Magnitude and sources of uncertainties in soil organic carbon (SOC) stock assessments at various scales *Eur. J Soil Sci* 60, 723-739,. <https://doi.org/10.1111/j.1365-2389.2009.01157.x>
- Guan, K., Jin, Z., Peng, B., Tang, J., DeLucia, E.H., West, P.C., Jiang, C., Wang, S., Kim, T., Zhou, W., Griffis, T., Liu, L., Yang, W.H., Qin, Z., Yang, Q., Margenot, A., Stuchiner, E.R., Kumar, V., Bernacchi, C., Coppess, J., Novick, K.A., Gerber, J., Jahn, M., Khanna, M., Lee, D., Chen, Z., Yang, S.-J., 2023. A scalable framework for quantifying field-level agricultural carbon outcomes. *Earth-Sci. Rev.* 243, 104462. <https://doi.org/10.1016/j.earscirev.2023.104462>
- Guilayn, F., Rouez, M., Crest, M., Patureau, D., Jimenez, J., 2020. Valorization of digestates from urban or centralized biogas plants: a critical review. *Rev. Environ. Sci. Biotechnol.* 19, 419–462. <https://doi.org/10.1007/s11157-020-09531-3>
- Guillaume, T., Makowski, D., Libohova, Z., Bragazza, L., Sallaku, F., Sinaj, S., 2022. Soil organic carbon saturation in cropland-grassland systems: Storage potential and soil quality. *Geoderma* 406, 115529. <https://doi.org/10.1016/j.geoderma.2021.115529>
- Gurung, R.B., Ogle, S.M., FJ, B., 2020. Bayesian calibration of the DayCent ecosystem model to simulate soil organic carbon dynamics and reduce model uncertainty. *Geoderma* 376, 114529. <https://doi.org/10.1016/j.geoderma.2020>.
- Guy, A.L., Siciliano, S.D., Lamb, E.G., 2015. Spiking regional vis-NIR calibration models with local samples to predict soil organic carbon in two High Arctic polar deserts using a vis-NIR probe. *Can. J. Soil Sci.* 95, 237–249. <https://doi.org/10.4141/cjss-2015-004>
- Guzman, J.G., Ussiri, D.A., Lal, R., 2019. Soil physical properties following conversion of a reclaimed minesoil to bioenergy crop production. *Catena* 176, 289–295.
- Hallama, M., Pekrun, C., Lambers, H., Kandeler, E., 2019. Hidden miners – the roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems. *Plant Soil* 434, 7–45. <https://doi.org/10.1007/s11104-018-3810-7>
- Hargreaves, P., Baker, K., Graceson, A., Bonnett, S., Ball, B., Cloy, J., 2019. Soil compaction effects on grassland silage yields and soil structure under different levels of compaction over three years. *Eur. J. Agron.* 109, 125916.
- Hargreaves, S.K., Hofmockel, K.S., 2014. Physiological shifts in the microbial community drive changes in enzyme activity in a perennial agroecosystem. *Biogeochemistry* 117, 67–79. <https://doi.org/10.1007/s10533-013-9893-6>
- Heuvelink, G., 2014. Uncertainty quantification of GlobalSoilMap products. In:GlobalSoilMap. CRC Press. <https://doi.org/10.1201/b16500-62>
- Heuvelink, G.B.M., Angelini, M.E., Poggio, L., 2021. Machine learning in space and time for modelling soil organic carbon change. *Eur J Soil Sci* 72, 1607–1623. <https://doi.org/10.1111/ejss.12998>
- Hillel, D., Hatfield, J.L. (Eds.), 2005. *Encyclopedia of soils in the environment*, 1st ed. ed. Elsevier/Academic Press, Oxford, UK ; Boston.
- Hillier, J., Walter, C., Malin, D., Garcia-Suarez, T., Mila-i-Canals, L., Smith, P., 2011. A farm-focused calculator for emissions from crop and livestock production. *Environ. Model. Softw.* 26, 1070–1078. <https://doi.org/10.1016/j.envsoft.2011.03.014>
- Hoffland, E., Kuyper, T.W., Comans, R.N.J., Creamer, R.E., 2020. Eco-functionality of organic matter in soils. *Plant Soil* 455, 1–22. <https://doi.org/10.1007/s11104-020-04651-9>
- Hu, Q., Liu, T., Ding, H., Li, C., Tan, W., Yu, M., Liu, J., Cao, C., 2023. Effects of nitrogen fertilizer on soil microbial residues and their contribution to soil organic carbon and total nitrogen in a rice-wheat system. *Appl. Soil Ecol.* 181, 104648. <https://doi.org/10.1016/j.apsoil.2022.104648>
- Hua, K., Wang, D., Guo, X., Guo, Z., 2014. Carbon Sequestration Efficiency of Organic Amendments in a Long-Term Experiment on a Vertisol in Huang-Huai-Hai Plain, China. *PLoS ONE* 9, e108594. <https://doi.org/10.1371/journal.pone.0108594>
- Impollonia, G., Croci, M., Ferrarini, A., Brook, J., Martani, E., Blandinières, H., Marcone, A., Awty-Carroll, D., Ashman, C., Kam, J., Kiesel, A., Trindade, L.M., Boschetti, M., Clifton-Brown, J., Amaducci, S., 2022a. UAV Remote Sensing for High-

Throughput Phenotyping and for Yield Prediction of *Miscanthus* by Machine Learning Techniques. *Remote Sens.* 14. <https://doi.org/10.3390/rs14122927>

Impollonia, G., Croci, M., Martani, E., Ferrarini, A., Kam, J., Trindade, L.M., Clifton-Brown, J., Amaducci, S., 2022b. Moisture content estimation and senescence phenotyping of novel *Miscanthus* hybrids combining UAV-based remote sensing and machine learning. *GCB Bioenergy* 14, 639–656. <https://doi.org/10.1111/gcbb.12930>

Islam, M., Ferrarini, A., Ali, A., Kam, J., Trindade, L.M., Clifton-Brown, J., Amaducci, S., 2023. Assessment of Drought and Zinc Stress Tolerance of Novel *Miscanthus* Hybrids and *Arundo donax* Clones Using Physiological, Biochemical, and Morphological Traits. *Biology* 12. <https://doi.org/10.3390/biology12121525>

Jackson Hammond, A.A., Motew, M., Brummitt, C.D., DuBuisson, M.L., Pinjuv, G., Harburg, D.V., Campbell, E.E., Kumar, A.A., 2021. Implementing the Soil Enrichment Protocol at Scale: Opportunities for an Agricultural Carbon Market. *Front. Clim.* 3, 686440. <https://doi.org/10.3389/fclim.2021.686440>

Jordon, M.W., Smith, P., 2022. Modelling soil carbon stocks following reduced tillage intensity: A framework to estimate decomposition rate constant modifiers for RothC-26.3, demonstrated in north-west Europe. *Soil Tillage Res.* 222, 105428. <https://doi.org/10.1016/j.still.2022.105428>

Jordon, M.W., Smith, P., Long, P.R., Bürkner, P.-C., Petrokofsky, G., Willis, K.J., 2022. Can Regenerative Agriculture increase national soil carbon stocks? Simulated country-scale adoption of reduced tillage, cover cropping, and ley-arable integration using RothC. *Sci. Total Environ.* 825, 153955. <https://doi.org/10.1016/j.scitotenv.2022.153955>

Kahle, P., Beuch, S., Boelcke, B., Leinweber, P., Schulten, H.-R., 2001. Cropping of *Miscanthus* in Central Europe: biomass production and influence on nutrients and soil organic matter. *Eur. J. Agron.* 15, 171–184. [https://doi.org/10.1016/S1161-0301\(01\)00102-2](https://doi.org/10.1016/S1161-0301(01)00102-2)

Kahle, P., Boelcke, B., Zacharias, S., 1999. Effects of *Miscanthus x giganteus* cultivation on chemical and physical soil properties. *J. Plant Nutr. Soil Sci.-Z. Pflanzenernahrung Bodenkd.* 162, 27–32.

Kakhani, N., Alamdar, S., Kebonye, N.M., Amani, M., Scholten, T., 2024. Uncertainty Quantification of Soil Organic Carbon Estimation from Remote Sensing Data with Conformal Prediction. *Remote Sens* 16, 438. <https://doi.org/10.3390/rs16030438>

Kallenbach, C.M., Grandy, A.S., Frey, S.D., Diefendorf, A.F., 2015. Microbial physiology and necromass regulate agricultural soil carbon accumulation. *Soil Biol. Biochem.* 91, 279–290. <https://doi.org/10.1016/j.soilbio.2015.09.005>

Kallenbach, C.M., Wallenstein, M.D., Schipanski, M.E., Grandy, A.S., 2019. Managing Agroecosystems for Soil Microbial Carbon Use Efficiency: Ecological Unknowns, Potential Outcomes, and a Path Forward. *Front. Microbiol.* 10, 1146. <https://doi.org/10.3389/fmicb.2019.01146>

Kane, J.L., Schartiger, R.G., Daniels, N.K., Freedman, Z.B., McDonald, L.M., Skousen, J.G., Morrissey, E.M., 2023. Bioenergy crop *Miscanthus x giganteus* acts as an ecosystem engineer to increase bacterial diversity and soil organic matter on marginal land. *Soil Biol. Biochem.* 186, 109178. <https://doi.org/10.1016/j.soilbio.2023.109178>

Kasraei, B., Heung, B., Saurette Daniel, D., Schmidt Margaret, V., Bulmer Chuck, E., Bethel, W., 2021. Quantile regression as a generic approach for estimating uncertainty of digital soil maps produced from machine-learning. *Environ. Model. Softw.* 144. <https://doi.org/10.1016/j.envsoft.2021.105139>

Kästner, M., Miltner, A., Thiele-Bruhn, S., Liang, C., 2021. Microbial Necromass in Soils—Linking Microbes to Soil Processes and Carbon Turnover. *Front. Environ. Sci.* 9, 756378. <https://doi.org/10.3389/fenvs.2021.756378>

Kell, D.B., 2011. Breeding crop plants with deep roots: their role in sustainable carbon, nutrient and water sequestration. *Ann. Bot.* 108, 407–418. <https://doi.org/10.1093/aob/mcr175>

Kennard, R.W., Stone, L.A., 1969. Computer aided design of experiments. *Technometrics* 11, 137–148.

Kirkby, C.A., Richardson, A.E., Wade, L.J., Batten, G.D., Blanchard, C., Kirkegaard, J.A., 2013. Carbon-nutrient stoichiometry to increase soil carbon sequestration. *Soil Biol. Biochem.* 60, 77–86. <https://doi.org/10.1016/j.soilbio.2013.01.011>

Kirkby, C.A., Richardson, A.E., Wade, L.J., Conyers, M., Kirkegaard, J.A., 2016. Inorganic Nutrients Increase Humification Efficiency and C-Sequestration in an Annually Cropped Soil. *PLOS ONE* 11, e0153698. <https://doi.org/10.1371/journal.pone.0153698>

Kirkby, C.A., Richardson, A.E., Wade, L.J., Passioura, J.B., Batten, G.D., Blanchard, C., Kirkegaard, J.A., 2014. Nutrient availability limits carbon sequestration in arable soils. *Soil Biol. Biochem.* 68, 402–409. <https://doi.org/10.1016/j.soilbio.2013.09.032>

Kirkegaard, J.A., Richardson, A.E., Kirkby, C.A., 2023. Fate and cost effectiveness of soil carbon sequestered using supplementary nutrients applied to crop residues under field conditions. *Nutr. Cycl. Agroecosystems* 126, 143–161. <https://doi.org/10.1007/s10705-023-10272-2>

Kok, M., Sarjant, S., Verweij, S., Vaessen Stefan, F.C., Ros Gerard, H., 2024. On-site soil analysis: A novel approach combining NIR spectroscopy, remote sensing and deep learning. *Geoderma* 446. <https://doi.org/10.1016/j.geoderma.2024.116903>

Kuang, B., Tekin, Y., Mouazen, A.M., 2015. Comparison between artificial neural network and partial least squares for

on-line visible and near infrared spectroscopy measurement of soil organic carbon, pH and clay content. *Soil Tillage Res.* 146, 243–252.

Kuzyakov, Y., 2002. Factors affecting rhizosphere priming effects. *J. Plant Nutr. Soil Sci.* 165, 382–396.

Kweon, G., 2012. Toward the Ultimate Soil Survey: Sensing Multiple Soil and Landscape Properties in One Pass. *Agron J* 104, 1547–1557.

Kyker-Snowman, E., Lombardozi, D.L., GB, B., 2022. Increasing the spatial and temporal impact of ecological research: a roadmap for integrating a novel terrestrial process into an Earth system model. *Glob Change Biol* 28, 665–684. <https://doi.org/10.1111/gcb.15894>

Lagacherie, P., Arrouays, D., Bourennane, H., Gomez, C., Nkuba-Kasanda, L., 2019. Analysing the impact of soil spatial sampling on the performances of Digital Soil Mapping models and their evaluation: A numerical experiment on Quantile Random Forest using clay contents obtained from Vis-NIR-SWIR hyperspectral imagery. *Geoderma* 375, 0016–7061. <https://doi.org/10.1016/j.geoderma.2020.114503>.

Lagomarsino, A., Moscatelli, M.C., Di Tizio, A., Mancinelli, R., Grego, S., Marinari, S., 2009. Soil biochemical indicators as a tool to assess the short-term impact of agricultural management on changes in organic C in a Mediterranean environment. *Ecol. Indic.* 9, 518–527. <https://doi.org/10.1016/j.ecolind.2008.07.003>

Lajtha, K., Driscoll, C.T., Jarrel, W.M., Elliott, E.T., 1999. Soil phosphorus: characterization and total element analysis., in: *Standard Soil Methods for Long-Term Ecological Research*. Oxford University Press, New York., pp. 115–142.

Lal, R., 2015. Restoring Soil Quality to Mitigate Soil Degradation. *Sustainability* 7, 5875–5895. <https://doi.org/10.3390/su7055875>

Lal, R., 2009. Challenges and opportunities in soil organic matter research. *Eur. J. Soil Sci.* 60, 158–169. <https://doi.org/10.1111/j.1365-2389.2008.01114.x>

Lal, R., 2004a. Soil Carbon Sequestration Impacts on Global Climate Change and Food Security. *Science* 304, 1623–1627. <https://doi.org/10.1126/science.1097396>

Lal, R., 2004b. Soil carbon sequestration to mitigate climate change. *Geoderma* 123, 1–22. <https://doi.org/10.1016/j.geoderma.2004.01.032>

Lavallee, J.M., McConkey, B.G., Kyker-Snowman, E., Dietze, M.C., Harrison, M.T., Liu, K., Smith, W., Grant, B., Karunaratne, S., Marklein, A., 2024. Modeling Soil Carbon and Greenhouse Gas Emissions: Identifying challenges and advancing guidance for using process-based models in soil emission reduction and removal projects.

Lavallee, J.M., Soong, J.L., Cotrufo, M.F., 2020. Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. *Glob. Change Biol.* 26, 261–273. <https://doi.org/10.1111/gcb.14859>

Lee, J., Hopmans, J.W., Rolston, D.E., Baer, S.G., Six, J., 2009. Determining soil carbon stock changes: Simple bulk density corrections fail. *Agric. Ecosyst. Environ.* 134, 251–256. <https://doi.org/10.1016/j.agee.2009.07.006>

Lemus, R., Lal, R., 2005. Bioenergy Crops and Carbon Sequestration. *Crit. Rev. Plant Sci.* 24, 1–21. <https://doi.org/10.1080/07352680590910393>

Lenth, R., Singmann, H., Love, J., Buerkner, P., Herve, M., 2018. Emmeans: Estimated marginal means. AKA Least-Sq. Means 2–1.

Lesschen, J.P., 2024. CARBON REMOVALS EXPERT GROUP TECHNICAL ASSISTANCE Agriculture on mineral soils – Technical Assessment Paper. EU climate action.

Li, J., Wu, X., Gebremikael, M.T., Wu, H., Cai, D., Wang, B., Li, B., Zhang, J., Li, Y., Xi, J., 2018. Response of soil organic carbon fractions, microbial community composition and carbon mineralization to high-input fertilizer practices under an intensive agricultural system. *PLOS ONE* 13, e0195144. <https://doi.org/10.1371/journal.pone.0195144>

Li, S., A., V.R.R., Richard, W., 2022. The cost-effectiveness of reflectance spectroscopy for estimating soil organic carbon. *Eur. J. Soil Sci.* 73.

Li, T., Xia, A., McLaren, T.I., Pandey, R., Xu, Z., Liu, H., Manning, S., Madgett, O., Duncan, S., Rasmussen, P., 2023. Preliminary Results in Innovative Solutions for Soil Carbon Estimation: Integrating Remote Sensing, Machine Learning, and Proximal Sensing Spectroscopy. *Remote Sens* 15, 5571. <https://doi.org/10.3390/rs15235571>

Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. *Nat. Microbiol.* 2, 17105. <https://doi.org/10.1038/nmicrobiol.2017.105>

Liang, Z., Cao, B., Jiao, Y., Liu, C., Li, X., Meng, X., Shi, J., Tian, X., 2022. Effect of the combined addition of mineral nitrogen and crop residue on soil respiration, organic carbon sequestration, and exogenous nitrogen in stable organic matter. *Appl. Soil Ecol.* 171, 104324. <https://doi.org/10.1016/j.apsoil.2021.104324>

Lilley, J.M., Kirkegaard, J.A., 2011. Benefits of increased soil exploration by wheat roots. *Field Crops Res.* 122, 118–130. <https://doi.org/10.1016/j.fcr.2011.03.010>

Liu, J., Qiu, T., Peñuelas, J., Sardans, J., Tan, W., Wei, X., Cui, Y., Cui, Q., Wu, C., Liu, L., Zhou, B., He, H., Fang, L., 2023. Crop residue return sustains global soil ecological stoichiometry balance. *Glob. Change Biol.* gcb.16584. <https://doi.org/10.1111/gcb.16584>

- Lobsey, C.R., Viscarra Rossel, R.A., 2016. Sensing of soil bulk density for more accurate carbon accounting. *Eur J Soil Sci* 67, 504–513. <https://doi.org/10.1111/ejss.12355>
- Lucà, F., Conforti, M., Castrignanò, A., Matteucci, G., Buttafuoco, G., 2017. Effect of calibration set size on prediction at local scale of soil carbon by Vis-NIR spectroscopy. *Geoderma* 288, 175–183.
- Lugato, E., Bampa, F., Panagos, P., Montanarella, L., Jones, A., 2014. Potential carbon sequestration of European arable soils estimated by modelling a comprehensive set of management practices. *Glob. Change Biol.* 20, 3557–3567. <https://doi.org/10.1111/gcb.12551>
- Ma, Y., Roudier, P., Kumar, K., Palmada, T., Grealish, G., Carrick, S., Lilburne, L., Triantafyllis, J., 2023. A soil spectral library of New Zealand. *Geoderma Reg.* v.35, 00726,. <https://doi.org/10.1016/j.geodrs.2023.e00726>.
- Magenau, E., Clifton-Brown, J., Awty-Carroll, D., Ashman, C., Ferrarini, A., Kontek, M., Martani, E., Roderick, K., Amaducci, S., Davey, C., Jurišić, V., Kam, J., Trindade, L.M., Lewandowski, I., Kiesel, A., 2022. Site impacts nutrient translocation efficiency in intraspecies and interspecies miscanthus hybrids on marginal lands. *GCB Bioenergy* 14, 1035–1054. <https://doi.org/10.1111/gcbb.12985>
- Magenau, E., Clifton-Brown, J., Parry, C., Ashman, C., Awty-Carroll, D., Ferrarini, A., Kontek, M., Martani, E., Amaducci, S., Davey, C., Dolstra, O., Jurišić, V., Kam, J., Trindade, L.M., Lewandowski, I., Kiesel, A., 2023. Spring emergence and canopy development strategies in miscanthus hybrids in Mediterranean, continental and maritime European climates. *GCB Bioenergy* 15, 559–574. <https://doi.org/10.1111/gcbb.13035>
- Majumdar, S., Smith, R., Conway, B.D., Lakshmi, V., 2022. Advancing remote sensing and machine learning-driven frameworks for groundwater withdrawal estimation in Arizona: Linking land subsidence to groundwater withdrawals. *Hydrol. Process.* 36, 14757. <https://doi.org/10.1002/hyp.14757>
- Mäkipää, raisa, Häkkinen, M., Muukkonen, P., Peltoniemi, M., 2008. The costs of monitoring changes in forest soil carbon stocks. *Boreal Environ. Res.* 13, 120–130.
- Manzoni, S., Trofymow, J.A., Jackson, R.B., Porporato, A., 2010. Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter. *Ecol. Monogr.* 80, 89–106. <https://doi.org/10.1890/09-0179.1>
- Martani, E., Ferrarini, A., Hastings, A., Amaducci, S., 2023. Soil Organic Carbon Significantly Increases When Perennial Biomass Plantations Are Reverted Back to Annual Arable Crops. *Agronomy* 13, 447. <https://doi.org/10.3390/agronomy13020447>
- Martani, E., Ferrarini, A., Serra, P., Pilla, M., Marcone, A., Amaducci, S., 2020. Belowground biomass C outweighs soil organic C of perennial energy crops: Insights from a long-term multispecies trial. *GCB Bioenergy* 00, 1–14. <https://doi.org/10.1111/gcbb.12785>
- Martin, M.P., Dimassi, B., Román Dobarco, M., Guenet, B., Arrouays, D., Angers, D.A., Blache, F., Huard, F., Soussana, J., Pellerin, S., 2021. Feasibility of the 4 per 1000 aspirational target for soil carbon: A case study for France. *Glob. Change Biol.* 27, 2458–2477. <https://doi.org/10.1111/gcb.15547>
- Mathers, C., Black, C.K., Segal, B.D., Gurung, R.B., Zhang, Y., Easter, M.J., Williams, S., Motew, M., Campbell, E.E., Brummitt, C.D., Paustian, K., Kumar, A.A., 2023. Validating DayCent-CR for cropland soil carbon offset reporting at a national scale. *Geoderma* 438, 116647. <https://doi.org/10.1016/j.geoderma.2023.116647>
- Mattila, T.J., Hagelberg, E., Söderlund, S., Joona, J., 2022. How farmers approach soil carbon sequestration? Lessons learned from 105 carbon-farming plans. *Soil Tillage Res.* 215.
- Meinshausen, N., 2006. Quantile regression forests. *J Mach Learn Res.*,7, 983–999,.
- Meinshausen, N., Schiesser, L., 2015. Quantregforest: Quantile Regression Forests.
- Minasny, B., Bandai, T., Ghezzehei, T.A., Huang, Y.-C., Ma, Y., McBratney, A.B., Ng, W., Norouzi, S., Padarian, J., Rudiyanto, S.A., Styc, Q., Widayastuti, M., 2024. Soil Science-Informed Machine Learning. *Geoderma* 452, 117094. <https://doi.org/10.1016/j.geoderma.2024.117094>
- Minasny, B., McBratney, A.B., 2006. A conditioned Latin hypercube method for sampling in the presence of ancillary information. *Comput. Geosci.* 32, 1378–1388. <https://doi.org/10.1016/j.cageo.2005.12.009>
- Minasny, B., McBratney, A.B., Arrouays, D., Chabbi, A., Field, D.J., Kopittke, P.M., Morgan, C.L.S., Padarian, J., Rumpel, C., 2023. Soil Carbon Sequestration: Much More Than a Climate Solution. *Environ. Sci. Technol.* 57, 19094–19098. <https://doi.org/10.1021/acs.est.3c07312>
- Moebius-Clune, B.N., Moebius-Clune, D.J., Gugino, B.K., Idow, O.J., Schindelbeck, R.R., Ristow, A.J., van Es, H.M., Thies, J.E., Shayler, H.A., McBride, M.B., Kurtz, K.S.M., Wolfe, D.W., Abawi, G.S., 2016. Comprehensive assessment of soil health: the Cornell framework manual.
- Mohammedzein, M.A., Csorba, A., Rotich, B., Justin, P.N., Melenya, C., Andrei, Y., Micheli, E., 2023. Development of Hungarian spectral library: Prediction of soil properties and applications. *Eurasian J. Soil Sci. EJSS* v.12, 244–256,. <https://doi.org/10.18393/ejss.1275149>.
- Moinet, G.Y.K., Hijbeek, R., Van Vuuren, D.P., Giller, K.E., 2023. Carbon for soils, not soils for carbon. *Glob. Change Biol.* 29, 2384–2398. <https://doi.org/10.1111/gcb.16570>
- Mondini, C., Cayuela, M.L., Sinicco, T., Fornasier, F., Galvez, A., Sánchez-Monedero, M.A., 2017. Modification of the

RothC model to simulate soil C mineralization of exogenous organic matter. *Biogeosciences* 14, 3253–3274. <https://doi.org/10.5194/bg-14-3253-2017>

Mooshammer, M., Wanek, W., Hämmerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch, A., Schnecker, J., Takriti, M., Watzka, M., Wild, B., Keiblinger, K.M., Zechmeister-Boltenstern, S., Richter, A., 2014. Adjustment of microbial nitrogen use efficiency to carbon:nitrogen imbalances regulates soil nitrogen cycling. *Nat. Commun.* 5, 3694. <https://doi.org/10.1038/ncomms4694>

Morari, F., Lugato, E., Berti, A., Giardini, L., 2006. Long-term effects of recommended management practices on soil carbon changes and sequestration in north-eastern Italy. *Soil Use Manag.* 22, 71–81. <https://doi.org/10.1111/j.1475-2743.2005.00006.x>

Morellos, A., Pantazi, X.E., Moshou, D., Alexandridis, T., Whetton, R., Tziotzios, G., Wiebensohn, J., Bill, R., Mouazen, 2016. A.Machine Learning Based Prediction of Soil Total Nitrogen, Organic Carbon and Moisture Content by Using VIS-NIR Spectroscopy. *Biosyst. Eng.* 152, 104–116.

Nabuurs, G.J., Mrabet, R., Abu Hatab, A., Bustamante, M., Clark, H., Havlík, P., House, J., Mbow, C., Ninan, K.N., Popp, A., Roe, S., Sohngen, B., Towprayoon, S., 2023. Agriculture, Forestry and Other Land Uses (AFOLU). In IPCC, 2022: Climate Change 2022: Mitigation of Climate Change. Contribution of Working Group III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [P.R. Shukla, J. Skea, R. Slade, A. Al Khourdajie, R. van Diemen, D. McCollum, M. Pathak, S. Some, P. Vyas, R. Fradera, M. Belkacemi, A. Hasija, G. Lisboa, S. Luz, J. Malley, (eds.)], in: *Climate Change 2022 - Mitigation of Climate Change: Working Group III Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, pp. 747–860. <https://doi.org/10.1017/9781009157926.009>

Norman, R.J., Edberg, J.C., Stucki, J.W., 1985. Determination of Nitrate in Soil Extracts by Dual-wavelength Ultraviolet Spectrophotometry. *Soil Sci. Soc. Am. J.* 49, 1182–1185. <https://doi.org/10.2136/sssaj1985.03615995004900050022x>

Oldfield, E.E., Eagle, A.J., Rubin, R.L., Rudek, J., Sanderman, J., Gordon, D.R., 2021. Agricultural soil carbon credits: Making sense of protocols for carbon sequestration and net greenhouse gas removals.

Ouattara, M.S., Laurent, A., Ferchaud, F., Berthou, M., Borujerdi, E., Butier, A., Malvoisin, P., Romelot, D., Loyce, C., 2021. Evolution of soil carbon stocks under *Miscanthus × giganteus* and *Miscanthus sinensis* across contrasting environmental conditions. *GCB Bioenergy* 13, 161–174. <https://doi.org/10.1111/gcbb.12760>

Padarian, J., Minasny, B., McBratney, A.B., 2022. Assessing the uncertainty of deep learning soil spectral models using Monte Carlo dropout. *Geoderma* 425, 116063. <https://doi.org/10.1016/j.geoderma.2022.116063>.

Pagliai, M., Vignozzi, N., Pellegrini, S., 2004. Soil structure and the effect of management practices. *Soil Tillage Res.* 79, 131–143. <https://doi.org/10.1016/j.still.2004.07.002>

Panagos, P., Rosa, D., Liakos, L., Labouyrie, M., Borrelli, P., Ballabio, C., 2024. Soil bulk density assessment in Europe. *Agric. Ecosyst. Environ.* 64, 108907. <https://doi.org/10.1016/j.agee.2024.108907>

Paul, C., Bartkowski, B., Dönmez, C., Don, A., Mayer, S., Steffens, M., Weigl, S., Wiesmeier, M., Wolf, A., Helming, K., 2023. Carbon farming: Are soil carbon certificates a suitable tool for climate change mitigation? *J. Environ. Manage.* 330, 117142. <https://doi.org/10.1016/j.jenvman.2022.117142>

Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G.P., Smith, P., 2016. Climate-smart soils. *Nature* 532, 49–57. <https://doi.org/10.1038/nature17174>

Pei, Y., Dong, J., Zhang, Y., Yuan, W., Doughty, R., Yang, J., Zhou, D., Zhang, L., Xiao, X., 2022. Evolution of light use efficiency models: Improvement, uncertainties, and implications. *Agric. For. Meteorol.* 317, 108905. <https://doi.org/10.1016/j.agrformet.2022.108905>

Peralta, G., Di Paolo, L.E., Luotto, I., Omuto, C., Mainka, M., Viatkin, K., Yigini, Y., 2022. Global soil organic carbon sequestration potential map (GSOCseq v1.1) – Technical manual. FAO. <https://doi.org/10.4060/cb9002en>

Petersson, T., Antoniella, G., Perugini, L., Chiriaco, M.V., Chiti, T., 2025. Carbon farming practices for European cropland: A review on the effect on soil organic carbon. *Soil Tillage Res.* 247, 106353. <https://doi.org/10.1016/j.still.2024.106353>

Pidlisnyuk, V., Stefanovska, T., Lewis, E.E., Erickson, L.E., Davis, L.C., 2014. *Miscanthus* as a Productive Biofuel Crop for Phytoremediation. *Crit. Rev. Plant Sci.* 33, 1–19. <https://doi.org/10.1080/07352689.2014.847616>

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., 2012. Nonlinear mixed-effects models. *R Package Version 3*, 1–89.

Pisani, O., Klick, S.A., Strickland, T.C., Pisarello, K.L., Coffin, A.W., 2024. Chemical Composition of the Aboveground Tissues of *Miscanthus × giganteus* and Relationships to Soil Characteristics. *BioEnergy Res.* <https://doi.org/10.1007/s12155-023-10718-z>

Poeplau, C., Don, A., 2015. Carbon sequestration in agricultural soils via cultivation of cover crops – A meta-analysis. *Agric. Ecosyst. Environ.* 200, 33–41. <https://doi.org/10.1016/j.agee.2014.10.024>

Poeplau, C., Don, A., 2014. Soil carbon changes under *Miscanthus* driven by C₄ accumulation and C₃ decomposition - toward a default sequestration function. *GCB Bioenergy* 6, 327–338. <https://doi.org/10.1111/gcbb.12043>

Poeplau, C., Helfrich, M., Dechow, R., Szoboszlai, M., Tebbe, C.C., Don, A., Greiner, B., Zopf, D., Thumm, U., Korevaar,

- H., Geerts, R., 2019. Increased microbial anabolism contributes to soil carbon sequestration by mineral fertilization in temperate grasslands. *Soil Biol. Biochem.* 130, 167–176. <https://doi.org/10.1016/j.soilbio.2018.12.019>
- Poggio, L., de Sousa Luis, M., Batjes, N.H., Heuvelink, G.B., Kempen, B., Ribeiro, E., Rossiter, D., 2021. SoilGrids 2.0: producing soil information for the globe with quantified spatial uncertainty. *SOIL* 7, 217–240. <https://doi.org/10.5194/soil-7-217-2021>
- Potash, E., Guan, K., Margenot, A.J., Lee, D., Boe, A., Douglass, M., Heaton, E., Jang, C., Jin, V.L., Li, N., Mitchell, R., Namoi, N., Schmer, M.R., Wang, S., Zumpf, C., 2023. Multisite evaluation of stratified and balanced sampling of soil organic carbon stocks in agricultural fields. *Geoderma* 438, 116587. <https://doi.org/10.1016/j.geoderma.2023.116587>
- Poulton, P., Johnston, J., Macdonald, A., White, R., Powlson, D., 2018. Major limitations to achieving “4 per 1000” increases in soil organic carbon stock in temperate regions: Evidence from long-term experiments at Rothamsted Research, United Kingdom. *Glob. Change Biol.* 24, 2563–2584. <https://doi.org/10.1111/gcb.14066>
- Prince, S.D., Goward, S.N., 1995. Global Primary Production: A Remote Sensing Approach. *J. Biogeogr.* 22, 815. <https://doi.org/10.2307/2845983>
- Prommer, J., Walker, T.W.N., Wanek, W., Braun, J., Zezula, D., Hu, Y., Hofhansl, F., Richter, A., 2020. Increased microbial growth, biomass, and turnover drive soil organic carbon accumulation at higher plant diversity. *Glob. Change Biol.* 26, 669–681. <https://doi.org/10.1111/gcb.14777>
- Prosser, J.A., Speir, T.W., Stott, D.E., 2011. Soil Oxidoreductases and FDA Hydrolysis, in: *Methods of Soil Enzymology*, SSSA Book Series. pp. 103–124. <https://doi.org/10.2136/sssabookser9.c6>
- Pulleman, M., Creamer, R., Hamer, U., Helder, J., Pelosi, C., Pérès, G., Rutgers, M., 2012. Soil biodiversity, biological indicators and soil ecosystem services—an overview of European approaches. *Curr. Opin. Environ. Sustain.* 4, 529–538. <https://doi.org/10.1016/j.cosust.2012.10.009>
- Ramirez-Lopez, L., Schmidt, K., Behrens, T., Wesemael, B., Demattê, J.A.M., Scholten, T., 2014. Sampling optimal calibration sets in soil infrared spectroscopy. *Geoderma* 226–227, 140–150.
- Renella, G., Landi, L., Ascher, J., Ceccherini, M.T., Pietramellara, G., Nannipieri, P., 2006. Phosphomonoesterase production and persistence and composition of bacterial communities during plant material decomposition in soils with different pH values. *Soil Biol. Biochem.* 38, 795–802.
- Reyes, J., Ließ, M., 2023. On-the-Go Vis-NIR Spectroscopy for Field-Scale Spatial-Temporal Monitoring of Soil Organic Carbon. *Agriculture* 13, 1611. <https://doi.org/10.3390/agriculture13081611>
- Rhine, E.D., Mulvaney, R.L., Pratt, E.J., Sims, G.K., 1998. Improving the Berthelot Reaction for Determining Ammonium in Soil Extracts and Water. *Soil Sci. Soc. Am. J.* 62, 473–480. <https://doi.org/10.2136/sssaj1998.03615995006200020026x>
- Richardson, A.E., Kirkby, C.A., Banerjee, S., Kirkegaard, J.A., 2014. The inorganic nutrient cost of building soil carbon. *Carbon Manag.* 5, 265–268. <https://doi.org/10.1080/17583004.2014.923226>
- Richter, G.M., Agostini, F., Redmile-Gordon, M., White, R., Goulding, K.W.T., 2015. Sequestration of C in soils under *Miscanthus* can be marginal and is affected by genotype-specific root distribution. *Agric. Ecosyst. Environ.* 200, 169–177. <https://doi.org/10.1016/j.agee.2014.11.011>
- Rossel, R.A.V., McBratney, A.B., Minasny, B., 2010. *Proximal soil sensing*. Springer Science & Business Media.
- Roudier, P., 2021. *clhs: Conditioned latin hypercube sampling*. R package version 0.7.0.
- Rowe, R.L., Keith, A.M., Elias, D., Dondini, M., Smith, P., Oxley, J., McNamara, N.P., 2016. Initial soil C and land-use history determine soil C sequestration under perennial bioenergy crops. *GCB Bioenergy* 8, 1046–1060. <https://doi.org/10.1111/gcbb.12311>
- Rudiyanto, Minasny, B., Setiawan, B.I., Arif, C., Saptomo, S.K., Chadirin, Y., 2016. Digital mapping for cost-effective and accurate prediction of the depth and carbon stocks in Indonesian peatlands. *Geoderma* 272, 20–31. <https://doi.org/10.1016/j.geoderma.2016.02.026>
- Ruf, T., Makselon, J., Udelhoven, T., Emmerling, C., 2018. Soil quality indicator response to land-use change from annual to perennial bioenergy cropping systems in Germany. *GCB Bioenergy* 10, 444–459. <https://doi.org/10.1111/gcbb.12513>
- Ruf, Th., Schmidt, A., Delfosse, P., Emmerling, C., 2017. Harvest date of *Miscanthus x giganteus* affects nutrient cycling, biomass development and soil quality. *Biomass Bioenergy* 100, 62–73. <https://doi.org/10.1016/j.biombioe.2017.03.010>
- Satrio, A.E., Gandaseca, S., Ahmed, O.H., Majid, N.M.A., 2009. Effect of logging operation on soil carbon storage of a tropical peat swamp forest. *Am. J. Environ. Sci.* 5, 748–752.
- Scharlemann, J.P., Tanner, E.V., Hiederer, R., Kapos, V., 2014. Global soil carbon: understanding and managing the largest terrestrial carbon pool. *Carbon Manag.* 5, 81–91. <https://doi.org/10.4155/cmt.13.77>
- Schipsanski, M.E., McClelland, S.C., Hughes, H.M., Jabbour, R., Malin, D., Hillier, J., Paustian, K., Reaves, E., 2024. Improving Decision Support Tools for Quantifying GHG Emissions from Organic Production Systems. *Org. Agric.* <https://doi.org/10.1007/s13165-024-00466-5>
- Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic

matter as an ecosystem property. *Nature* 478, 49–56. <https://doi.org/10.1038/nature10386>

Schnürer, J., Rosswall, T., 1982. Fluorescein Diacetate Hydrolysis as a Measure of Total Microbial Activity in Soil and Litter. *Appl. Environ. Microbiol.* 43, 1256–1261. <https://doi.org/10.1128/aem.43.6.1256-1261.1982>

Schrama, M., Vandecasteele, B., Carvalho, S., Muylle, H., Van Der Putten, W.H., 2016. Effects of first- and second-generation bioenergy crops on soil processes and legacy effects on a subsequent crop. *GCB Bioenergy* 8, 136–147. <https://doi.org/10.1111/gcbb.12236>

Shepherd, A., Awty-Carroll, D., Kam, J., Ashman, C., Magenau, E., Martani, E., Kontek, M., Ferrarini, A., Amaducci, S., Davey, C., Jurišić, V., Petrie, G.-J., Al Hassan, M., Lamy, I., Lewandowski, I., de Maupéou, E., McCalmont, J., Trindade, L., van der Crujisen, K., van der Pluijm, P., Rowe, R., Lovett, A., Donnison, I., Kiesel, A., Clifton-Brown, J., Hastings, A., 2023. Novel Miscanthus hybrids: Modelling productivity on marginal land in Europe using dynamics of canopy development determined by light interception. *GCB Bioenergy* 15, 444–461. <https://doi.org/10.1111/gcbb.13029>

Sherrod, S.K., Belnap, J., Miller, M.E., 2002. COMPARISON OF METHODS FOR NUTRIENT MEASUREMENT IN CALCAREOUS SOILS: ION-EXCHANGE RESIN BAG, CAPSULE, MEMBRANE, AND CHEMICAL EXTRACTIONS: *Soil Sci.* 167, 666–679. <https://doi.org/10.1097/00010694-200210000-00005>

Siddique, I.A., Grados, D., Chen, J., Lærke, P.E., Jørgensen, U., 2023. Soil organic carbon stock change following perennialization: a meta-analysis. *Agron. Sustain. Dev.* 43, 58. <https://doi.org/10.1007/s13593-023-00912-w>

Silvero, N.E.Q., Demattê, J.A.M., Minasny, B., Rosin, N.A., Nascimento, J.G., Rodríguez Albarracín, H.S., Bellinaso, H., Gómez, A.M.R., 2023. Chapter Three - Sensing technologies for characterizing and monitoring soil functions: A review, in: Sparks, D.L. (Ed.), *Advances in Agronomy*. Academic Press, pp. 125–168. <https://doi.org/10.1016/bs.agron.2022.08.002>

Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biol. Biochem.* 42, 391–404. <https://doi.org/10.1016/j.soilbio.2009.10.014>

Sinsabaugh, R.L., Hill, B.H., Follstad Shah, J.J., 2009. Eoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. *Nature* 462, 795–798. <https://doi.org/10.1038/nature08632>

Sinsabaugh, R.L., Manzoni, S., Moorhead, D.L., Richter, A., 2013. Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. *Ecol. Lett.* 16, 930–939. <https://doi.org/10.1111/ele.12113>

Six, J., Elliott, E.T., Paustian, K., 2000. Soil Structure and Soil Organic Matter II. A Normalized Stability Index and the Effect of Mineralogy. *Soil Sci. Soc. Am. J.* 64, 1042–1049. <https://doi.org/10.2136/sssaj2000.6431042x>

Smith, L.J., Torn, M.S., 2013. Ecological limits to terrestrial biological carbon dioxide removal. *Clim. Change* 118, 89–103. <https://doi.org/10.1007/s10584-012-0682-3>

Smith, P., 2012. Soils and climate change. *Curr. Opin. Environ. Sustain.* 4, 539–544. <https://doi.org/10.1016/j.cosust.2012.06.005>

Smith, P., 2005. An overview of the permanence of soil organic carbon stocks: influence of direct human-induced, indirect and natural effects. *Eur. J. Soil Sci.* 56, 673–680. <https://doi.org/10.1111/j.1365-2389.2005.00708.x>

Smith, P., 2004. How Long before a Change in Soil Organic Carbon Can Be Detected?: Time taken to measure a change in soil organic carbon. *Glob Change Biol* 10, 1878–1883.

Smith, P., Bustamante, M., Ahammad, H., Clark, H., Dong, H., Tubiello, F., 2014. Agriculture, Forestry and Other Land Use (AFOLU). In: *Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Edenhofer, O., R. Pichs-Madruga, Y. Sokona, E. Farahani, S. Kadner, K. Seyboth, A. Adler, I. Baum, S. Brunner, P. Eickemeier, B. Kriemann, J. Savolainen, S. Schlömer, C. von Stechow, T. Zwickel and J.C. Minx (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. <https://doi.org/10.1017/CBO9781107415416>

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., McCarl, B., Ogle, S., O'Mara, F., Rice, C., Scholes, B., Sirotenko, O., Howden, M., McAllister, T., Pan, G., Romanenkov, V., Schneider, U., Towprayoon, S., Wattenbach, M., Smith, J., 2007. Greenhouse gas mitigation in agriculture. *Philos. Trans. R. Soc. B Biol. Sci.* 363, 789–813. <https://doi.org/10.1098/rstb.2007.2184>

Smith, P., Soussana, J., Angers, D., Schipper, L., Chenu, C., Rasse, D.P., Batjes, N.H., Egmond, F., McNeill, S., Kuhnert, M., Arias-Navarro, C., Olesen, J.E., Chirinda, N., Fornara, D., Wollenberg, E., Álvaro-Fuentes, J., Sanz-Cobena, A., Klumpp, K., 2020. How to measure, report and verify soil carbon change to realize the potential of soil carbon sequestration for atmospheric greenhouse gas removal. *Glob. Change Biol.* 26, 219–241. <https://doi.org/10.1111/gcb.14815>

Sokol, N.W., Sanderman, J., Bradford, M.A., 2019. Pathways of mineral-associated soil organic matter formation: Integrating the role of plant carbon source, chemistry, and point of entry. *Glob. Change Biol.* 25, 12–24. <https://doi.org/10.1111/gcb.14482>

Sokol, N.W., Slessarev, E., Marschmann, G.L., Nicolas, A., Blazewicz, S.J., Brodie, E.L., Firestone, M.K., Foley, M.M., Hestrin, R., Hungate, B.A., Koch, B.J., Stone, B.W., Sullivan, M.B., Zablocki, O., LLNL Soil Microbiome Consortium, Trubl, G., McFarlane, K., Stuart, R., Nuccio, E., Weber, P., Jiao, Y., Zavarin, M., Kimbrel, J., Morrison, K., Adhikari, D., Bhattacharaya, A., Nico, P., Tang, J., Didonato, N., Paša-Tolić, L., Greenlon, A., Sieradzki, E.T., Dijkstra, P., Schwartz, E., Sachdeva, R., Banfield, J., Pett-Ridge, J., 2022. Life and death in the soil microbiome: how ecological processes influence

biogeochemistry. *Nat. Rev. Microbiol.* <https://doi.org/10.1038/s41579-022-00695-z>

Song, X., Liu, X., Liang, G., Li, S., Li, J., Zhang, M., Zheng, F., Ding, W., Wu, X., Wu, H., 2022. Positive priming effect explained by microbial nitrogen mining and stoichiometric decomposition at different stages. *Soil Biol. Biochem.* 175, 108852. <https://doi.org/10.1016/j.soilbio.2022.108852>

Spearman, C., 1904. General Intelligence, Objectively Determined and Measured. *Am. J. Psychol.* 15, 201–292. <https://doi.org/10.2307/1412107>

Spohn, M., Pötsch, E.M., Eichorst, S.A., Woebken, D., Wanek, W., Richter, A., 2016. Soil microbial carbon use efficiency and biomass turnover in a long-term fertilization experiment in a temperate grassland. *Soil Biol. Biochem.* 97, 168–175. <https://doi.org/10.1016/j.soilbio.2016.03.008>

Stanley, P., Spertus, J., Chiartas, J., Stark, P.B., Bowles, T., 2023. Valid inferences about soil carbon in heterogeneous landscapes. *Geoderma* 430, 116323. <https://doi.org/10.1016/j.geoderma.2022.116323>.

Stevens, A., Udelhoven, T., Denis, A., Tychon, B., Lioy, R., Hoffmann, L., Wesemael, B., 2010. Measuring soil organic carbon in croplands at regional scale using airborne imaging spectroscopy. *Geoderma* 158, 32–45.

Stevens, Ramirez-Lopez, 2022. Package ‘prospectr’ version 0.2.7 in R.

Strullu, L., Cadoux, S., Preudhomme, M., Jeuffroy, M.-H., Beaudoin, N., 2011. Biomass production and nitrogen accumulation and remobilisation by *Miscanthus x giganteus* as influenced by nitrogen stocks in belowground organs. *Field Crops Res.* 121, 381–391. <https://doi.org/10.1016/j.fcr.2011.01.005>

Stutter, M.I., Shand, C.A., George, T.S., Blackwell, M.S.A., Dixon, L., Bol, R., MacKay, R.L., Richardson, A.E., Condrón, L.M., Haygarth, P.M., 2015. Land use and soil factors affecting accumulation of phosphorus species in temperate soils. *Spec. Issue Dev. Soil Org. Phosphorus Cycl. Nat. Agric. Ecosyst.* 257–258, 29–39. <https://doi.org/10.1016/j.geoderma.2015.03.020>

Sulman, B.N., Moore, J.A.M., Abramoff, R., Averill, C., Kivlin, S., Georgiou, K., Sridhar, B., Hartman, M.D., Wang, G., Wieder, W.R., Bradford, M.A., Luo, Y., Mayes, M.A., Morrison, E., Riley, W.J., Salazar, A., Schimel, J.P., Tang, J., Classen, A.T., 2018. Multiple models and experiments underscore large uncertainty in soil carbon dynamics. *Biogeochemistry* 141, 109–123. <https://doi.org/10.1007/s10533-018-0509-z>

Swetha, R.K., Chakraborty, S., 2021. Combination of soil texture with Nix color sensor can improve soil organic carbon prediction. *Geoderma* 382, 0016-7061. <https://doi.org/10.1016/j.geoderma.2020.114775>.

Szatmári, G., Pásztor, L., 2019. Comparison of various uncertainty modelling approaches based on geostatistics and machine learning algorithms. *Geoderma* 337, 1329–1340. <https://doi.org/10.1016/j.geoderma.2018.09.008>

Szatmári, G., Pirkó, B., Koós, S., Laborczy, A., Bakacsi, Z., Szabó, J., Pásztor, L., 2019. Spatio-temporal assessment of topsoil organic carbon stock change in Hungary. *Soil Tillage Res.* 195, 104410. <https://doi.org/10.1016/j.still.2019.104410>

Tarocco, A., Aprea, A., 2021. Carta dei suoli della regione emilia-romagna.

Tian, L., Shi, W., 2014. Soil peroxidase regulates organic matter decomposition through improving the accessibility of reducing sugars and amino acids. *Biol. Fertil. Soils* 50, 785–794. <https://doi.org/10.1007/s00374-014-0903-1>

Tiemann, L.K., Grandy, A.S., 2015. Mechanisms of soil carbon accrual and storage in bioenergy cropping systems. *GCB Bioenergy* 7, 161–174. <https://doi.org/10.1111/gcbb.12126>

Ungaro, F., Staffilani, F., Tarocco, P., 2010. Assessing and mapping topsoil organic carbon stock at regional scale: A scorpan kriging approach conditional on soil map delineations and land use. *Land Degrad. Dev.* 21, 565–581.

Van der Voort, T.S., Verweij, S., Fujita, Y., 2023. Enabling soil carbon farming: presentation of a robust, affordable, and scalable method for soil carbon stock assessment. *Agron Sustain Dev* 43, 22. <https://doi.org/10.1007/s13593-022-00856-7>

van Es, H.M., Schindelbeck, R.R., 2003. Field procedures and data analysis for the Cornell sprinkle infiltrometer [Online]. Available by Cornell University.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19, 703–707. [https://doi.org/10.1016/0038-0717\(87\)90052-6](https://doi.org/10.1016/0038-0717(87)90052-6)

Vaudour, E., Gholizadeh, A., Castaldi, F., Saberioon, M., Boruvka, L., Urbina-Salazar, D., Fouda, Y., Arrouays, D., RicherdeForges, A.C., Biney, J.K.M., 2022. Satellite Imagery to Map Topsoil Organic Carbon Content over Cultivated Areas: An Overview. *Remote Sens* 14, 2917.

Vaudour, E., Gomez, C., Loiseau, T., Baghdadi, N., Loubet, B., Arrouays, D., Ali, L., Lagacherie, P., 2019. The impact of acquisition date on the prediction performance of topsoil organic carbon from Sentinel-2 for croplands. *Remote Sens* 11, 2143.

Vaysse, K., Lagacherie, P., 2017. Using quantile regression forest to estimate uncertainty of digital soil mapping products. *Geoderma* 291, 55–64. <https://doi.org/10.1016/j.geoderma.2016.12.017>.

Verra, 2024. VCS Methodology (VM0042) - IMPROVED AGRICULTURAL LAND MANAGEMENT, version 2.1.

Verra, TerraCarbon LLC, 2023. VCS Module (VMD0053) - MODEL CALIBRATION, VALIDATION AND UNCERTAINTY GUIDANCE FOR THE METHODOLOGY FOR IMPROVED AGRICULTURAL LAND MANAGEMENT, version 2.0.

Villarino, S.H., Talab, E., Contisciani, L., Videla, C., Di Geronimo, P., Mastrángelo, M.E., Georgiou, K., Jackson, R.B.,

- Piñeiro, G., 2023. A large nitrogen supply from the stable mineral-associated soil organic matter fraction. *Biol. Fertil. Soils* 59, 833–841. <https://doi.org/10.1007/s00374-023-01755-z>
- Viscarra Rossel, R.A., Brus, D.J., 2018. The cost-efficiency and reliability of two methods for soil organic C accounting. *Land Degrad. Dev.* 29, 506–520.
- Viscarra Rossel, R.A.V., Shen, Z., Lopez, L.R., Behrens, T., Shi, Z., Wetterlind, J., Orellano, C., 2024. An imperative for soil spectroscopic modelling is to think global but fit local with transfer learning. *Earth-Sci. Rev.* 104797.
- Vranova, V., Rejsek, K., Formanek, P., 2013. Proteolytic activity in soil: A review. *Appl. Soil Ecol.* 70, 23–32. <https://doi.org/10.1016/j.apsoil.2013.04.003>
- Wadoux, A.M.-C., Brus, D.J., Heuvelink, G.B.M., 2019. Sampling design optimization for soil mapping with random forest. *Geoderma* 355, 113913. <https://doi.org/10.1016/j.geoderma.2019.113913>
- Wadoux, A.M.J.-C., McBratney, A.B., 2021. Digital soil science and beyond. *Soil Sci. Soc. Am. J.* 85, 1313–1331.
- Wadoux, A.M.J.-C., Minasny, B., McBratney, A.B., 2020. Machine Learning for Digital Soil Mapping: Applications, Challenges and Suggested Solutions. *Earth-Sci. Rev.* 210, 103359.
- Weatherburn, M.W., 1967. Phenol-hypochlorite reaction for determination of ammonia. *Anal. Chem.* 39, 971–974. <https://doi.org/10.1021/ac60252a045>
- Wellington, M.J., Kuhnert, P., Renzullo, L.J., Lawes, R., 2022. Modelling Within-Season Variation in Light Use Efficiency Enhances Productivity Estimates for Cropland. *Remote Sens.* 14. <https://doi.org/10.3390/rs14061495>
- Wendt, J.W., Hauser, S., 2013. An equivalent soil mass procedure for monitoring soil organic carbon in multiple soil layers. *Eur. J. Soil Sci.* 64, 58–65. <https://doi.org/10.1111/ejss.12002>
- Whittaker, C., McManus, M.C., Smith, P., 2013. A comparison of carbon accounting tools for arable crops in the United Kingdom. *Environ. Model. Softw.* 46, 228–239. <https://doi.org/10.1016/j.envsoft.2013.03.015>
- Wiesmeier, M., Spörlein, P., Geuß, U., Hangen, E., Haug, S., Reischl, A., Schilling, B., Lutzow, M., Kögel-Knabner, I., 2019. Soil organic carbon stocks in Southeast Germany (Bavaria) as affected by land use, soil type, and sampling depth. *Agric. Ecosyst. Environ.* 281, 90–102. <https://doi.org/10.1016/j.agee.2019.04.021>
- Winkler, B., Mangold, A., von Cossel, M., Clifton-Brown, J., Pogrzeba, M., Lewandowski, I., Iqbal, Y., Kiesel, A., 2020. Implementing miscanthus into farming systems: A review of agronomic practices, capital and labour demand. *Renew. Sustain. Energy Rev.* 132, 110053. <https://doi.org/10.1016/j.rser.2020.110053>
- Wittmann, C., Kähkönen, M.A., Ilvesniemi, H., Kurola, J., Salkinoja-Salonen, M.S., 2004. Areal activities and stratification of hydrolytic enzymes involved in the biochemical cycles of carbon, nitrogen, sulphur and phosphorus in podsolized boreal forest soils. *Soil Biol. Biochem.* 36, 425–433. <https://doi.org/10.1016/j.soilbio.2003.10.019>
- Wu, H., Cai, A., Dong, W., Xing, T., Xu, M., Lu, C., 2023. Nutrient stoichiometric management promotes carbon sequestration by improving microbial nutrient availability and metabolic efficiency in straw-amended soil. *J. Soils Sediments* 23, 1182–1192. <https://doi.org/10.1007/s11368-022-03396-5>
- Wu, H., Cai, A., Xing, T., Huai, S., Zhu, P., Han, X., Xu, M., Lu, C., 2022. Integrated management of crop residue and nutrient enhances new carbon formation by regulating microbial taxa and enzymes. *J. Integr. Agric.* 21, 1772–1785. [https://doi.org/10.1016/S2095-3119\(21\)63752-8](https://doi.org/10.1016/S2095-3119(21)63752-8)
- Wu, J., Cheng, X., Liu, G., 2022. Increased soil organic carbon response to fertilization is associated with increasing microbial carbon use efficiency: Data synthesis. *Soil Biol. Biochem.* 171, 108731. <https://doi.org/10.1016/j.soilbio.2022.108731>
- Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass C by fumigation-extraction—an automated procedure. *Soil Biol. Biochem.* 22, 1167–1169. [https://doi.org/10.1016/0038-0717\(90\)90046-3](https://doi.org/10.1016/0038-0717(90)90046-3)
- Wu, W., Ma, B., 2015. Integrated nutrient management (INM) for sustaining crop productivity and reducing environmental impact: A review. *Sci. Total Environ.* 512–513, 415–427. <https://doi.org/10.1016/j.scitotenv.2014.12.101>
- Wu, Y., Huang, H., Chen, F., Tan, T., Xu, Y., 2024. Soil organic carbon sequestration rate and spatiotemporal dynamics under perennial energy crops cultivation: A global meta-analysis. *Soil Tillage Res.* 240, 106064. <https://doi.org/10.1016/j.still.2024.106064>
- Xia, Y., Sanderman, J., Watts, J.D., Machmuller, M.B., Ewing, S., Rivard, C., 2024. Leveraging legacy data with targeted field sampling for low-cost mapping of soil organic carbon stocks on extensive rangeland properties. *Geoderma* 448, 116952. <https://doi.org/10.1016/j.geoderma.2024.116952>
- Xu, Y., Zheng, C., Liang, L., Yi, Z., Xue, S., 2021. Quantitative assessment of the potential for soil improvement by planting *Miscanthus* on saline-alkaline soil and the underlying microbial mechanism. *GCB Bioenergy* 13, 1191–1205. <https://doi.org/10.1111/gcbb.12845>
- Xu, Y., Zhou, J., Feng, W., Jia, R., Liu, C., Fu, T., Xue, S., Yi, Z., Guillaume, T., Yang, Y., Peixoto, L., Zeng, Z., Zang, H., 2022. Marginal land conversion to perennial energy crops with biomass removal enhances soil carbon sequestration. *GCB Bioenergy* 14, 1117–1127. <https://doi.org/10.1111/gcbb.12990>
- Yu, W., Huang, W., Weintraub-Leff, S.R., Hall, S.J., 2022. Where and why do particulate organic matter (POM) and

mineral-associated organic matter (MAOM) differ among diverse soils? *Soil Biol. Biochem.* 172, 108756. <https://doi.org/10.1016/j.soilbio.2022.108756>.

Yuzugullu, O., Fajraoui, N., Don, A., Liebisch, F., 2024. Satellite-based soil organic carbon mapping on European soils using available datasets and support sampling. *Sci. Remote Sens.* 9, 100118. <https://doi.org/10.1016/j.srs.2024.100118>

Zatta, A., Clifton-Brown, J., Robson, P., Hastings, A., Monti, A., 2014. Land use change from C3 grassland to C4 *Miscanthus* : effects on soil carbon content and estimated mitigation benefit after six years. *GCB Bioenergy* 6, 360–370. <https://doi.org/10.1111/gcbb.12054>

Zechmeister-Boltenstern, S., Keiblinger, K.M., Mooshammer, M., Peñuelas, J., Richter, A., Sardans, J., Wanek, W., 2015. The application of ecological stoichiometry to plant–microbial–soil organic matter transformations. *Ecol. Monogr.* 85, 133–155. <https://doi.org/10.1890/14-0777.1>

Zhang, Y., Lavalley, J.M., Robertson, A.D., Even, R., Ogle, S.M., Paustian, K., Cotrufo, M.F., 2021. Simulating measurable ecosystem carbon and nitrogen dynamics with the mechanistically defined MEMS 2.0 model. *Biogeosciences* 18, 3147–3171. <https://doi.org/10.5194/bg-18-3147-2021>

Zhao, D., Liu, Z., Xu, Y., Wang, Z., Li, Z., Ling, J., Wu, G., Wen, Y., 2024. Subsoil SOC increased by high C:N ratio straw application with optimized nitrogen supplementation. *Soil Use Manag.* 40, e13020. <https://doi.org/10.1111/sum.13020>

Zhao, H., Sun, B., Lu, F., Wang, X., Zhuang, T., Zhang, G., Ouyang, Z., 2017. Roles of nitrogen, phosphorus, and potassium fertilizers in carbon sequestration in a Chinese agricultural ecosystem. *Clim. Change* 142, 587–596. <https://doi.org/10.1007/s10584-017-1976-2>

Zhou, J., Li, E., Wei, H., Li, C., Qiao, Q., Armaghani, D.J., 2019. Random forests and cubist algorithms for predicting shear strengths of rockfill materials *Appl. Sci* 9, 1621.

Zhou, T., Geng, Y., Ji, C., Xu, X., Wang, H., Pan, J., Bumberger, J., Haase, D., Lausch, A., 2021. Prediction of soil organic carbon and the C:N ratio on a national scale using machine learning and satellite data: A comparison between Sentinel-2, Sentinel-3 and Landsat-8 images. *Sci. Total Environ.* 755, 142661. <https://doi.org/10.1016/j.scitotenv.2020.142661>

Zhu, E., Liu, Z., Ma, L., Luo, J., Kang, E., Wang, Y., Zhao, Y., Jia, J., Feng, X., 2024. Enhanced Mineral Preservation Rather Than Microbial Residue Production Dictates the Accrual of Mineral-Associated Organic Carbon Along a Weathering Gradient. *Geophys. Res. Lett.* 51, e2024GL108466. <https://doi.org/10.1029/2024GL108466>

Zimmermann, J., Dondini, M., Jones, M.B., 2013. Assessing the impacts of the establishment of *Miscanthus* on soil organic carbon on two contrasting land-use types in Ireland. *Eur. J. Soil Sci.* 64, 747–756. <https://doi.org/10.1111/ejss.12087>

Ringraziamenti

Giungere alla fine di questo percorso di dottorato è stato possibile grazie al supporto, alla guida e all'incoraggiamento di molte persone, alle quali desidero esprimere la mia più profonda gratitudine.

In primo luogo, vorrei ringraziare il mio supervisore, Andrea Ferrarini, per la sua costante disponibilità, per i preziosi consigli e per avermi guidato con rigore scientifico e grande passione nella ricerca. Il suo entusiasmo e la sua visione sono stati per me una fonte continua di ispirazione.

Un sincero grazie anche a Stefano Amaducci, per il suo supporto e per aver reso possibile questa ricerca grazie al sostegno finanziario dei progetti.

Desidero inoltre ringraziare la mia prima supervisor e mentore, Giovanna Visioli, per avermi trasmesso la passione per la ricerca e per avermi sempre supportata, incoraggiata e dato fiducia in ciò che facevo. I suoi insegnamenti, sia a livello scientifico che umano, sono stati inestimabili e hanno lasciato un segno profondo nel mio percorso.

Un grazie di cuore ai miei colleghi e alle mie colleghe, sia passati che presenti, del gruppo di ricerca – Michele Croci, Giorgio Impollonia, Irena Ymeti, Andre Marcone, Angelica Barone, Yuri Bellone, Manuele Ragazzi, Lorenzo Cremonesi, Enrico Martani, Henri Blandinières – per il costante supporto, i preziosi consigli e il clima di collaborazione che ha reso questo percorso più stimolante e arricchente. Un ringraziamento speciale va soprattutto ai co-autori dei capitoli e a coloro che hanno contribuito alla loro finalizzazione, partecipando alla raccolta dati, offrendo suggerimenti, revisioni e discussioni preziose.

Allo stesso modo, desidero ringraziare i nostri collaboratori Edoardo Volpato, Levyn Eric Perez Alvarado e Luis Brayan Lopez Lazo per le lunghe ore trascorse in campo e in laboratorio, senza le quali non sarei riuscita a raccogliere la grande mole di dati che ha reso possibile questo lavoro. Un grazie particolare a Cornelia Rottensteiner, per la sua disponibilità e il supporto nell'esperimento proposto nel Capitolo 4.

Un pensiero speciale va ai miei colleghi e alle mie colleghe del XXXVII ciclo di dottorato, e alle ragazze del DIPROVES – Alessandra Lezzi, Letizia Ottaviani, Monica Fittipaldi Broussard, Florencia Asinari, Maria Chiara Rosace – con cui ho condiviso momenti di confronto, risate e incoraggiamento reciproco durante questi anni. Grazie a chi ha reso i viaggi in treno più leggeri, tra chiacchiere, sfoghi e battute, trasformando anche le giornate più impegnative in un'esperienza più piacevole.

Grazie anche a chi mi ha offerto un posto per dormire, quando ancora vagavo senza fissa dimora tra Bologna, Parma e Piacenza: Angelica e Giorgio, per avermi aperto la porta di casa e risparmiato sveglie all'alba, e Lisa e Peter, per avermi ospitato e accolto come una figlia.

Infine, il mio ringraziamento più profondo va alla mia famiglia – mamma Vale, papà Carlo e il Pedrin – per aver sempre creduto in me e per avermi sostenuto incondizionatamente in questo percorso, con pazienza e affetto. Grazie per avermi aiutata in tutti i traslochi affrontati durante la mia carriera universitaria e per aver reso ogni ritorno a casa un momento di calore e serenità. Senza di voi, nulla di questo sarebbe stato possibile.

A Ivan, grazie di tutto: per avermi sempre ascoltata e supportata, per credere in me e nelle mie capacità e per avermi insegnato a guardare il mondo con un inguaribile ottimismo.

Alle amicizie di una vita, quelle che esistono da sempre: Cristina e Rachele, semplicemente grazie. Non servono altre parole, sapete già.

Grazie, infine, a tutte le persone che, in un modo o nell'altro, hanno contribuito a rendere questi anni di dottorato un'esperienza indimenticabile.