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Multiple ecosystem services provision from perennial bioenergy crops

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. . . to my parents, Giovanni and Rosanna, who always encouraged studying, pursuing research, and whose only requisite was that I do my best. I hope to continue to make them proud.

"*Learn from yesterday, live for today, hope for tomorrow. The important thing is to not stop questioning*."
Albert Einstein

Abstract

The 21st century will challenge agriculture to feed and fuel a growing world while conserving the environment. In this thesis an alternative bioenergy land use scenario to the conversion of marginal land has been tested: the bioenergy buffers. Given the environmental issues related to "food-energy-environment" trilemma, the Millennium Ecosystem Assessment framework on ES provides an opportunity to examine the environmental impacts of this new bioenergy land use scenario. In this thesis I aimed to determine to what extent do the perennial bioenergy crops affect the delivery of multiple ES when cultivated as bioenergy buffers. To reach this aim, I combined a systematic revision of literature on ES provided by perennial bioenergy crops with a field experiment on bioenergy buffers.

Applying an impact scoring methodology to the effects on ES extracted from literature, I showed that, cultivating perennial bioenergy crops along field margins of former croplands offer a great opportunity to sustain the provision of multiple ES. The cultivation of perennial bioenergy crops on field margins can improve climate, biodiversity and water regulation services, sustain soil health and provide biomass for energetic purposes. On the contrary, grassland conversion showed a net negative impact on multiple ES provision.

Nevertheless, I found two main shortcomings related to bioenergy buffers establishment and management. First, several site-specific factors along field margins must be taken into account, because they can affect crop establishment and buffers long-term productivity. Second, regarding to biomass supply chain, a limited working space for the farm machinery operations has been recognized as the main disadvantages of bioenergy buffers compared to large-scale bioenergy plantations. This spatial logistics constraint may inevitably increase harvest and collection operation times and fossil fuel consumption.

Conducting a field experiment with bioenergy buffers in a nitrate-enriched shallow groundwater, I showed that miscanthus and willow buffers are able to efficiently intercept and remove from groundwater the incoming NO_3-N as much as buffer strips with spontaneous species. Yet, due to their deep rooting systems, bioenergy buffers promote significant plant-microbial linkages along the soil profile. At deeper soil layers, a higher fine root biomass led perennial bioenergy crops to outperform patches of adventitious vegetation in terms of biological N removal from soil and belowground GHG mitigation potential. The results on biomass production and N removal via harvesting further confirmed that the cultivation of perennial bioenergy crops along watercourses is an effective win-win strategy: biomass production and protection of the environment.

In conclusion, the revealed potential of perennial bioenergy crops on multiple ES provision implies that their cultivation as perennial landscape elements in strategic locations within landscape is a promising option to promote the ecological sustainable intensification of agroecosystems.

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Chapter 1

General introduction

General Introduction

1.1 Relevance of land use transition to perennial bioenergy crops

The 21st century will challenge agriculture to feed and fuel a growing world while conserving the environment (Foley *et al.*, 2005; Tilman *et al.*, 2011). Energy security, economic development and environmental protection have become three recurrent and closely intertwined policy themes globally (Lal, 2010). Fossil fuel combustion is recognized as the most important driver of anthropogenic climate change (IPCC, 2007, 2014). Dependence on non-renewable fossil fuels as well as environmental concerns related to greenhouse gas (GHG) effects contributing to global warming and climate change have stimulated interests of policy makers and industry to promote bioenergy as part of energy security and climate change mitigation strategies (Joly *et al.*, 2015). In an attempt to lower the EU's reliance on fossil energy sources and to mitigate climate change, several renewable energy sources have been introduced into the EU market during the last few decades (EU, 2009). In particular a great interest has been developed around the use of second-generation bioenergy crops (Lewandowski *et al.*, 2003; Del Grosso *et al.*, 2014), which include a variety of perennial grasses and woody crops grown purely for energy production (Karp & Richter, 2011; Carneiro & Ferreira, 2012). The possibility of using biomass for energetic purposes allows a wide range of candidate crops: perennial C4 crops, short rotation coppices (SRC) (Karp & Shield, 2008; Zegada-Lizarazu *et al.*, 2010; Hastings *et al.*, 2014; López-bellido *et al.*, 2014). These crops require fewer inputs, have greater energy ratios, and increase GHG savings more than annual C4 and C3 bioenergy crops (Tilman *et al.*, 2006; Davis *et al.*, 2010; Gelfand *et al.*, 2013; Harris *et al.*, 2015). The lignocellulose in these second-generation crops is a more energy-dense material than the starch and sugars used from annual bioenergy crops (Cadoux *et al.*, 2014). It represents a potentially vast and renewable source of biomass feedstock (Creutzig *et al.*, 2014). Strong incentives have been put in place to increase the use of bioenergy from perennial crops from both in the transport as well as in the energy sector, mainly in the form of mandatory targets (Bruell, 2007; Umbach, 2010).

However energy security, economic development and environmental protection are causing concerns over indirect land-use change and conflicts between bioenergy and food production (Karp & Richter, 2011; Dauber *et al.*, 2012; Valentine *et al.*, 2012; Manning *et al.*, 2015). The question is whether and how agriculture can provide sustainably yields to meet the needs of bioenergy and food in a growing population and within the context of a world’s changing climate (Dauber *et al.*, 2012; Valentine *et al.*, 2012; Manning *et al.*, 2015). Cultivation of biomass crops has fuelled several debates on the environmental impacts of their diffusion on arable lands previously occupied by food crops and natural ecosystems (Fargione *et al.*, 2008).

For this reasons, various issues have been associated with bioenergy sustainability. Hill *et al.*, (2006) and Tilman *et al.* (2009) suggest that bioenergy land use need to be net energy provider, environmentally sustainable, economically competitive and not compete with food production. Several decades of research have revealed the environmental impacts of land use (Vitousek *et al.*, 1997; Tilman *et al.*, 2011). Globally, land use thus presents us with a dilemma (Foley *et al.*, 2005): "by considering that agricultural practices are absolutely essential for humanity, because they provide critical provisioning ecosystem services (such as food, fiber, energy and freshwater), are agricultural activities degrading the global environment in ways that may ultimately undermine ecosystem services, human wellbeing, and the long-term sustainability of human societies"?. Over the last decade, numerous initiatives are aiming to promote an environmental-friendly bioenergy production and use (Souza *et al.*, 2015). The potential consequences of land use transition to bioenergy crops on GHG balance through food crop displacement or 'indirect' land use change (iLUC) is currently the main issue for bioenergy research (Searchinger *et al.*, 2008; Dauber *et al.*, 2012; Del Grosso *et al.*, 2014). As a consequence, much effort is now focussed on determining the climate regulation service provided by bioenergy cropping systems (soil C sequestration and GHG savings) (see e.g. Creutzig *et al.*, 2014; Hudiburg *et al.*, 2014; Agostini *et al.*, 2015; Harris *et al.*, 2015). On the contrary, less research has been undertaken on the impacts of bioenergy land use on a wider range of other ecosystem services (ES) essential for human well-being (Holland *et al.*, 2015; Milner *et al.*, 2015). Only in the recent years, it is emerging the needs to put ES in the bioenergy narrative. This is being done in terms of study the impacts of an increased cultivation of perennial bioenergy crops and resulting impacts on ES (Manning *et al.*, 2015). Increasingly, our society is demanding that farmlands produce bioenergy in a sustainable way. One of the key questions of primary importance to food/energy security is how to optimize sustainable intensification to balance competing demands on land for food and energy production, while ensuring the provision of ES and maintaining or increasing yields. The main background of this paper is represented by the idea that perennial bioenergy crops can provide part of the solution to this key question, as they may be allocated on strategic locations within landscape so they do not compete with those lands required for food production. An hypothetical sustainable bioenergy landscape is the one in which a land use is explicitly replaced with the aim to provide biomass for energetic purposes and explicitly support a broad set of key ES for human wellbeing (Figure 1.1c). Experiences from bioenergy development have shown that sustainable production of biomass is an important condition for its public acceptance (van der Horst & Evans, 2010; Lupp *et al.*, 2011; Carneiro & Ferreira, 2012; Dale *et al.*, 2013; Ssegane *et al.*, 2015). Therefore, nowadays the main challenge to the growth of bioenergy is a sustainable production and a sufficient supply of biomass (Karp & Shield, 2008), which can be an important contribution towards sustainable agriculture (López-bellido *et al.*, 2014).

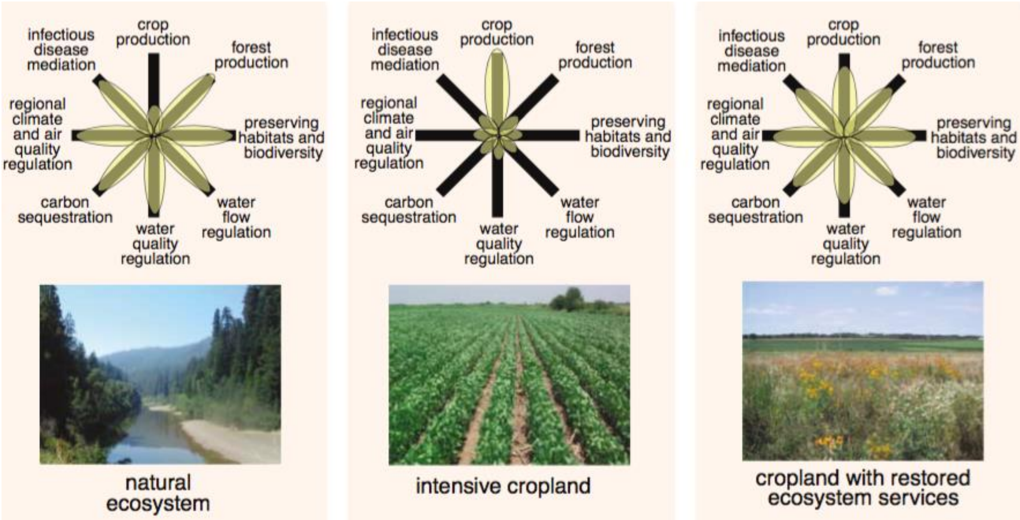

Figure 1.1 Conceptual framework for comparing land use and trade-offs of ecosystem services as proposed by Foley *et al.* (2005). With "flower" diagrams is represented the provisioning of multiple ecosystem services under different land-use regimes. The level of provisioning of each ecosystem service is indicated along each axis. (source: Foley *et al.*, 2005).

1.2 On multiple ecosystems services provision

Regarding the growing human pressure on the global ecosystem, as expressed in such phenomena as the loss of biological diversity or climate problems, it is becoming ever more urgent to control the increasing claims upon limited resources, and to ensure sustainable land use (Tilman *et al.*, 2011). On this regard, the ES topic is currently largely determining the debate in the area of sustainable land use management (Carpenter *et al.*, 2009). Agriculture occupies a substantial proportion of the European land, and consequently agroecosystems play an important role in maintaining ES and cultural landscapes (Schröter *et al.*, 2005). Unsustainable farming practices and land use have an adverse impact on biodiversity and thus on the overall functioning of agroecosystems (Phalan *et al.*, 2011) (Figure 1.1b). The increases in yields have relied heavily on intensive use of fertilizer and pesticides (Tilman *et al.*, 2002), which have polluted some ground and surface waters. Intensification of agricultural practices have depleted C stocks in agricultural soils (Lal, 2011). This simultaneous intensification and expansion of agriculture has caused losses in belowground biodiversity (de Vries *et al.*, 2013; Tsiafouli *et al.*, 2014) and reduced habitat for beneficial organisms like insect pollinators and predators (Klein *et al.*, 2007; Tscharntke *et al.*, 2007; Phalan *et al.*, 2011). To face these issues, the ecosystems approach to sustainable development ("the ecosystems approach") has been promoted by many international organizations including: the Conference of the Parties to the Convention on Biological Diversity (CBD), the Food and Agriculture Organization of the United Nations (FAO), The Organisation for Economic Co-operation and Development and the United

Nations Environment Programme. The CBD defined the "ecosystem approach" as the "importance of managing ecosystems in a socio-economic context in order to maintain ES for humans and that conservation of resources must be balanced with their use". The Millennium Ecosystem Assessment (MEA, 2003, 2005a) developed this principle into a framework of ES (Figure 1.2) in which was promoted the concept that "ecosystem processes insure agroecosystems health and functioning".
This framework assessed the consequences of ecosystem change for human well-being, defining ES as "the benefits people obtain from ecosystems" (MEA, 2005a, p. 40). According to this framework (MEA, 2003), ES are classified in four categories (Figure 1.2): *supporting services* ("services necessary to the production of all other ES"), *provisioning services* ("products obtained from ecosystems"), *regulating services* ("benefit obtained from regulation of ecosystem processes") and *cultural services* ("non material benefit obtained from ecosystems"). Influenced by agronomic practices, ecosystem processes within agroecosystems can provide services that support the biomass provisioning services, including pollination, pest control, regulation of soil fertility and soil erosion and water quality regulation (Power, 2010). Management practices also influence the potential for "disservices" from agriculture, including loss of habitat for beneficial wildlife, water pollution, sedimentation of watercourses and pesticide poisoning of biological species (Zhang *et al.*, 2007).
Soil management is fundamental to all agroecosystems, yet there is evidence for widespread degradation of agricultural soils in the form of erosion, loss of organic matter, contamination, compaction, salinization and other harms (European Commision, 2002). Soil is a non-renewable resource, which provides a number of ecosystem, social and economic services (Doran & Zeiss, 2000; Karlen *et al.*, 2003). Because food and energy production depend on soil for the provision of these services (Costanza *et al.*, 1997; Daily, 1997; de Groot *et al.*, 2002) it is essential to include soil ES into the MEA framework to inform agri-environmental policies (Robinson *et al.*, 2013).
The reason for this is that soil is a living system and as such is distinguished from parent material mainly by its biology. Agricultural soils are the habitat for many different key functional organisms (Brussaard *et al.*, 2007) which collectively contribute to a variety of soil-based ES (Wall *et al.*, 2004; de Vries *et al.*, 2013) (Figure 1.3). These ES can be easily recognized and included within the categories identified by the MEA (Dominati *et al.*, 2010).

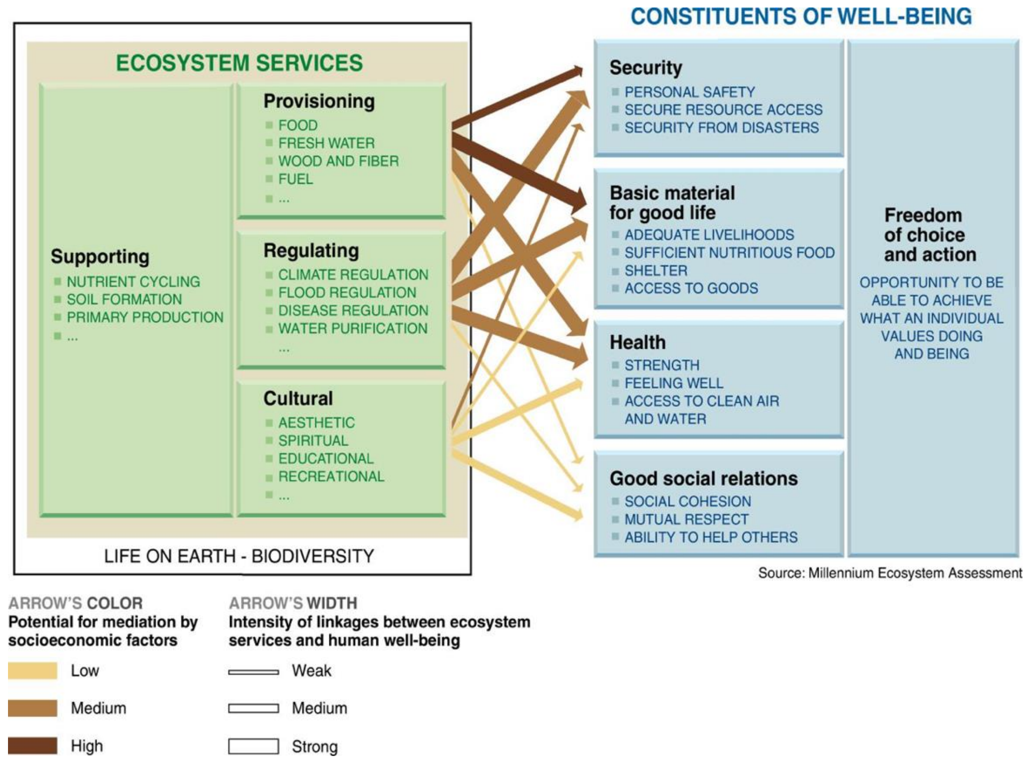

Figure 1.2 Ecosystem services and their links to human well-being, as described in the conceptual framework of the Millennium Ecosystem Assessment (source: MEA, 2005a).

These soil-specific ES include the ecosystem processes that support the production of food and energy (*supporting services* of Figure 1.2), such as nutrient cycling, and sustain the regulation of water flow and quality, the biological control of pests and diseases and the regulation of the soil GHG emissions with its implications for the control of the global climate (*regulating services* of Figure 1.2). In reality, these services are all functional outputs of soil biological processes (Brussaard *et al.*, 2007; Kibblewhite *et al.*, 2008).

In the last decade, a lot of research was needed to better characterize the ES provided by soils along with a better understanding of the interrelationships of different ES supplied by soils and soil management practices (Dominati *et al.*, 2010; Robinson *et al.*, 2013). Many authors, mainly working on a broad range of different agroecosystems, have detailed services provided by soils (Lavelle *et al.*, 2006; Barrios, 2007; Zhang *et al.*, 2007; Sandhu *et al.*, 2008; Porter *et al.*, 2009). Moreover, it has been underlined how management of soil biodiversity is a keystone for multiple provision of soil-based ES (Barrios, 2007; Brussaard *et al.*, 2007; Kibblewhite *et al.*, 2008; Pulleman *et al.*, 2012; de Vries *et al.*, 2013). Recently, a conceptual framework for classifying, quantifying and modelling soil natural capital and ES (Figure 1.3) has been proposed (Dominati *et al.*, 2010) and applied to national scale (Orwin *et al.*, 2015). This framework is based on the classification of ES as described in MEA

(2003), but provides a more holistic approach to identify ES by linking soil ES to soil natural capital and soil processes and especially how ES are affected by soil management. This is fundamental because the major impacts on soil functions and consequently the provision of ES are derived from land-use change and soil management practices (Powlson *et al.*, 2011). The knowledge of soil functioning in relation to management practices and climate change issue has led, indeed, soil scientists to evaluate a set of soil health indicators representative of fundamental soil-based ES (Bastida *et al.*, 2008; Faber & van Wensem, 2012; Pulleman *et al.*, 2012). Within the debate over the perspectives of creating sustainable bioenergy landscapes, the concept of soil health in response to increased bioenergy crops cultivation play a pivotal role for making this debate effective. Soil health, as formulated by Kibblewhite *et al.* (2008), is as an integrative property that reflects the capacity of soil to respond to agricultural intervention, so that it continues to support both the agricultural production and the provision of multiple ES. On this context, the MEA framework of ES (Figure 1.2) is applied in this thesis for studying the impacts of land use transition to perennial bioenergy crops on multiple ES provision, taking into account also the soil-based ES as described by Dominati et al. (2010) (Figure 1.3).

Another reason for which ES were used as main assessment tool to study environmental impacts of bioenergy crops is because of the attractiveness of the concept of ES, which is based on its integrative, interdisciplinary character, as well as its linking of environmental and socio-economic aspect (MEA, 2005b). Using the concept of ES when discussing the food-energy-environment trilemma (Tilman *et al.*, 2009) can bring the interrelations and dynamics between food and bioenergy cropping systems into the picture while at the same time maintain a certain degree of simplicity (Dale *et al.*, 2011a; Gasparatos *et al.*, 2011).

This is because the concept of ES directly links ecosystem impact and human wellbeing, which are two key elements of the bioenergy debate evoked by supporters and critics alike (Gasparatos *et al.*, 2011; Dale *et al.*, 2014; Milner *et al.*, 2015). Additionally, ES have gained popularity in the academic community (Fisher *et al.*, 2009) and have been widely accepted by soil science community (Dominati *et al.*, 2010; Robinson *et al.*, 2013) and by policy makers. The concept of ES is, indeed, a matter of great political relevance since it has been adopted by multilateral environmental agreements such as the Global Bioenergy Partnership (GBEP) and the FAO-Global Soil Partnership. European Union has expressed the "ES approach" in several policies, e.g. in the EU Soil Thematic Strategy and in Common Agricultural Policy 2014-2020. Moreover, both European and member states policies explicitly address a number of environmental and societal goals on different levels related with bioenergy production, e.g. protection of natural resources, enhancement of ES, creating regional added value and employment in rural and marginal areas. Impacts and effects of these strategies are to be assessed regarding all levels of sustainability.

The concept of multiple ES (Figure 1.2-1.3) addresses all these levels of sustainability, and it can be used as a stimulus and as a tool to find appropriate solutions to balance the production of renewable energy with other regulating ES provided by multifunctional bioenergy landscapes. Even though the MEA framework of ES (MEA, 2003,205a) has been used extensively to understand the impact of numerous human activities on diverse social–ecological systems, there is still little literature explicitly linking bioenergy production, land use change and multiple ES (e.g. Donnelly *et al.*, 2011; Gasparotos *et al.*, 2011; Holland *et al.*, 2015, Milner *et al.*, 2015). As demonstrated in the following chapters, the land use transition to perennial bioenergy crops can affect various ecosystem processes and ultimately delivery a broad variety of key ES. In particular, it will be addressed here the possibility to maintain and in some cases enhance, through a careful spatial design of bioenergy crops into landscape, the provision of a large set of regulating ES (climate, water and biodiversity regulation) that may ultimately promote the creation of multifunctional bioenergy landscapes.

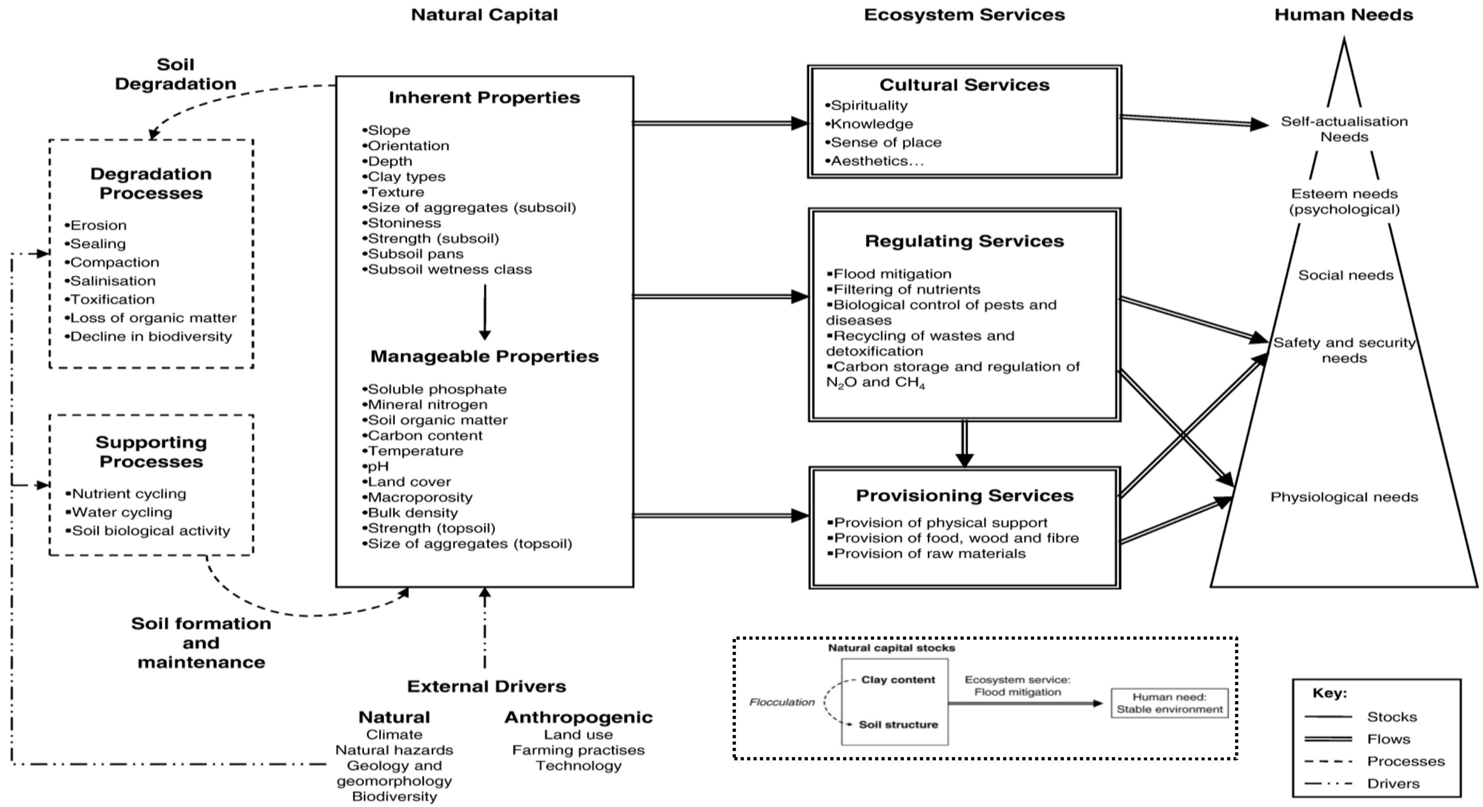

Figure 1.3 Framework for the provision of ecosystem services from soil natural capital as proposed by Dominati *et al.* (2010). The “process” is defined as the transformation of input into outputs. Ecosystem services are not processes but flows (amount per unit time), as opposed to stocks (amount). See the dotted box as an example: flocculation is the physical-chemical processes where cations and water molecules bound themselves to negatively charged clay particles. As results of this process, the provision of the ecosystem service ‘flood mitigation’ depends on the amount of water a soil can store (stock).

1.3 Bioenergy buffers: a case study for multiple ES provision from perennial bioenergy crops

Increasing the cultivation of bioenergy crops introduces the difficulty of reconciling food and energy production, and conservation of the environment (Tilman et al., 2009). Hence, to ensure a sustainable development of bioenergy crops, the area dedicated to their production must be limited to minimize the competition with food production, the energy production per unit area must be high enough to replace significant amounts of fossil resources, and their impacts on ES must be as low as possible (Karp & Shield, 2008; Haughton *et al.*, 2009; Rowe *et al.*, 2013; Werling *et al.*, 2013; Del Grosso *et al.*, 2014). This suggests seeking the optimal perennial bioenergy crop for a given environment, its optimal allocation into the landscape and its suitable cropping practices that could simultaneously fulfill all these requirements.

The paradigm of the current research on bioenergy production is based on the "food *vs.* fuel" debate (Anderson-Teixeira *et al.*, 2012; Valentine *et al.*, 2012). The key point is that the current policies rely on the old-fashioned agricultural paradigm of cultivating bioenergy crops on large-scale cultivations that are spatially distinct with intensive agriculture dedicated to food production (Manning *et al.*, 2015). As a consequence of the adoption of this "land sparing" approach (Anderson-Teixeira *et al.*, 2012), the ES provision is threatened if natural or semi natural area are converted to intensive bioenergy production (Fargione *et al.*, 2008; Creutzig *et al.*, 2014). Research is focusing, indeed, on developing bioenergy systems that avoid land use conflicts (Fargione *et al.*, 2008; Karp & Shield, 2008; Valentine *et al.*, 2012). A common response to the potential competition between energy and food crops is to suggest that marginal lands rather than cropland be targeted for bioenergy production (Dauber *et al.*, 2012) (Figure 1.1a). Marginal lands are those lands poorly suited to field crops because of low crop productivity due to inherent edaphic or climatic limitations or because they are located in areas that are vulnerable to erosion or other environmental risks when cultivated (Gopalakrishnan *et al.*, 2006; Valentine *et al.*, 2012; Shortall, 2013). If bioenergy crops are cultivated on marginal lands, there are two major drawbacks of this. First, bioenergy production still needs to be economically viable on low-yielding marginal lands. This because it would not be cost-effective to establish bioenergy crops on areas where conditions are too unfavorable, water supplies are limited, or the logistic constraints are too high (e.g. distance to the power plant) (Allen *et al.*, 2014). Moreover, Bryngelsson & Lindgren (2013) showed that the economic incentives would be strong for owners of more productive cropland to grow bioenergy anyway and out-compete the more costly production on low yielding marginal lands. Second, marginal land, if not being used for agricultural production, is likely to have a high biodiversity and ES value.

Moving bioenergy production to marginal lands is often associated with the conversion of natural or semi-natural ecosystems, resulting in greater biodiversity and C losses than when compared to the conversion of arable lands (Fargione *et al.*, 2008; Phalan *et al.*, 2011; Immerzeel *et al.*, 2014).

Current policy advice largely ignores the potential to better manage bioenergy crops to reduce their impacts on biodiversity and ES and for strategic deployment of perennial bioenergy crops within agricultural landscapes (Manning *et al.*, 2015). Recently published papers showed that the linkage between bioenergy production and multiple ES is dependent not only on the choice of bioenergy crop but also on its location relative to other land uses (Glover *et al.*, 2010; Young-Mathews *et al.*, 2010; Rowe *et al.*, 2013; Werling *et al.*, 2013). On this context, science-based policies based on new bioenergy land use scenarios are needed to inform sustainable bioenergy landscape design. Werling *et al.* (2013) and Manning *et al.* (2014) claimed that careful design of bioenergy landscapes has the potential to enhance multiple ES in food and bioenergy cropping systems, leading to important synergies that have not yet informed the ongoing bioenergy debate. Such research would contribute to the current trend to develop “ecological intensification” strategies that foster synergies between land uses and attempt to reduce the trade-offs between the delivery of multiple ES (Garnett *et al.*, 2013) within limited land resources (Allen *et al.*, 2014). On this regard, new bioenergy land use scenarios are being formulated in which food and bioenergy plantations are spatially mixed within the same farmland (Figure 1.4b) (Asbjornsen *et al.*, 2012; Christen & Dalgaard, 2013; Manning *et al.*, 2015; Golkowska *et al.*, 2016). For example, the results of several modelling studies (Gopalakrishnan *et al.*, 2012; Meehan *et al.*, 2013; Ssegane *et al.*, 2015) show that the cultivation of bioenergy crops e.g. along watercourses may achieve yields that are comparable to those obtained for food cropping systems while simultaneously providing multiple ES. Within this framework, an excellent case study area in which to explore the possibility to optimize land use for food, energy, and ES is the European agricultural landscape. Linear elements such as ditches, grass margins, buffers strips and hedgerows are landscape elements widely adopted across EU member states (Marshall & Moonen, 2002; Van Der Zanden *et al.*, 2013). Buffer strips and grass margins, for example, have been widely recognized for their ecological perfomances (Le Cœur *et al.*, 2002; De Cauwer *et al.*, 2005) in terms of mitigation of disservices of the agricultural activities via climate mitigation (Falloon *et al.*, 2004), biodiversity regulation (Smith *et al.*, 2007; Ernoult *et al.*, 2013) and erosion regulation (Panagos *et al.*, 2015).

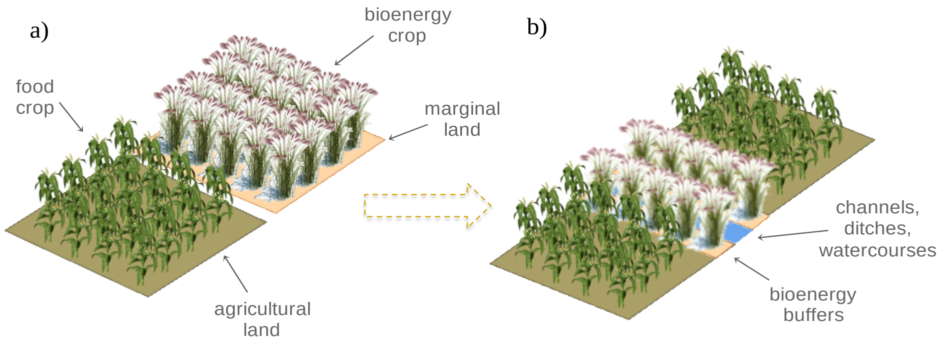

Figure 1.4 Schematic representation of the current bioenergy land use with marginal land conversion (a) and the alternative bioenergy land use scenario proposed in this thesis with "bioenergy buffers" (b). Bioenergy buffers are in our view perennial landscape elements, consisting of narrow bands (as wide as national recommendations e.g. 5-10 m wide) placed along arable field margins and watercourses, and cultivated with perennial herbaceous (switchgrass or miscanthus) or woody (poplar or willow SRC) dedicated to bioenergy production.

A recent paper proved that grass margins are not only beneficial for wildlife but also for the productivity over the long term of the adjacent cropped areas (Pywell *et al.*, 2015). Buffer strips were mainly thought as landscape elements aiming at reducing in lowland areas the agricultural non point source pollution such pesticides and nitrate water pollution (Mayer *et al.*, 2007; Borin *et al.*, 2010). In the EU environmental policy context, indeed, buffer strips were made mandatory among member states in order to fulfill the obligations to maintain and improve Good Ecological Status under the EU Water Framework Directive (EC 2000/60). However, if properly vegetated and managed, buffer strips can also produce biomass for energetic purposes (Golkowska *et al.*, 2016). On the other hand, farming restrictions in the management of buffer strips led to different decisions among EU member states regarding subsidies and management schemes for buffer strips (Brown *et al.*, 2012; Stutter *et al.*, 2012). If no harvest bans on the buffer strips exist, the biomass could generate additional incomes that might contribute to bioenergy supply while maintaining buffers ecological functioning (Golkowska *et al.*, 2016).

Targeting perennial bioenergy crops along buffer strips (hereinafter "bioenergy buffers" – Figure 1.4b) could be used to design new sustainable bioenergy landscapes. The cultivation of perennial bioenergy crops has already shown that on large-scale plantations multiple ES can be provided in a larger extent compared to annual food crops (Rowe *et al.*, 2013; Werling *et al.*, 2013; Holland *et al.*, 2015; Milner *et al.*, 2015). To see if the productive and ecological performances of perennial bioenergy crops are significant also in a bioenergy buffers scenario, new research is required. For this reason, in this thesis, bioenergy buffers are considered as a stimulating case study for seeking an alternative bioenergy land use scenario within the food-energy-environment trilemma.

1.4 Objectives of the thesis

Given the issues of “food-energy-environment” trilemma (Tilman *et al.*, 2009) and the implications of land use transition to bioenergy crops on climate regulation (Creutzig *et al.*, 2014; Agostini *et al.*, 2015; Harris *et al.*, 2015) and biodiversity (Dauber *et al.*, 2010; Immerzeel *et al.*, 2014), the MEA framework on ES provides an opportunity to examine the impacts of new bioenergy land use scenarios (Gasparatos *et al.*, 2011; Holland *et al.*, 2015). In this thesis an alternative bioenergy land use scenario has been tested: the bioenergy buffers (Figure 1.4b). Despite natural riparian buffers provide multiple functions in agricultural landscapes (Marshall & Moonen, 2002; Borin *et al.*, 2010; Pywell *et al.*, 2015), the debate evoked by Christen & Dalgaard (2013) to establish and manage buffers with a dual purpose (biomass production and environment protection) has never been repeated. Applied research on bioenergy buffers is still lacking both in terms of multiple ES provision, biomass yield potential and biomass logistic management. In particular, given the role attributed to buffer strips in mitigating groundwater pollution, is still unclear, for bioenergy buffers, to what extent, under field conditions, bioenergy crops remove N from groundwater as compared to natural riparian buffers. Yet, there are no available information for bioenergy buffers on the role of plant-derived C inputs and belowground biomass on the biological removal of N from soil. Hence, the main research question of this thesis is: *“To what extent do the perennial bioenergy crops affect the delivery of multiple ecosystem services when cultivated as bioenergy buffers?”*

To answer this question, the main objectives of the thesis are:

1. to synthetize the current state of knowledge on the impact of land use transition to bioenergy buffers on multiple ES provision (climate, water and biodiversity regulation, soil health and biomass provisioning);
2. to identify the opportunities and shortcomings related to the implementation of bioenergy crops along buffers and to the biomass logistics management in bioenergy buffers;
3. to evaluate bioenergy buffers effectiveness (BSE) in removing N from groundwater;
4. to identify the biogeochemical processes and key factors governing N removal in bioenergy buffers;
5. to quantify below- and above-ground biomass production and plant N removal in bioenergy buffers.

1.5 Outline and experimental approach

To address the objectives of the thesis a combination of systematic literature review (Chapter 2) and field studies (Chapter 3) was used. The following hypothesis resulted from the objectives of the thesis:

H1 Perennial bioenergy crops could be grown as bioenergy buffers to produce bioenergy, sustain multiple ES and diversify agricultural landscapes

H2 Bioenergy buffers may challenge the sustainability of the biomass supply chain

H3 Perennial bioenergy crops, if cultivated adjacent to watercourses, may intercept and remove efficiently N from groundwater as much as buffers strips with spontaneous species

H4 Deep-rooted crops such as perennial bioenergy crops lead to significant plant-microbial linkages activating soil microbial biomass and, in turn, biological N removal from soil

H5 Miscanthus and willow buffers produce a significant amount of below- and above-ground biomass if cultivated in nitrate-enriched shallow groundwater

Chapter 2 addresses the first two hypotheses. Combining the Millenium Ecosystem Assessment framework on ES (MEA, 2003) (Figure 1.2) with the framework on soil ES proposed by Dominati *et al.* (2010) (Figure 1.3), the literature on ES provided by perennial bioenergy crops replacing cropland and grassland was systematically reviewed to answer hypothesis H1. The literature search is conducted on four candidate bioenergy crops for Europe namely miscanthus, swicthgrass, poplar and willow as short-rotation coppice (SRC). The impacts on multiple ES of land use transition to herbaceous or woody bioenergy buffers was synthetized by applying to 237 effects on ES (extracted from 127 studies) an impact scoring methodology to reveal direction and level of confidence of the impacts. Comparing the different ES provided by bioenergy buffers, the following roles of soil in the provision of ES from bioenergy buffers have been addressed in *Chapter 2*: climate regulation role; water quality and soil erosion regulation role, aboveground biodiversity conservation role, role in supporting soil health and biomass/energy provisioning role.

Chapter 2 address also the establishment of bioenergy buffers and their biomass logistics management. By reviewing the existing literature that investigated in large-scale bioenergy plantations the crop establishment issues and the biomass harvest, storage and transports operations, the available technologies and the potential logistic and management options that could also apply to bioenergy buffers have been investigated (hypothesis H2).
Particular attention is given to search for the environmental implications and the potential logistic and biophysical constraints that may emerge from the management of bioenergy and food crops within the same farmland.

Chapter 3 is focused on a very important issue for bioenergy sustainability: use of perennial bioenergy crops not only for bioenergy production, but explicitly for the protection of groundwater from the nitrate leaching from agricultural fields. *Chapter 3* is based on an experimental field trial aiming at studying the productive and environmental performances of bioenergy buffers at farm-scale.

In a N-enriched shallow groundwater, 5 and 10 m wide miscanthus and willow buffers (Figure 1.5c and 1.5d) are established along a ditch of an agricultural field in Po valley (Italy). A control treatment consisting of field margins left revegetating with spontaneous species is included (Figure 1.5e) to compare differences in removing N between spontaneous and bioenergy crop species. The hypothesis H3, H4 and H5 were addressed in *Chapter 3* through the measurement of the below- and above-ground biomass production and the N removal from groundwater and soil. The study allows to answer the question about the dual purpose of bioenergy buffers: biomass production (H5) and environment protection: in this case, mitigation of groundwater N pollution (H3) and active biological removal of N from soil (H4).

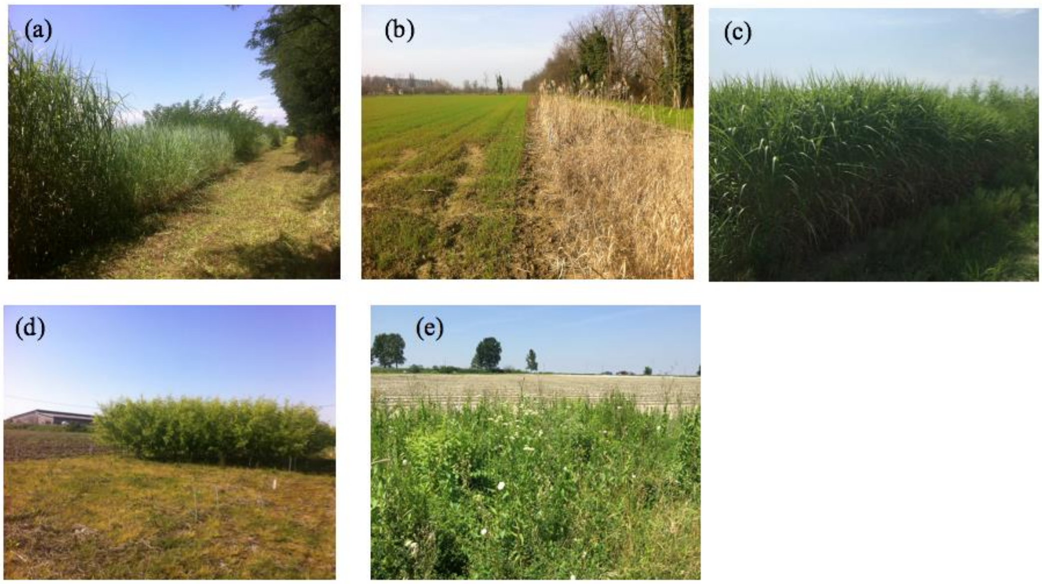

Figure 1.5 Examples of bioenergy buffers established in Po valley (northern Italy): (a) panoramic of a field trial with arable field margins cultivated with miscanthus (*Miscanthus x giganteus* L.), switchgrass (*Panicum virgatum* L.) and black locust (*Robinia pseudoacacia* L.) (July 2013); (b) miscanthus and switchgrass during the winter periods (December 2014) offering soil cover and habitat for wildlife along buffers; (c-d) bioenergy buffers experimental trial (June 2015) with miscanthus (c) and willow (*Salix matsudana* Koidz) (d) set up along a ditch where 5m wide buffer strips are mandatory under the EU Water Framework Directive (2000/60/EC); (e) spontaneous species as control treatment to compare in *Chapter 3* the N removal efficiency between naturally vegetated buffers (e) and bioenergy buffers (c-d).

Chapter 2

Multiple ecosystem services provision and biomass logistics management in bioenergy buffers: a state-of-the-art review

The chapter is submitted to *Renewable & Sustainable Energy Reviews* as:

Ferrarini A.[1], Serra P.[1], Almagro M.[2], Trevisan M.[3], Amaducci S.[1*]. Multiple ecosystem services provision and biomass logistics management in bioenergy buffers: a state-of-the-art review

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Multiple ecosystem services provision and biomass logistics management in bioenergy buffers: a state-of-the-art review

Abstract

Bioenergy buffers are linear landscape elements cultivated with perennial herbaceous or woody crops dedicated to bioenergy production placed along arable field margins and watercourses. In this study, we sought to provide an evidence base for potential impacts of bioenergy buffers on multiple ecosystem services (ES) while identifying the opportunities and shortcomings related to the biomass logistics in bioenergy buffers compared to that derived from large-scale bioenergy plantations. We synthetize the current state of knowledge on the impacts of land use transition to bioenergy crops on regulating (climate, water and biodiversity regulation), supporting (soil health) and provisioning services (biomass provision and energy yield). Using an Impact Assessment (IA) methodology we evaluated the short- and long-term impacts of woody and herbaceous bioenergy buffers on previous croplands and grasslands on the provision of those ES. The results of the IA revealed that the implementation of bioenergy buffers on previous croplands rather than on grasslands sustains long-term provision of ES such as climate, water quality, and biodiversity regulation. Moreover, herbaceous rather than woody buffers were found to be more effective in the provision of multiple ES. Nevertheless, some research gaps were identified relative to the impacts of bioenergy buffers during the establishment phase on climate and water quality regulation services. Regarding biomass logistics, the limited working space for the farm machinery operations may be considered as the main shortcoming for bioenergy buffers compared to large-scale bioenergy plantations. The intra- and inter-farm spatial fragmentation of biomass supply areas may increase environmental costs related to biomass collection and transport operations. In order to address this logistic constraint and to stimulate the scientific debate on the ES benefits rendered by bioenergy buffers to agroecosystems, their implementation as Ecological Focus Area within 2014-2020 CAP and as mandatory buffer strips under the EU Water Framework Directive is encouraged.

Keywords: land use conflicts, bioenergy buffers, miscanthus, switchgrass, willow, poplar, ecosystem services, climate regulation, water quality regulation, biodiversity regulation, biomass supply chain

2.1 Introduction

Food security, climate change, and energy use are widely recognized to be the main challenges faced by humankind in the 21st century (Lal, 2010; Karp & Richter, 2011). The increasing demand for energy and food (Umbach, 2010; Tilman *et al.*, 2011) and the negative implications of climate change on agricultural production are fuelling the debate over the land use conflicts between bioenergy and food production (Fargione *et al.*, 2008; Dauber *et al.*, 2012; Valentine *et al.*, 2012). This situation is a direct consequence of the old-fashioned paradigm of an intensive cultivation on arable lands of bioenergy crops as large-scale plantations (Shortall, 2013; Manning *et al.*, 2015).

The paradigm should shift from "food *vs.* fuel" debate to one more challenging target: Where and how bioenergy crops could be established within intensively managed agricultural landscapes? Given that land management should focus on developing bioenergy systems that avoid land use conflicts (Fargione *et al.*, 2008; Karp & Shield, 2008; Valentine *et al.*, 2012), to grow bioenergy crops on marginal lands could be a good option (Dauber *et al.*, 2012) (Figure 2.1a). The sustainable use of land for bioenergy production is inextricably linked to energy savings and yield potentials of bioenergy crops, biomass supply chain management, and multiple ecosystem services (ES) provision, with potentially positive or negative consequences depending on how these linkages are managed (Dale *et al.*, 2011b; Del Grosso *et al.*, 2014; López-bellido *et al.*, 2014). Besides energy savings of bioenergy crops (Rettenmaier *et al.*, 2010) and their yield potential (Laurent *et al.*, 2015), a particular interest revolves around the potential impacts of land use transition to bioenergy crops on multiple ES provision (Milner *et al.*, 2015). This is because herbaceous and short rotation coppices (SRC) woody crops are being considered promising carbon-neutral options due to their potential for greenhouse gas (GHG) emission savings (Rettenmaier *et al.*, 2010; Felten *et al.*, 2013; Gelfand *et al.*, 2013; Creutzig *et al.*, 2014) and long-term soil carbon (C) sequestration (Agostini *et al.*, 2015; Harris *et al.*, 2015; Chimento *et al.*, 2016). Furthermore, from different review papers emerged that other ES could be delivered cultivating bioenergy crops such as biodiversity and water quality regulation (Blanco-Canqui, 2010; Immerzeel *et al.*, 2014; Holland *et al.*, 2015). Identifying the direction of the impacts on multiple ES provision can fuel the discussion over the synergies which could be achieved between bioenergy production and other land uses.

Along with ES provision, the optimization of the biomass supply chain is becoming a crucial issue within the sustainability framework of land use transition to bioenergy production (Smeets *et al.*, 2009; Dale *et al.*, 2011b; Gold & Seuring, 2011; van der Hilst *et al.*, 2012). An optimal allocation of bioenergy plantations within the landscape is needed in order to harmonize biomass logistic management (Gold & Seuring, 2011; Mafakheri & Nasiri, 2014; Dale *et al.*, 2016). In particular, the following aspects should be considered: i) field location and relative land-use conflicts (Smeets *et al.*, 2009); ii) available technologies associated with biomass logistics from harvest to transport (Gold & Seuring, 2011; Cattaneo *et al.*, 2014a); iii) spatial and temporal combination of biomass supply areas and energy demand within the landscape (Howard *et al.*, 2012); iv) farmer's acceptance of new bioenergy crops (van der Horst & Evans, 2010; Rizzo *et al.*, 2014); and v) existing environmental protected areas (Gopalakrishnan *et al.*, 2006).

In order to reduce the logistic issues and find the best trade-offs with multiple ES provision, new land use scenarios for bioenergy production are needed. Unlike the land sparing approach focused on marginal land conversion (Figure 1a), new scenarios are being formulated in which food and bioenergy plantations are spatially mixed within the landscape (Asbjornsen *et al.*, 2012; Christen & Dalgaard, 2013; Manning *et al.*, 2015) and spatially simulated (Gopalakrishnan *et al.*, 2012; Meehan *et al.*, 2013; Ssegane *et al.*, 2015). In this study we propose to spatially mix food crops with bioenergy plantations within the same farmland (Figure 1b). In particular, we propose to grow biomass on "bioenergy buffers". Those are considered perennial landscape elements, consisting of narrow bands (5 - 10 m width) placed along arable field margins and watercourses, and cultivated with perennial herbaceous or woody SRC crops dedicated to bioenergy production (Figure 1c-e). Although the impact assessment of large-scale bioenergy cultivations on ES provision has received considerable attention, the same is not true for the implementation of bioenergy buffers. Furthermore, to our knowledge, apart from a review paper on ecological functioning of buffers dedicated to general biomass production purposes (Christen & Dalgaard, 2013), no synthesis studies have been performed to assess the net effects of bioenergy buffer establishment on multiple ES provision considering also their logistic features along the biomass supply chain.

The main scope of this paper is to review the consequences of land use transition to bioenergy buffers on multiple ES provision and identify the opportunities and shortcomings related to the logistics of biomass from bioenergy buffers. The specific objectives are: 1) to assess the potential impacts of herbaceous and woody bioenergy buffers on the provision of ES such as climate, water, and biodiversity regulation, and biomass provision, at the establishment phase as well as during perennial crop lifespan (for this purpose bioenergy buffers replacing croplands or grasslands were considered as main land use transitions); 2) to identify biophysical and management factors affecting the implementation of bioenergy buffers along field margins; and 3) to identify the logistic features along the biomass supply chain of bioenergy buffers and compare them to those derived from large-scale bioenergy plantations.

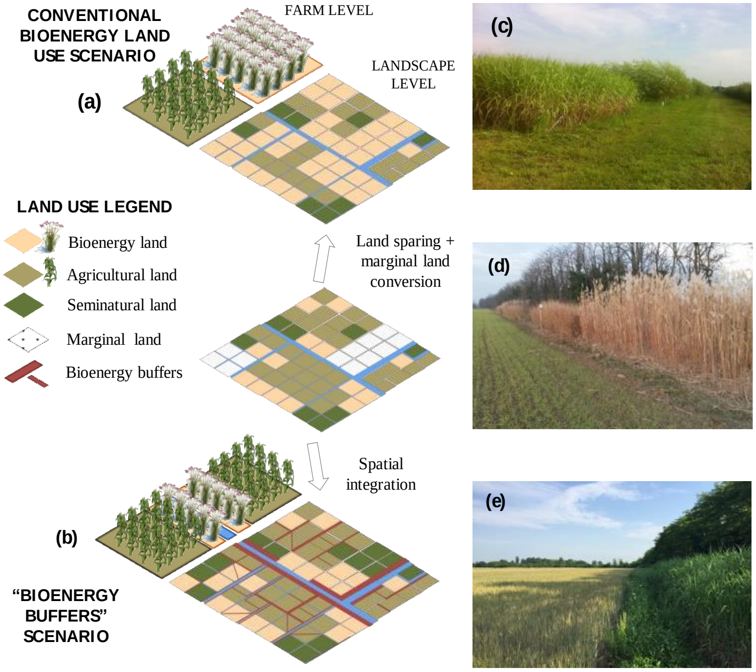

Figure 2.1 Conventional and "bioenergy buffers" land use scenarios for bioenergy crops at farm and landscape scale. (a) The conventional "land-sparing" scenario in which intensive agriculture and bioenergy plantations are spatially separated in different land use units. (b) The alternative land use scenario with "bioenergy buffers" located along watercourses and arable field margins. (c-e) Bioenergy buffer experimental field trials with miscanthus, switchgrass and willow established in the Po valley (Italy). (c): grass and woody buffers strips alongside watercourses (d-e): narrow arable field margins converted to herbaceous or woody strips in flatlands.

2.2 Methods of systematic literature review

The objective of this synthesis study is to assess the implications for ecosystem service provision and biomass supply chain management of land-use change associated with conversion to bioenergy buffers. The systematic literature review process was performed from December 2014 to May 2015, and followed three steps to collect, classify and evaluate the existing literature body on bioenergy buffers (Figure 2.2). English-written peer-reviewed scientific papers and reviews were selected as main unit of analysis. The major databases and library services were used: Google scholar, Scopus, Elsevier, Springer and Wiley. As a first step, the literature body was collected by searching papers that focused on land-use change to bioenergy crops (Figure 2.2 – Step 1). The references were filtered based on the presence of the combination of "land use change" and "bioenergy crops" in their title, abstract and keywords.

Due to the inconsistent use of the term “marginal” land within the literature (Shortall, 2013; Holland *et al.*, 2015), the keyword search was restricted to studies in which bioenergy crops replaced croplands or grasslands. This search returned 665 references. In the second step, two classification contexts were created to pool and classify the literature body previously collected. The context “Bioenergy large-scale plantations” included the references addressing land use change to bioenergy crops at open-field scale and focused on the land sparing approach (n_{ref} = 420). The context “Bioenergy buffers” referred to those references that addressed specifically the use of bioenergy crops as riparian buffer strips, filter strips, grassed waterways and shelterbelts (n_{ref} = 56).

In the third step, all the material collected was further filtered to synthesize the state of the art knowledge on bioenergy buffers according to the objectives 1 and 3. Regarding objective 1, we used the framework of the Millennium Ecosystem Assessment (MEA, 2003, 2005a) that divides ecosystem services into provisioning, regulating, supporting and cultural services in combination with the framework on soil ES proposed by Dominati *et al.* (2010). Here we reviewed the most relevant ES of bioenergy production (Gasparatos *et al.*, 2011; Holland *et al.*, 2015; Milner *et al.*, 2015), agroecosystem (Zhang *et al.*, 2007; Power, 2010) and soil natural capital (Dominati *et al.*, 2010; Powlson *et al.*, 2011; Robinson *et al.*, 2013): provisioning services (biomass provision and energy yield), regulating services (climate, water quality, and aboveground biodiversity regulation) and supporting services (soil health). Studies measuring changes over time in the ES provision during conversion of cropland or grassland sites to bioenergy crops (using either a reference state or a space-for-time substitution approach) were included in the analysis following the approach of Holland *et al.* (2015) (Table S2.2). On these studies, the following combinations of main descriptors (and their relative keywords) were used to search for the potential impacts of the land use transition to bioenergy buffers on ES provision: “impact” or “effect” as well as “ecosystem services” such as “climate regulation”, “water quality regulation”, “soil health”, “belowground biodiversity”, “aboveground biodiversity”, and “pollination and pest control”. In the case that only few studies within the “Bioenergy buffers” context met our selection criteria for a certain ES, the studies grouped into the “Bioenergy large-scale cultivations” context were considered. This was based on the assumption that bioenergy crops grown in bioenergy buffers would have the same impact on a specific ES than those grown at open-field scale.

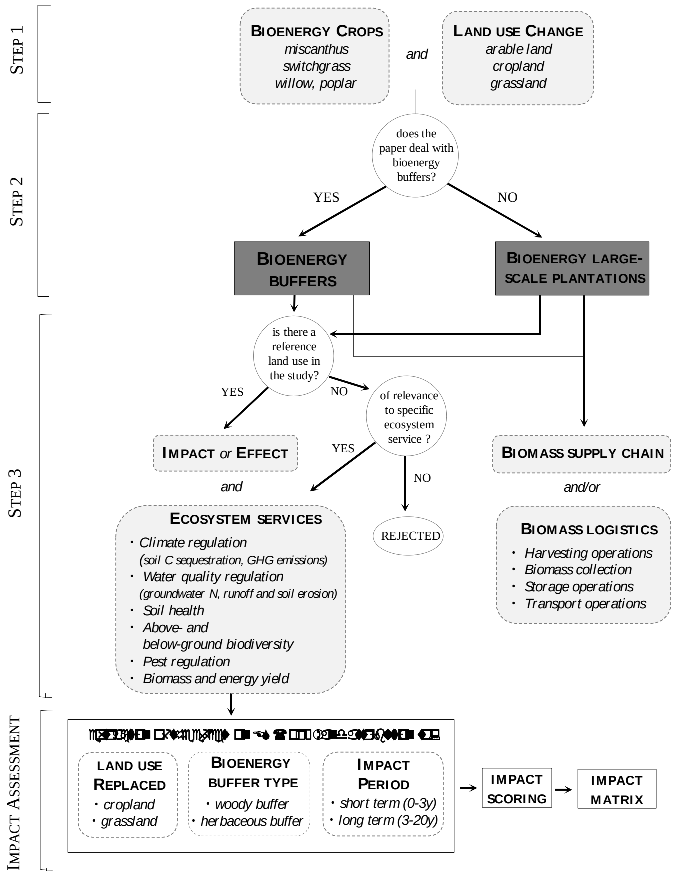

Figure 2.2 Flow chart of steps taken in conducting the systematic literature review process. Dashed grey boxes indicate the main descriptors (capital bold) and keywords (italic) used along the systematic revision. White circles indicate the filters used to refine the literature search. Dark grey boxes indicate the classification contexts where material collected were stored to be further analysed. White boxes indicate the steps involved in the impact assessment on ES provision of land use transition to bioenergy buffers.

Afterwards, the potential impacts on ES provision were derived through the application of an Impact Assessment (IA) methodology (Figure 2.2). The main goal of the IA is to identify where the impact on multiple ES provision would most probably be placed depending on different combinations of land use transitions to bioenergy buffers (*i.e.,* short- and long-term impacts of cropland or grassland conversion to woody or herbaceous bioenergy buffers). The details of the IA methodology are described in the Supporting Information (Appendix S2.1). Briefly, IA followed three steps (Figure 2.2): 1) extraction from each study of the ES examined, the land-use replaced, bioenergy buffer type, the impact period and the direction of the impact (Table S2.2). If a study reported several ES, land-use transitions, buffer types, impact periods, or combinations of these, the information was disaggregated to capture individual reported effects; 2) application of an impact scoring system (Eq. S2.1) to determine the direction of the impacts on ES provision of a particular land use transition and its level of confidence (Table S2.1); and 3) creation of an impact matrix as main data mining tool which provides an overall evidence base of the potential impacts of the implementations of bioenergy buffers on multiple ES provision (Figure 2.5).

Concerning biomass logistics in bioenergy buffers (objective 3), the material collected in step 2 was analysed by conducting a literature search based on the combinations of the descriptors "biomass supply chain" and/or "biomass logistics" (Figure 2.2 - Step 3). Afterwards, the main text of the selected studies was further examined through an iterative search according to the following keywords: "harvesting operations", "biomass collection", "biomass storage", and "handling and transport operations". The material collected was then analysed to compare the logistic features between the two following scenarios: "bioenergy buffers" and "large-scale bioenergy plantations" (Figure 2.3). The comparison was performed analysing the potential differences of harvest and collection, storage, and transport operations between the two scenarios, as reported in the dark grey boxes in Figure 2.3.

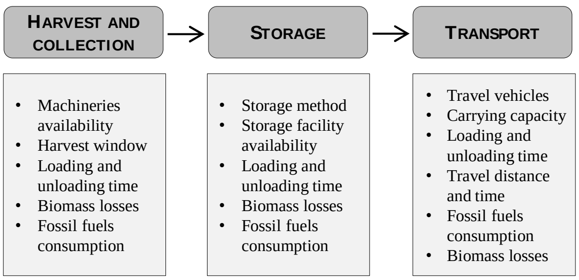

Figure 2.3 Framework used for the comparison of the biomass supply chain between the "bioenergy buffers" and "large-scale bioenergy plantations" scenarios. Light grey boxes represent the main logistic operations (used as main descriptors in Figure 2.2 – step 3), and dark grey boxes show the logistic features that were addressed in the comparison between the two scenarios.

2.3 Overview of research on multiple ecosystem services provision from bioenergy buffers

In total 127 references addressing the effects of different land use transitions to bioenergy crops on ES provision were found to meet our selection criteria (Figure 2.2 - step 3). 77 of these were derived from studies on large-scale cultivations of bioenergy crops and 50 (39% of the total) from studies that specifically addressed the land use transition to bioenergy buffers. In these studies, a total of 237 effects were found for the seven key ES reported in Figure 2.4. The whole list of effects on ES provision and their references are reported in the Supporting Information (Table S2.2). A higher number of effects on ES were reported for cropland (*n*=178, with 26% on bioenergy buffers) compared to grassland conversion (*n*=59, with 12% on bioenergy buffers) (Figure 2.4a). A higher number of effects on ES were found for herbaceous crops (*n*=156, with 20% on bioenergy buffers) compared to SRC woody crops (*n*=81, 26% on bioenergy buffers) (Figure 2.4b).

In terms of temporal impact, effects over long-term periods are widely studied (n=184, 25% on bioenergy buffers) in comparison with the ones over short-term periods (n=53, 17% on bioenergy buffers) (Figure 2.4c). Among the different ES, “soil C sequestration” was the ES with the highest number of effects reported for all the three categories considered (Figure 2.4 a-c), followed by “Aboveground biodiversity and pest regulation”, “GHG emissions”, and “nutrient runoff and soil erosion regulation”. Regarding specific studies on bioenergy buffers, a high number of effects were found in studies that addressed bioenergy buffers ability to provide “water quality regulation” services. On average, for all these ES, 42% of the total number of effects recorded derive from studies on bioenergy buffers.

Overall, the impact matrix (Figure 2.5) indicates that implementing bioenergy buffers (either herbaceous or woody crops) on field margins of cropland has a positive impact on the provision of the seven ecosystem services with a high level of confidence (Table S2.1). Similar outcomes, either in terms of the direction of their impact and level of confidence, were recently reported in two review studies (Holland *et al.*, 2015; Milner *et al.,* 2015) assessing the effects of cropland conversion to large-scale bioenergy plantations on water, climate, and biodiversity, although different IA methodologies and selection criteria for the references were used.

Examining the different impacts on ES between woody and herbaceous buffers, the results of our IA for cropland conversion confirmed that bioenergy buffers with herbaceous crops such as miscanthus and switchgrass have overall beneficial effects on many ecosystem services from the crop establishment phase (0-3 years): i) aboveground biodiversity and pest regulation (Meehan *et al.*, 2012; Werling *et al.*, 2013), ii) soil health (Glover *et al.*, 2010), iii) runoff and soil erosion (Lee *et al.*, 1998), and iv) groundwater quality (Gopalakrishnan *et al.*, 2012). Nevertheless, there are still knowledge gaps regarding the provision of climate regulation services in the short term (Figure 2.5 and Table S2.1). In a recent meta-analysis (Harris *et al.*, 2015) addressed similar knowledge gaps on the effects of land use change to bioenergy crops on greenhouse gas balance.

Concerning the land use transition from grassland to bioenergy buffers, it seems that the intensive soil disturbance occurring during the crop establishment phase negatively affects the provision of several ES (Figure 2.5). Our results show that all the ES considered were strongly and negatively affected by the establishment of bioenergy buffers on former grassland soils. Similar general conclusions were drawn by (Donnelly *et al.*, 2011) for grasslands converted to miscanthus. However, in the long term, herbaceous bioenergy buffers replacing grassland can impact positively the provision of ES like water and climate regulation (Figure 2.5 and Table S2.1). This is slightly in contrast with the low level of confidence for grassland conversion reported by Milner *et al*. (2015) and Harris *et al.* (2015) for ES of “water quality” and “climate regulation”, respectively. A likely explanation for these discrepancies is that no distinction between short- and long- term impact was performed in those studies.

2.4 Multiple ecosystem services provision

In the following sections (from 2.4.1 to 2.4.5), the differences of the impacts on ES provision (direction and science behind) between cropland and grassland conversion to bioenergy buffers are discussed separately for each ES. In the case that the number of effects on ES extracted from the literature provided a high level of confidence (Table S2.1), a summary figure showing the different performance of herbaceous compared to woody bioenergy buffers is presented. Likewise, critical knowledge gaps relative to ES provision of bioenergy buffers are highlighted to stimulate further research.

2.4.1 Climate regulation

2.4.1.1 Soil C sequestration and CO_2 emission mitigation

The impact matrix suggests that the conversion of croplands to bioenergy buffers has long-term positive impacts on soil C sequestration (Figure 2.5), which is in agreement with findings in other systematic revisions (Holland *et al.*, 2015; Milner *et al.*, 2015).

Likewise, a positive impact on soil C sequestration (although with low level of confidence – Table S2.1) was found in the long-term when herbaceous (Hansen *et al.*, 2004; Zimmerman *et al.*, 2012; Poeplau & Don, 2013; Harris *et al.*, 2015; Richter *et al.*, 2015) or woody (Ens *et al.*, 2013; Harris *et al.*, 2015; Walter *et al.*, 2015) bioenergy crops were established on former grasslands. Agostini *et al.* (2015) recently showed that the mean annual soil C sequestration rate under herbaceous crops (1.51 Mg C ha^{-1} $year^{-1}$), for the high leaf and root litter C-inputs, would largely exceed the minimum mitigation requirement (0.25 Mg C ha^{-1} $year^{-1}$), compared to 0.68 Mg C ha^{-1} $year^{-1}$ calculated for woody SRC crops.

During cropland conversion to bioenergy crops, a more active soil microbial community is triggered by the increase of leaf and root litter C-inputs into the soil and thus favouring net soil C sequestration (Rubino *et al.*, 2010; Anderson-Teixeira *et al.*, 2013; Cotrufo *et al.*, 2015). The plant-derived C inputs are mainly found in the root- and leaf-litter derived particulate organic matter (POM), as revealed by ^{13}C natural abundance studies (Garten & Wullschleger, 2000; Hansen *et al.*, 2004; Felten & Emmerling, 2012; Cattaneo *et al.*, 2014a). The POM fraction proved to be a sensitive indicator of land-use change to bioenergy crops (Chimento *et al.*, 2016) and its physical protection within soil aggregates plays a key role in the stabilization of soil C (Dondini *et al.*, 2009; Wienhold *et al.*, 2013; Tiemann & Grandy, 2014).

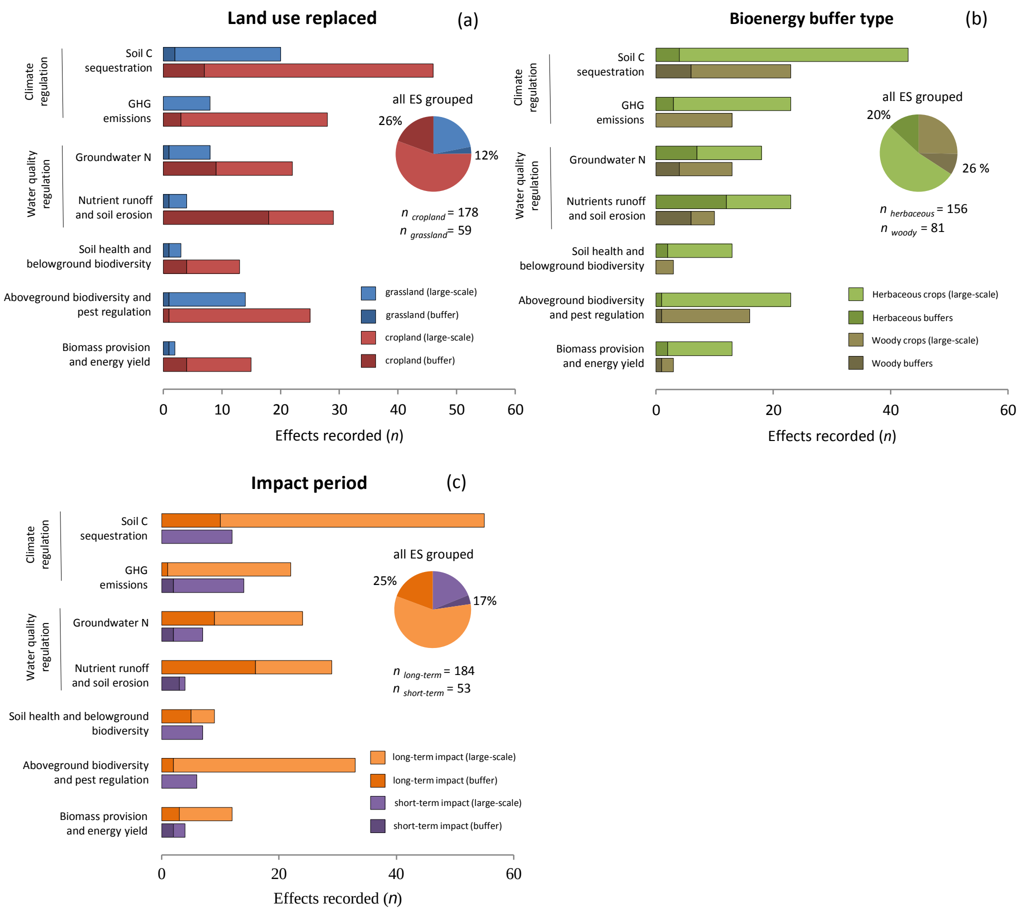

Figure 2.4 Number of effects recorded for the seven ecosystem services (ES) used in the systematic revision. The effects are grouped by land use replaced (a), bioenergy buffer type (b) and impact period (c). All the effects were extracted from the papers that met our selection criteria (Figure 2.2; Table S2.2). Shaded bars and darker colours in the pie charts represent the number of effects recorded within papers pooled into “Bioenergy buffers” classification context.

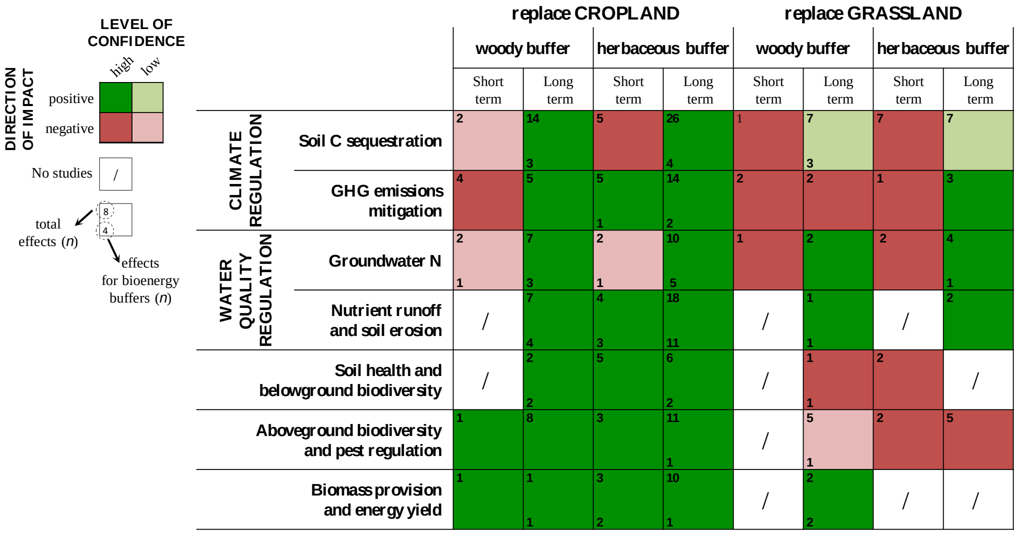

Figure 2.5 Impact matrix reporting the impacts of cropland and grassland conversion to bioenergy buffers on the provision of ecosystem services (ES). Impacts were scored according to their direction, and classified according to their level of confidence (Table S2.1). See Supporting Information (section 2.8) for the impact assessment methodology. In each cell, the total number of effects on ES recorded in literature (top left) and those specifics for bioenergy buffers (bottom left) are further reported. The list of the effects used for compiling the impact matrix is reported in the Supporting Information (Table S2.2).

In the limited number of studies that have been carried out to assess the potential soil C storage capacity under herbaceous and woody bioenergy buffers (Table S2.2), these proved to have positive effects on soil C sequestration (Tufekcioglu *et al.*, 2003; Falloon *et al.*, 2004; Fortier *et al.*, 2010a, 2015). However, on the short-term, negative impacts on the provision of the "Climate regulation" services were found for bioenergy buffers (Figure 2.5). During the establishment phase of bioenergy crops (0-3 years), two factors can negatively affect the short-term soil C balance: the so called "rhizosphere priming effect" (Kuzyakov, 2002) and the interactions between land use legacies and the new bioenergy crop management (Kallenbach & Grandy, 2015). For example, (Harris *et al.*, 2015) found an average increase of soil CO_2 emissions of 6.6 Mg CO_2 ha^{-1} y^{-1} after the establishment of SRC crops on former grassland soils.

The high fine root turnover and rhizodeposition rates, as reported for willow and poplar (Rytter, 2001; Berhongaray *et al.*, 2013), can stimulate microbial biomass and increase the turnover rate of native SOC (Neergaard *et al.*, 2002; Gielen *et al.*, 2005; Abou Jaoudé *et al.*, 2010; Berhongaray & Ceulemans, 2015). As a result of this initial C losses, soil C sequestration rates of SRC crops established in former grasslands and croplands is generally negative in the short-term (Walter *et al.*, 2015).

The occurrence of the priming effect in the rhizosphere has also been reported in herbaceous bioenergy crops (Zatta *et al.*, 2014; Richter *et al.*, 2015). In those studies, it was demonstrated that the decomposition of SOC stabilized in former grasslands was triggered by easily available new C sources derived from miscanthus, explaining that soil C sequestration resulted not significant in the short-term.

2.4.1.2 Soil N_2O emission mitigation

In general, perennial bioenergy crops result in lower soil N_2O emissions than annual cropping systems in the long-term (Davis *et al.*, 2010, 2014; Drewer *et al.*, 2012; Gauder *et al.*, 2012; Smith *et al.*, 2013; Zona *et al.*, 2013a). Although no significant comparisons among the different land use transitions to bioenergy crops can be done, Harris *et al.* (2015) reported a general reduction in soil N_2O emissions for cropland and grassland transitions to herbaceous and SRC crops (-0.2 Mg CO_2-eq ha^{-1} y^{-1}), except for grassland conversion to SRC (+2.5 Mg CO_2-eq ha^{-1} y^{-1}). These findings together with the absence of N fertilization in bioenergy buffers might further increase their N_2O emission reduction potential, as it has been already demonstrated for miscanthus growing in large-scale plantations (Behnke *et al.*, 2012; Davis *et al.*, 2014).

Plant–microbes interactions play an important role in lowering soil N_2O emissions from bioenergy crops. This is due to the enhancement of a diversified and stable archea-dominated denitrifier community (Mao *et al.*, 2011), which promotes a soil rhizosphere where microbial N immobilization occurs (Hargreaves & Hofmockel, 2013). However, a ranking for annual and perennial bioenergy crops based on soil N_2O emission mitigation potential is not available so far (Don *et al.*, 2012; Del Grosso *et al.*, 2014).

To date and to our knowledge, only three modelling studies have dealt with soil N_2O emissions from bioenergy buffers (Gopalakrishnan *et al.*, 2012; Meehan *et al.*, 2013; Ssegane *et al.*, 2015). Results from those studies revealed that soil N_2O emissions might be reduced by50%-90% compared with those in the adjacent field with continuous corn-soybean rotation by establishing 50-m width herbaceous bioenergy buffers (Gopalakrishnan *et al.*, 2012; Meehan *et al.*, 2013). However, (Ssegane *et al.*, 2015) calculated that the annual soil N_2O emissions could be reduced only for up to 11% by establishing 30-m width switchgrass and willow buffers. Despite general evidence of a positive long-term impact on soil N_2O emissions, significant knowledge gaps still exist for bioenergy buffers concerning the relevance of site- and crop-specific factors for soil N_2O emissions (Vidon *et al.*, 2010; Christen & Dalgaard, 2013). The main uncertainties arise from a low understanding of the denitrification processes and of the role played by: (1) leaf-litter quality and its relationship with rainfall events and groundwater table dynamics; and (2) the relationship between plant-derived C inputs and denitrification pathways in subsoil layers.

2.4.2 Water quality regulation

2.4.2.1 Groundwater Nitrogen regulation

The impact matrix clearly shows that both woody and herbaceous buffers without fertilization can impact positively on the provision of “groundwater N regulation” service (Figure 2.5) in the long-term, regardless of whether bioenergy buffers are cultivated adjacent to cropland or grassland sites.

The effectiveness of narrow unfertilized buffer strips to remove the groundwater NO_3-N input coming from adjacent agricultural fields via denitrification (van Beek *et al.*, 2007; Balestrini *et al.*, 2008, 2011; Gumiero *et al.*, 2011) and plant uptake (Sabater *et al.*, 2003; Hefting *et al.*, 2005) has been thoroughly investigated and the factors affecting N removal extensively reviewed (Mayer *et al.*, 2007). For bioenergy buffers a high effectiveness (>60%) in removing NO_3-N has been reported (Zhou *et al.*, 2010; Gopalakrishnan *et al.*, 2012; Christen & Dalgaard, 2013; Ssegane *et al.*, 2015). In studies in which a comparison between woody and herbaceous buffers was performed (e.g. Haycock & Pinay, 1993; Young & Briggs, 2005), the woody buffers proved more efficient in removing the incoming NO_3-N compared to the herbaceous ones (on average, 90% and 70%, respectively).

Three main reasons can explain the high N removal effectiveness of bioenergy buffers: 1) the high nitrogen use efficiency of bioenergy crops (Owens *et al.*, 2013; Wilson *et al.*, 2013) and their ability to immobilize N in vegetative components in the long-term (Tufekcioglu *et al.*, 2003; Fortier *et al.*, 2015); 2) their well-known deep rooting system (Fortier *et al.*, 2013a; Chimento & Amaducci, 2015) that contributes to increase the depth of the active denitrification zone along the soil profile (Hill & Cardaci, 2000; Balestrini *et al.*, 2008) since denitrification at deeper soil layers is highly dependent on root exudation (Senbayram *et al.*, 2012); and 3)the lower nitrate leaching rates reported for bioenergy crops in comparison with those under continuous maize or maize-soybean rotations (McIsaac *et al.*, 2010; Smith *et al.*, 2013; Sarkar & Miller, 2014). However, miscanthus was reported to have a high risk of nitrate leaching during the establishment phase (0-2 years) when planted on sandy soils with shallow groundwater, or with poor crop establishment (Lesur *et al.*, 2014). The same risk during the establishment phase can also occur with SRC crops (Goodlass *et al.*, 2007; Nikièma *et al.*, 2012).

Despite the positive impact on “groundwater N regulation” service (Figure 2.5), some research questions need still to be answered concerning the direction of impact during the establishment phase (0-3 years) of bioenergy buffers. From our systematic revision on the studies addressing N removal efficiency by bioenergy crops, it emerged that the magnitude of N leaching processes should be assessed under different pedological conditions, and that the contribution of root rhizodeposition to nitrate removal via denitrification deserves further research in order to minimize mineral N loadings to water bodies.

2.4.2.2 Nutrient runoff and soil erosion regulation

Bioenergy crops can increase water infiltration, prevent soil erosion and reduce nutrients surface runoff (Kort *et al.*, 1998; Blanco-Canqui *et al.*, 2006; Dabney *et al.*, 2009; Blanco-Canqui, 2010). If planted as herbaceous buffers, bioenergy crops were effective in reducing water run-off and trapping eroded sediment coming from adjacent cropland or grassland (Figure 2.5 and Table S2.1). High trapping efficiencies of the incoming sediments were reported for switchgrass buffers adjacent to conventional cropping systems (66%, 77% and 95% of trapping efficiency in 3-, 6- and 7-m width buffers, respectively) (Lee *et al.*, 1998, 2003). Dabney *et al.* (2012) and Dabney *et al.* (2009) estimated a reduction by 25% and 50% in sediment yield coming from adjacent fields due to the presence of 1 m width buffers of switchgrass and miscanthus . Compared to reduction values of other conservation practices addressed in the Common Agricultural Policy at European level (Panagos *et al.*, 2015), herbaceous bioenergy buffers confirm to be optimal land conserving practices.

In addition to the ability of bioenergy buffers to prevent soil erosion, a consistent and positive impact in reducing nutrient runoff to surface waters was found in bioenergy buffers (Figure 2.5). Lee *et al.*, (1998) showed that a 7 m width switchgrass buffer efficiently remove sediment-bound runoff nutrients by 80% for total-N and by 78% for total-P. A mixed switchgrass-poplar buffer showed greater efficiency in removing runoff nutrients than a simple switchgrass buffer (Lee *et al.*, 2003). At watershed scale, Meehan *et al.* (2013) calculated that a reduction of 29% in annual P loading to surface waters could be achieved replacing annual crops with herbaceous bioenergy buffers along waterways.

The effectiveness in trapping phosphorous (P) over-time could however turn buffer strips into diffused sources of bioavailable P (Stutter *et al.*, 2009; Stutter & Richards, 2012; Noij *et al.*, 2013). This is a consequence of the biologically active soil-litter interface which is able to remobilize the trapped P into dissolved P forms that can be easily leached out (Roberts *et al.*, 2012). On this regard, herbaceous crops such as switchgrass, being harvested annually, offer a greater potential of P removal via harvesting (14-28 kg P ha^{-1} y^{-1}) (Kelly *et al.*, 2007; Lemus *et al.*, 2009; Silveira *et al.*, 2012) than woody crops with 3-4 years harvest cycles (4-26 kg P ha^{-1} y^{-1}) such as willow and poplar hybrids (Heilman & Norby, 1998; Adegbidi *et al.*, 2001; Kauter *et al.*, 2003; Amichev *et al.*, 2014; Fortier *et al.*, 2015). Miscanthus indeed showed lower performances, ranging from 2 to 5 kg P ha^{-1} y^{-1} (Kering *et al.*, 2012). An increase in the number of P sink moments given by annual harvesting of herbaceous bioenergy crops can favour the reduction for bioenergy buffers of the abovementioned risks of P losses from dissolved P sources to surface waters (Stutter *et al.*, 2009).

2.4.3 Soil health and belowground biodiversity

Most of the benefits of the provision of ES discussed so far derive from the tight and positive relationships between soil biota abundance and diversity and biogeochemical processes (Wardle *et al.*, 2004; Brussaard *et al.*, 2007; Kibblewhite *et al.*, 2008). For this reason soil community composition is a key factor in regulating soil health and ecosystem functioning (Wagg *et al.*, 2014). The positive influence of bioenergy crops on belowground biodiversity (Figure 2.5) ultimately affects biogeochemical processes such as litter decomposition and nutrient cycling (Young-Mathews *et al.*, 2010; Liang *et al.*, 2012; Cattaneo *et al.*, 2014b; Kallenbach & Grandy, 2015), C sequestration (Tiemann & Grandy, 2014; Bach & Hofmockel, 2015), N cycling (Glover *et al.*, 2010; Mao *et al.*, 2011; Hargreaves & Hofmockel, 2013) and natural belowground pest-suppression (Rowe *et al.*, 2010; Meehan *et al.*, 2012; Chauvat *et al.*, 2014). Belowground food web under bioenergy crops was also showed to be more complex and resilient than that under annual crop fields (Glover *et al.*, 2010). Literature on bioenergy crops shows that cropland conversion to perennial herbaceous crops led to an increase of the following biotic components of soil health: enzymes activities associated with C, N and P-cycling (Udawatta *et al.*, 2008; Paudel *et al.*, 2011; Cattaneo *et al.*, 2014b); richness of N transforming microbes (Mao *et al.*, 2011); diversity of soil rRNA (Jesus *et al.*, 2010), arbuscular mycorrhizal fungi (Liang *et al.*, 2012), and richness of microarthropods, nematodes and earthworms communities (Smith *et al.*, 2008; Felten & Emmerling, 2011; Robertson *et al.*, 2012; Zangerl *et al.*, 2013). On the contrary, much less is known about the impacts on soil health of grassland and cropland conversion to SRC woody crops (Figure 2.5).

2.4.4 Aboveground biodiversity and pest regulation

Our systematic revision indicates that the conversion of cropland to bioenergy crops has a positive and consistent impact on the provision of "aboveground biodiversity and pest regulation" service (Figure 2.5 and Table S2.1). There is a well-developed literature at the farm level showing that perennial bioenergy crops can promote the abundance of numerous taxonomic groups (arthropods, ground beetles, small mammal and birds) in comparison with adjacent annual cropping systems (Dauber *et al.*, 2010; Immerzeel *et al.*, 2014). Clear examples of this increase are reported for willow (Rowe *et al.*, 2013) and for switchgrass (Robertson *et al.*, 2012; Werling *et al.*, 2013). Bioenergy buffers can provide nesting and food resources to key ecosystem service providers (ESPs) such as pollinators and predators of pests (Haughton *et al.*, 2009; Rowe *et al.*, 2010; Campbell *et al.*, 2012; Nackley *et al.*, 2013; Immerzeel *et al.*, 2014). (Manning *et al.*, 2015) stated that if perennial bioenergy crops are strategically planted like bioenergy buffers, the potential spillover of beetles, hoverflies, and various wasps, could support the pollination service in the surrounding croplands.

Concerning the grassland conversion to bioenergy crops some evidences were found for a negative impact on aboveground biodiversity (Figure 2.5) (Donnelly *et al.*, 2011; Bourke *et al.*, 2014; Immerzeel *et al.*, 2014).

To date, and at the farm level, it seems clear that bioenergy buffers can provide field margin habitats for key ESPs. For this reason, bioenergy buffers falls entirely within the definition of field margin habitat given by (Marshall & Moonen, 2002) likewise of other semi-natural linear landscape elements such as hedgerows, lines of trees and grass margins. These elements are widely present across many EU member states (Van Der Zanden *et al.*, 2013). Since bioenergy buffers act as ecological infrastructure in the same way as do linear landscape elements (Ernoult *et al.*, 2013) and late-winter harvest has no effects on biodiversity (Smith *et al.*, 2009), thus their impacts on biodiversity at larger spatial scales might be even lower than expected compared to those predicted for large-scale bioenergy plantations by Dauber *et al.* (2012) and Immerzeel *et al.* (2014). A recent analyses at regional- and national-scale (Haughton *et al.*, 2015) showed that a strategic planting of dedicated bioenergy crops can increase landscape heterogeneity and biodiversity and thus creating a multifunctional agricultural landscape. For this reason, future research should focus on the potential interactions of the network of bioenergy buffers with the ecosystem service/disservice provision from and to the surrounding landscape units (Bourke *et al.*, 2014; Dauber & Bolte, 2014).

2.4.5 Biomass provision and energy yield

Little research on biomass production has been done on woody and herbaceous bioenergy buffers (Table S1 and S2) compared to the extensive dataset available for perennial bioenergy crops cultivated on large-scale plantations or marginal lands (Laurent *et al.*, 2015; Amaducci *et al.*, 2016). Nevertheless, according to Fortier *et al.* (2010b), hybrid poplar buffers represent a sustainable landscape element to produce high amounts of biomass in the short-term. It was predicted by Christen & Dalgaard (2013) that the maximum biomass yield of bioenergy buffers, cultivated in soil without nutrient or water limitation as expected in buffer strips, can range from 11 to 16 Mg ha^{-1} y^{-1} for willow and poplar hybrids under 3-5 years SRC regime, respectively. Willow buffers e.g. in sandy loam soil with shallow groundwater showed values of biomass yield up to 17 Mg ha^{-1} y^{-1} (Ferrarini *et al.*, 2016). Regarding herbaceous bioenergy buffers with miscanthus or switchgrass, only limited information is available (Tufekcioglu *et al.*, 2003; Falloon *et al.*, 2004; Kelly *et al.*, 2007; Gopalakrishnan *et al.*, 2012) with a biomass yields on average of 4 and 12 Mg ha^{-1} y^{-1} respectively at 2nd and 3rd year after establishment.

Likewise biomass provision, data on energy yield in bioenergy buffers can be inferred from the literature on large-scale bioenergy plantations (e.g. Rettenmaier *et al.*, 2010; Felten *et al.*, 2013; Amaducci *et al.*, 2016). The heat and power generation can be the most promising option for energy and GHG savings (Rettenmaier *et al.*, 2010).

Herbaceous rather than SRC woody crops showed the greater potential in terms of GHG and energy savings (Rettenmaier *et al.*, 2010; Don *et al.*, 2012; Monti *et al.*, 2012), with miscanthus having a higher energy yield (GJ ha^{-1}) than switchgrass (Boehmel *et al.*, 2008). Among woody crops, poplar showed higher GHG savings but lower energy yields compared to willow (Heller *et al.*, 2004; Aylott *et al.*, 2008; Djomo *et al.*, 2011). However, it is difficult to point out the most energy-efficient crop on bioenergy buffers, mainly because, to date, no direct comparison between herbaceous and woody bioenergy buffers has been carried out. Furthermore, it has to be taken into consideration that an energy balance for bioenergy buffers will differ from that for large-scale bioenergy plantations. This is because of the absence of fertilization and pesticide use as well as the potential different harvesting and chipping machinery which can be used in a linear restricted working space like that of bioenergy buffers (see sections 2.5 and 2.6).

2.5 Considerations for implementation, establishment and management of bioenergy buffers

Bioenergy buffers are linear landscape elements whose spatial arrangement on farmlands should be carefully designed (position, length and width) taking into consideration the following features of field margins: 1) soil characteristics (e.g. compaction and poor soil drainage); 2) micro topographic conditions (e.g. zones susceptible to waterlogging, shallow groundwaters, lowlands with high nutrient runoff loads); 3) presence of sub irrigation and drainage systems; 4) the boundary of field margins (Marshall & Moonen, 2002) that may encompass hedge bank, fences, farm track, waterways (e.g. stream, channel, headlands) or natural ecological corridors such as windbreaks, hedge tree, grass or wildflower strips. All these features should be considered to avoid low germinability and soil crusting during the crop establishment phase (Lewandowski *et al.*, 2003; Zimmerman *et al.*, 2013a) and yield losses due to shading by existing natural riparian vegetation. On the other hand, surface and subsurface nutrient loads coming from adjacent fields (feature 2-4) might explain the high biomass yields observed in poplar and willow buffers respectively by Fortier *et al.* (2013b) and Ferrarini *et al.* (2016). However, the relevance of the hypothesis that soil N and P trapping mechanisms observed in bioenergy buffers (sections 2.4.2.1 and 2.4.2.2) can be considered a valuable natural fertilization has to be fully tested yet. Another potential benefit on yields, especially for willow buffers, might be the presence of a shallow groundwater (Jackson & Attwood, 1996).

Since no fertilisation or irrigation is foreseen, the main management practice on established bioenergy buffers is biomass harvesting. Harvesting time, especially for herbaceous crops, can drastically affect biomass quality and the net energy yield as a consequence (Lewandowski & Heinz 2003).

Delaying harvest after killing frost has been strongly recommended to minimize mineral concentrations of harvested biomass for combustion (Adler *et al.*, 2006; Monti *et al.*, 2008). In addition, harvesting bioenergy buffers in late winter is optimal as it favours plant nutrient recycling (Wilson *et al.*, 2013), reduces soil GHG emissions (Hudiburg *et al.*, 2015), increases abscised leaf C input into the soil (Amougou *et al.*, 2012; Woo *et al.*, 2014) and N-P runoff retention (Lee *et al.*, 1998) and it enhances the positive role of bioenergy buffers on biodiversity (Dauber *et al.*, 2010).

In addition to the site-specific features of field margins discussed above, Christen & Dalgaard (2013) reported other factors that can affect the implementation of bioenergy buffers: yield target, harvesting technology, local markets, farmer personal preferences and priorities on ES provision. Related to the latter factor, an interesting option for the implementation of bioenergy buffers comes from buffer strips that were made mandatory for many EU member states under the EU Water Framework Directive (EC 2000/60). On this regard, the so called buffer scenario "*High energy yield buffers on very low slopes*" as proposed by Christen & Dalgaard (2013) might help to promote bioenergy buffers within intensively managed agricultural landscapes. On flat farmlands, bioenergy buffers can be designed along waterways as wide as national recommendations (5-10 m) (Figure 2.1C-E). This would allow the mechanisation of harvest operations, while simultaneously providing biomasses for energy purposes (section 2.4.5) and sustaining a multiple ES provision (Figure 2.5).

2.6 Biomass supply chain of bioenergy buffers

When designing and managing bioenergy buffers special attention should be devoted to the logistic constraints, from biomass harvest to transport (Figure 2.6), that could represent a major barrier for a sustainable exploitation of the bioenergy buffers. The main constraint to biomass logistics on a bioenergy buffer is the presence of an adjacent arable field (Figures 2.1 b-e). Several studies have reviewed and modelled the different logistics constrains for large-scale bioenergy plantations (Allen *et al.*, 1998; Smeets *et al.*, 2009; Gold & Seuring, 2011; Bravo *et al.*, 2012; Rizzo *et al.*, 2014). However, to our knowledge, no studies have been carried out on the logistics issues related to bioenergy buffers. Hereinafter the potential different logistic features between large-scale bioenergy plantations and bioenergy buffers are discussed. Particular attention is given to search for the opportunities (e.g. coexistence of logistic operations for food and bioenergy crops within the same farmland) and the potential shortcomings (e.g. implications on fossil fuels consumption from harvest and transport operations) coming from biomass logistic management in bioenergy buffers.

2.6.1 Harvest and biomass collection

Given the linear spatial arrangement of bioenergy buffers, the following two bottlenecks regarding harvesting and collecting phases are identified:

i) Bioenergy buffer's width. A buffer width of 5-10 meters (mandatory for buffer strips in many EU countries) strongly affects the working capacity of harvest machineries. Biomass collection and harvest operations are hampered by the restricted working space along narrow bioenergy buffers. Specific machineries should be selected or designed to overcome this problem.

For example, if the harvest and collection of switchgrass and miscanthus buffers is performed by using the single-pass system proposed in Martelli & Bentini (2015), the biomass might be simultaneously shredded and baled. With this system the number of passages is reduced and with it the operating costs and working time. Concerning woody SRC crops, the use of a self-propelled chopper during harvesting operations proved to be a valuable option (Verani *et al.*, 2010; Costa *et al.*, 2014). Nevertheless, this method has still to be tested on a bioenergy buffer scenario.

ii) Presence of obstacles within inter-field road network. Elements of discontinuity within farmlands such as streams, channels, headlands, farm tracks, fences and hedge banks can interrupt the continuity of the bioenergy buffers network. An increase in buffers fragmentation will inevitably increase harvest operation times, fossil fuel consumption and therefore the operating costs. For this reason, a suitable inter-field road network is essential to minimize agricultural machineries downtime and consequently optimize logistics field operations. The presence of elements of discontinuity can be considered as one of the main logistics constraints that might affect the implementation of bioenergy buffers even at field scale. An optimal spatial configuration of arable field margins converted to bioenergy crops has to be matched with the existing inter-field road network in order to minimize the logistics constraints that can derive from the presence of elements of discontinuity.

2.6.2 Biomass storage

The choice of the correct storage method and location for the storage facilities is fundamental to minimize biomass quality degradation, dry matter losses and costs (Allen *et al.*, 1998). For example, biomass bales can be stored in stacks in open-fields using headland uncovered or covered with plastic (Huisman *et al.*, 1997; Gold & Seuring, 2011), or temporarily stored inside specific farm structures (e.g. sheds) or in one or more satellite storage facilities (SSF). Under bioenergy buffers scenario, storage methodology strongly depends on the following factors: linear spatial arrangement and accessibility of bioenergy buffers, the presence of inter-field roads of sufficient dimensions to allow a correct biomass loading, and availability of intermediate storage sites or farm sheds close to the power plant. The possibility of using SSF or farm sheds (Figure 2.6-C1) as biomass storage facilities would be a reasonable solution in narrow bioenergy buffers to avoid the storage in the open-field given the restricted working space. These storage solutions have the disadvantage that biomass has to be transported twice by road transport, resulting in additional travel costs (Allen *et al.*, 1998) and higher fossil fuel consumptions with respect to a system in which there is only one road transport (e.g., from the biomass supply source to the power plant station).

2.6.3 Biomass transport

The choice of a certain storage solution can affect transportation options. Transport operations start from in-field biomass loading but how the biomass is loaded are dependent on the choice of storage solution (Mafakheri & Nasiri, 2014). The choice of transport vehicles is also related to travelling distance, biomass density, storage sites accessibility, carrying capacity and travelling speed (Perpiñá *et al.*, 2009; Gold & Seuring, 2011). Both agricultural machineries (e.g. tractor-trailer combination) and conventional trucks can be used in large-scale bioenergy plantations for biomass on-field loading. In bioenergy buffers, however, the working space for trucks manoeuvring is reduced and therefore the use of agricultural machineries is preferable (Figure 2.6-B), also to prevent physical damages to the adjacent fields. Two transport options can be considered in bioenergy buffers (Figure 2.6-C). If bioenergy buffers are located far away from the biomass power plant, biomass can be transported to SSF or farm sheds using agricultural machineries, and subsequently to the biomass power plant using conventional trucks (Figure 2.6-C1). On the contrary, if bioenergy buffers are close to the power plant, transport operations should be preferably performed using agricultural machineries (Figure 2.6-C2). By using this options biomass is directly stored in sheds at the biomass power plant.

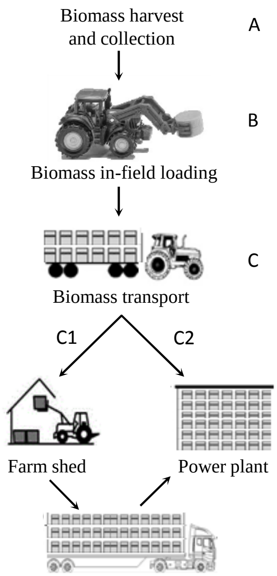

Figure 2.6 Biomass supply chain in bioenergy buffers. Note the two type of transport options (C1-C2) that can be adopted in bioenergy buffers.

2.7 Concluding remarks and future research perspectives

Our impact assessment indicated that the implementation of bioenergy buffers within farmlands can sustain the provision of multiple ecosystem services such as climate, water quality and biodiversity regulation, while producing biomass for bioenergy purposes (Figure 2.5). This highlights that the integration of perennial bioenergy buffers into agricultural landscapes would bring important long-term benefits for the functioning of agroecosytems (Glover *et al.*, 2010; Young-Mathews *et al.*, 2010; Rowe *et al.*, 2013; Werling *et al.*, 2013). The impacts of bioenergy buffers on ES provision were found to be strongly dependent on previous land use. The conversion of intensively managed croplands to bioenergy buffers is preferable for the provision of multiple ES compared to grassland conversion. Our analyses also suggest that herbaceous bioenergy buffers of switchgrass or miscanthus had more positive impacts on several ES in the long-term than woody SRC buffers when established on previous croplands. However, during the first crop establishment phases (0-3 years), the impacts on climate and water quality regulation services remain poorly understood. This is probably due to initial soil disturbance and for the time required until a new equilibrium is reached in the plant-soil system. Three main research gaps were identified in bioenergy buffers related to the following biogeochemical processes: 1) denitrification process in soil and litter and derived-soil N_2O emissions; 2) P biogeochemistry responsible for dissolved P delivery to surface waters; and 3) the magnitude of soil carbon losses caused by the priming effect after bioenergy crop establishment on previous land uses and its contribution to the carbon balance. How long it takes after establishment and which are the soil/management conditions for making bioenergy buffers an efficient C-stocking, N-removing and P-trapping land use practice are interesting research questions to be tested in future.

In most of the cases, reviewing ecological, productive and logistic issues of bioenergy buffers, as noted also by Stutter *et al.* (2012) and Christen and Dalgaard (2013) means to transfer knowledge and findings from different experimental conditions (e.g. from natural riparian zones or large-scale bioenergy cultivations) to managed buffers for biomass production (e.g. see the low percentages of effects on some ES found for bioenergy buffers in Figure 2.2 a-c). Thus transferring this knowledge at broader scales, like watershed or administrative regions, would mean ending up within the existing policy debate in supporting the achievement of multiple economic and environmental benefits (Dale *et al.*, 2016). On this regard, two interesting options for the implementation of bioenergy buffers could be: 1) their inclusion as part of the Ecological Focus Area (EFA) regulated in the 2014-2020 EU Common Agricultural Policy and 2) their implementation as mandatory buffer strips under the EU Water Framework Directive (EC 2000/60).

From the implementation of bioenergy buffers, there would be also perspectives for increasing landscape connectivity as demanded by "EU biodiversity strategy to 2020" (Bourke *et al.*, 2014), "good ecological status" demanded by EU water framework directive (Balana *et al.*, 2012) and address EU GHG savings policies (Falloon *et al.*, 2004).

In the diverse European farming landscapes, trade-offs and synergies between a successful implementation and multiple ES provision may vary depending on farmer's acceptance, legislative restrictions, local energy demand, biomass yield potential and biomass logistic issues. Despite promising findings were found regarding potential yields of bioenergy buffers (Gopalakrishnan *et al.*, 2012; Christen & Dalgaard, 2013), several logistic factors could limit the success of biomass harvest and collection operations along field margins (sections 2.6.1 and 2.6.2). Regarding biomass management and logistics, the limited working space for the farm machinery operations may be considered as the main shortcoming for bioenergy buffers compared to large-scale bioenergy crop cultivation. Intra- and inter-farm spatial fragmentation of biomass supply areas may increase environmental and economic costs related to biomass collection and transport to the power plant. To address these logistic constrains, the combination of Life Cycle Assessment and multi-criteria GIS-based approaches can be useful to explore the environmental advantages and logistic shortcomings derived from managing bioenergy buffers in fragmented mosaic of biomass supply areas within agricultural landscapes.

Addressing the logistic issues together with an optimal allocation of bioenergy buffers into agricultural landscapes, could help to develop a scientific debate over new sustainable bioenergy land use scenarios. Future work will be fundamental to prove whether bioenergy buffers are able to contribute to bioenergy demand and to optimize the trade-offs between multiple ES provision, land-use conflicts and farmer incomes within limited land resources.

2.8 Supporting information

2.8.1 Impact assessment (IA) methodology

The objective of the IA is to provide the state of the art knowledge of the impacts on ecosystem services (ES) provision associated with different land use transitions to bioenergy buffers (cropland or grassland conversion to woody or herbaceous buffers). These impacts were evaluated in the short-term (0-3 years; from soil preparation to crop establishment) and in the long-term (15-20 years; considering the whole bioenergy crop lifespan). The IA followed three steps:

1) *Extraction from the literature of the effects on ES*: following an inductive approach, the IA was carried out by first performing an iterative process based on category building and extraction of the number of effects on ES (Figure 2.2 – impact assessment) and by finally attributing effects to their own category. The effects were extracted from the collected papers at the step 3 and attributed to the following five categories (Table S2.2):
   - ✓ *Ecosystem service* (from MEA, 2003;Dominati *et al.*, 2010): “Soil C sequestration”, “GHG emissions”, “Groundwater quality N regulation”, “Nutrient runoff and soil erosion regulation”, “Soil health and belowground biodiversity”, “Aboveground biodiversity and pest regulation”, “Biomass provision and energy yield”;
   - ✓ *Land use replaced*: “cropland” or “grassland”;
   - ✓ *Bioenergy buffer type*: “herbaceous buffer” or “woody buffers”;
   - ✓ *Impact period*: “short-term impact” or “long-term impact”;
   - ✓ *Direction of the impact*: “positive” or “negative”. Here the “neutral” effects were considered as “negative”. Only the effects that included statistically significant or non-significant result, as tested in the experimental part of the paper were used as well as relevant findings coming from systematic reviews.

2) *Impact scoring*: an impact scoring system was applied for each land use transition x bioenergy buffer type x impact period combination to determine the direction of the impact on the provision of each ES considered in this study as well as its level of confidence. The impact matrix resulted in a total of 56 combinations (7 ecosystem services x 2 land use replaced x 2 bioenergy buffer types x 2 impact periods). For each of these combinations, an impact score (IS) was calculated by dividing the relative frequencies of the positive and negative effects recorded (Table S2.1).

The IS was calculated as:

$$\mathrm{IS}_{i,L} = \frac{N^{+}/N_{tot}}{N^{-}/N_{tot}} \qquad \begin{array}{cc} \mathrm{IS} > 1.5 & \text{positive effect} \\ 0.5 \leq \mathrm{IS} \geq 1 & \text{uncertain effect} \\ \mathrm{IS} < 0.5 & \text{negative effect} \end{array} \qquad \text{(Eq. S2.1)}$$

where *i* is a particular ES, L the specific land use transition, N^+ and N^- are the numbers of positive or negative effects extracted from the literature for that specific *i* and *L*, respectively, and N_{tot} is the sum of effects recorded (N_{tot}=N^+ + N^-). To indicate the direction of the impact two different thresholds for $IS_{i,L}$ were selected (1.5 and 0.5). If IS is higher than 1.5 indicates that the transition to bioenergy buffer increased the ES provision compared to the reference land use. This means that for a specific land use transition the number of positive effects found in the literature was consistently higher than the number of negative effects. For example, when assessing the long-term impacts of herbaceous bioenergy buffers on “aboveground biodiversity and pest regulation” service, 9 out of 11 total effects were reported as positive (N^+=0.82, N^-=0.18). Hence, the relative IS value of 4.55 can be considered as a clear indication of a positive effect. When only positive effects were recorded (N^+=100), a value of 0.01 for N^- was used to avoid getting an infinite value for $IS_{i,L}$; thus an $IS_{i,L}$ of 100 was obtained and it represents a positive effect with a high level of confidence. On the contrary, if IS is lower than 0.5 indicates that the land use transition affected negatively the ES provision. This is the case of the short-term impact of herbaceous buffers on soil C sequestration (Table S2.1). For IS values ranging between 0.5 and 1, the effect was considered as negative but uncertain, reflecting a low level of confidence on the ES provision because contrasting findings reported in literature. Positive effects with a low level of confidence were instead attributed to IS ranging from 1.5 to 1.

3) *Creation of the Impact Matrix*: to synthesize the IS obtained for each land use transition and bioenergy buffer type combination, an impact matrix was created as main data mining tool (Figure 2.5). The main goal of the impact matrix is to easily identify where the impact on multiple ES provision would most probably be placed with regard to different land use transition to bioenergy buffers. The IS used to create the impact matrix are reported in Table S2.1.

Table S2.1 Impact scores (bold numbers) calculated applying the Eq. S2.1 to the list of effects recorded from the literature review (Table S2.2). The structure of the matrix is the same to that used for the impact matrix (Figure 2.5). On the top of each cell, the relative frequencies of the positive (+) and negative (-) effects are reported.

		replace CROPLAND				replace GRASSLAND			
		woody buffer		herbaceous buffer		woody buffer		herbaceous buffer	
		Short term	Long term	Short term	Long term	Short term	Long term	Short term	Long term
CLIMATE REGULATION	Soil C sequestration	(+) (-) 0.5 0.5 **0.5**	(+) / (-) 0.93 0.07 **13.3**	(+) / (-) 0.2 0.8 **0.25**	(+) / (-) 1 0.01 **100**	(+) / (-) 0 1 **0**	(+) / (-) 0.57 0.43 **1.33**	(+) / (-) 0.14 0.86 **0.16**	(+) / (-) 0.57 0.43 **1.33**
	GHG emissions	(+) / (-) 0 1 **0**	(+) / (-) 0.8 0.2 **4**	(+) / (-) 0.8 0.2 **4**	(+) / (-) 1 0.01 **100**	(+) / (-) 0 1 **0**	(+) / (-) 0 1 **0**	(+) / (-) 0 1 **0**	(+) / (-) 1 0.01 **100**
WATER QUALITY REGULATION	Groundwater N	(+) (-) 0.5 0.5 **0.5**	(+) (-) 0.86 0.14 **6.1**	(+) (-) 0.5 0.5 **0.5**	(+) / (-) 1 0.01 **100**	(+) / (-) 0 1 **0**	(+) / (-) 1 0.01 **100**	(+) / (-) 0 1 **0**	(+) / (-) 1 0.01 **100**
	Nutrient runoff and soil erosion	/	(+) / (-) 1 0.01 **100**	(+) (-) 0.75 0.25 **3**	(+) / (-) 1 0.01 **100**	/	(+) / (-) 1 0.01 **100**	/	(+) / (-) 1 0.01 **100**
Soil health and belowground biodiversity (supporting service)		/	(+) / (-) 1 0.01 **100**	(+) / (-) 1 0.01 **100**	(+) (-) 0.83 0.17 **4.88**	/	(+) / (-) 0 1 **0**	(+) / (-) 0 1 **0**	/
Aboveground biodiversity and pest regulation		(+) / (-) 1 0.01 **100**	(+) (-) 0.75 0.25 **3**	(+) / (-) 0.66 0.33 **2**	(+) (-) 0.82 0.18 **4.55**	/	(+) (-) 0.4 0.6 **0.66**	(+) / (-) 0 1 **0**	(+) (-) 0.2 0.8 **0.25**
Biomass provisioning and energy yield		(+) / (-) 1 0.01 **100**	(+) / (-) 1 0.01 **100**	(+) / (-) 0.66 0.33 **2**	(+) / (-) 1 0.01 **100**	/	(+) / (-) 1 0.01 **100**	/	/

Table S2.2 List of studies used in the impact assessment of bioenergy buffers on ecosystem services provision.

Reference	Ecosystem service (ES)	Land use replaced	Bioenergy buffer type (width)	Specific crop type	Impact period	Country	Direction of the impact
(Agostini *et al.*, 2015)	Soil C sequestration	cropland	herbaceous	switchgrass/ miscanthus	long-term	Europe/ USA	↑
(Asbjornsen *et al.*, 2012)	Soil C sequestration	cropland	herbaceous	herbaceous crops	long-term	USA	↑
(Bach & Hofmockel, 2015)	Soil C sequestration	cropland	herbaceous	switchgrass	long-term	USA	↑
(Garten & Wullschleger, 2000)	Soil C sequestration	cropland	herbaceous	switchgrass	long-term	USA	↑
(Garten, 2012)	Soil C sequestration	cropland	herbaceous	switchgrass	long-term	USA	↑
(Harris *et al.*, 2015)	Soil C sequestration	cropland	herbaceous	switchgrass/ miscanthus	long-term	Global	↑
(Holland *et al.*, 2015)	Soil C sequestration	cropland	herbaceous	herbaceous crops	long-term	global	↑
(Milner *et al.*, 2015)	Soil C sequestration	cropland	herbaceous	miscanthus	long-term	global	↑
(Monti *et al.*, 2012)	Soil C sequestration	cropland	herbaceous	switchgrass	long-term	global	↑
(Poeplau & Don, 2013)	Soil C sequestration	cropland	herbaceous	miscanthus	long-term	Europe	↑
(Schmer *et al.*, 2011)	Soil C sequestration	cropland	herbaceous	switchgrass	long-term	USA	↑
(Tiemann & Grandy, 2014)	Soil C sequestration	cropland	herbaceous	switchgrass/ miscanthus	long-term	USA	↑
(van der Hilst *et al.*, 2012)	Soil C sequestration	cropland	herbaceous	miscanthus	long-term	Netherla nd	↑
(Wienhold *et al.*, 2013)	Soil C sequestration	cropland	herbaceous	switchgrass	long-term	USA	↑
(Lemus & Lal, 2005)	Soil C sequestration	cropland	herbaceous	herbaceous crops	long-term	global	↑
(Amougou *et al.*, 2012)	Soil C sequestration	cropland	herbaceous	miscanthus	long-term (4-5y)	France	↑
(Chimento *et al.*, 2016)	Soil C sequestration	cropland	herbaceous	switchgrass/ miscanthus	long-term (6y)	Italy	↑
(Cattaneo *et al.*, 2014a)	Soil C sequestration	cropland	herbaceous	miscanthus	long-term (9y)	Italy	↑
(Felten & Emmerling, 2012)	Soil C sequestration	cropland	herbaceous	miscanthus	long-term (15y)	Germany	↑
(Hansen *et al.*, 2004)	Soil C sequestration	cropland	herbaceous	miscanthus	long-term (16y)	Denmark	↑
(Bonin & Lal, 2012a)	Soil C sequestration	cropland	herbaceous	switchgrass	long-term (7y)	USA	↑

(Poeplau & Don, 2013)	Soil C sequestration	cropland	herbaceous	miscanthus	short-term	Europe	=
(Anderson-Teixeira *et al.*, 2013)	Soil C sequestration	cropland	herbaceous	switchgrass/ miscanthus	short-term (1.5-3y)	USA	↑
(Zimmerman *et al.*, 2012)	Soil C sequestration	cropland	herbaceous	miscanthus	short-term (2-3y)	Ireland	=
(Zimmerman *et al.*, 2013b)	Soil C sequestration	cropland	herbaceous	miscanthus	short-term (3y)	Ireland	=
(Felten & Emmerling, 2012)	Soil C sequestration	cropland	herbaceous	miscanthus	short-term (2.5y)	Germany	=
(Meehan *et al.*, 2013)	Soil C sequestration	cropland	herbaceous buffer (100m)	herbaceous crops	long-term	USA	↑
(Falloon *et al.*, 2004)	Soil C sequestration	cropland	herbaceous buffer (2-6-20m)	herbaceous crops	long-term	UK	↑
(Tufekcioglu *et al.*, 1999)	Soil C sequestration	cropland	herbaceous buffer (7m)	switchgrass	long-term	USA	↑
(Tufekcioglu *et al.*, 2003)	Soil C sequestration	cropland	herbaceous buffer (7m)	switchgrass	long-term	USA	↑
(Agostini *et al.*, 2015)	Soil C sequestration	cropland	woody	poplar, willow	long-term	Europe/ USA	↑
(Harris *et al.*, 2015)	Soil C sequestration	cropland	woody	poplar, willow	long-term	Global	=
(Holland *et al.*, 2015)	Soil C sequestration	cropland	woody	SRC crops	long-term	global	↑
(Milner *et al.*, 2015)	Soil C sequestration	cropland	woody	SRC crops	long-term	global	↑
(Walter *et al.*, 2015)	Soil C sequestration	cropland	woody	poplar, willow	long-term	Europe	↑
(Lemus & Lal, 2005)	Soil C sequestration	cropland	woody	SRC crops	long-term	global	↑
(Kahle *et al.*, 2010)	Soil C sequestration	cropland	woody	poplar, willow	long-term (15y)	Germany	↑
(Arevalo *et al.*, 2011)	Soil C sequestration	cropland	woody	poplar	long-term (3-11y)	Canada	↑
(Chimento *et al.*, 2016)	Soil C sequestration	cropland	woody	poplar, willow	long-term (6 y)	Italy	↑
(Bonin & Lal, 2012b)	Soil C sequestration	cropland	woody	willow	long-term (7y)	USA	↑
(Abou Jaoudé *et al.*, 2010)	Soil C sequestration	cropland	woody	poplar	long-term (9y)	Italy	↑
(Arevalo *et al.*, 2011)	Soil C sequestration	cropland	woody	poplar	short-term (0-3y)	Canada	↓
(Berhongaray & Ceulemans, 2015)	Soil C sequestration	cropland	woody	poplar, willow	short-term (2 y)	Belgium	↑
(Falloon *et al.*, 2004)	Soil C sequestration	cropland	woody buffer (2,6,20m)	tree rows	long-term	UK	↑

(Tufekcioglu *et al.*, 1999)	Soil C sequestration	cropland	woody buffer (9m)	poplar	long-term	USA	↑
(Tufekcioglu *et al.*, 2003)	Soil C sequestration	cropland	woody buffer (9m)	poplar	long-term	USA	↑
(Harris *et al.*, 2015)	Soil C sequestration	grassland	herbaceous	switchgrass/ miscanthus	long-term	global	↓
(Holland *et al.*, 2015)	Soil C sequestration	grassland	herbaceous	herbaceous crops	long-term	global	↑
(Milner *et al.*, 2015)	Soil C sequestration	grassland	herbaceous	miscanthus	long-term	global	=
(Poeplau & Don, 2013)	Soil C sequestration	grassland	herbaceous	miscanthus	long-term	Europe	↑
(Richter *et al.*, 2015)	Soil C sequestration	cropland	herbaceous	miscanthus	long-term (14y)	UK	↑
(Donnelly *et al.*, 2011)	Soil C sequestration	grassland	herbaceous	miscanthus	long-term (4-15y)	Ireland	↑
(Zatta *et al.*, 2014)	Soil C sequestration	grassland	herbaceous	miscanthus	long-term (6y)	UK	=
(Poeplau & Don, 2013)	Soil C sequestration	grassland	herbaceous	miscanthus	short-term	Europe	↓
(Ma *et al.*, 2000a)	Soil C sequestration	grassland	herbaceous	switchgrass	short-term (0-1y)	USA	↑
(Donnelly *et al.*, 2011)	Soil C sequestration	grassland	herbaceous	miscanthus	short-term (0-3y)	Ireland	=
(Ma *et al.*, 2000b)	Soil C sequestration	grassland	herbaceous	switchgrass	short-term (0-3y)	USA	=
(Ma *et al.*, 2000b)	Soil C sequestration	grassland	herbaceous	switchgrass	short-term (2-3y)	USA	=
(Zimmerman *et al.*, 2012)	Soil C sequestration	grassland	herbaceous	miscanthus	short-term (2-3y)	Ireland	=
(Zimmerman *et al.*, 2013b)	Soil C sequestration	grassland	herbaceous	miscanthus	short-term (3y)	Ireland	=
(Harris *et al.*, 2015)	Soil C sequestration	grassland	woody	poplar, willow	long-term	global	=
(Holland *et al.*, 2015)	Soil C sequestration	grassland	woody	SRC crops	long-term	global	↑
(Milner *et al.*, 2015)	Soil C sequestration	grassland	woody	SRC crops	long-term	global	=
(Walter *et al.*, 2015)	Soil C sequestration	grassland	woody	poplar, willow	long-term	Europe	↓
(Ens *et al.*, 2013)	Soil C sequestration	grassland	woody	willow	short-term (0-3y)	Canada	↓
(Young-Mathews *et al.*, 2010)	Soil C sequestration	grassland	woody buffer	SRC crops	long-term	USA	↑
(Fortier *et al.*, 2010a)	Soil C sequestration	grassland	woody buffer (4.5 m)	poplar	long-term (6y)	Canada	↑

(Fortier *et al.*, 2013c)	Soil C sequestration	grassland	woody buffer (4.5 m)	poplar	long-term (9y)	Canada	↑
(Davis *et al.*, 2010)	GHG emissions	cropland	herbaceous	switchgrass/ miscanthus	long-term	USA	↑
(Harris *et al.*, 2015)	GHG emissions	cropland	herbaceous	switchgrass/ miscanthus	long-term	Global	↑
(Anderson-Teixeira *et al.*, 2012)	GHG emissions	cropland	herbaceous	switchgrass/ miscanthus	long-term	Global	↑
(Creutzig *et al.*, 2014)	GHG emissions	cropland	herbaceous	switchgrass/ miscanthus	long-term	Global	↑
(Don *et al.*, 2012)	GHG emissions	cropland	herbaceous	switchgrass/ miscanthus	long-term	Europe	↑
(Monti *et al.*, 2012)	GHG emissions	cropland	herbaceous	switchgrass	long-term	global	↑
(Smeets *et al.*, 2009)	GHG emissions	cropland	herbaceous	switchgrass/ miscanthus	long-term	Europe	↑
(van der Hilst *et al.*, 2012)	GHG emissions	cropland	herbaceous	miscanthus	long-term	Netherla nds	↑
(Felten *et al.*, 2013)	GHG emissions	cropland	herbaceous	miscanthus	long-term	Germany	↑
(Drewer *et al.*, 2012)	GHG emissions	cropland	herbaceous	miscanthus	long-term (4-6y)	USA	↑
(Hudiburg *et al.*, 2015)	GHG emissions	cropland	herbaceous	switchgrass/ miscanthus	long-term (5-15y)	USA	↑
(Gauder *et al.*, 2012)	GHG emissions	cropland	herbaceous	willow	long-term (7y)	Germany	↑
(Zeri *et al.*, 2011)	GHG emissions	cropland	herbaceous	switchgrass/ miscanthus	short-term (0-3y)	USA	↑
(Davis *et al.*, 2010)	GHG emissions	cropland	herbaceous	miscanthus	short-term (0-3 y)	USA	↑
(Smith *et al.*, 2013)	GHG emissions	cropland	herbaceous	switchgrass/ miscanthus	short-term (0-3y)	USA	↑
(Anderson-Teixeira *et al.*, 2013)	GHG emissions	cropland	herbaceous	miscanthus, switchgrass	short-term (1.5-3y)	USA	↑
(Bradley *et al.*, 2011)	GHG emissions	cropland	herbaceous buffer	switchgrass/ miscanthus	short-term	Canada	↓
(Meehan *et al.*, 2013)	GHG emissions	cropland	herbaceous buffer (100m)	herbaceous crops	long-term	USA	↑
(Gopalakrishnan *et al.*, 2012)	GHG emissions	cropland	herbaceous buffer (50m)	miscanthus	long-term	USA	↑
(Don *et al.*, 2012)	GHG emissions	cropland	woody	poplar, willow	long-term	Europe	↑

(Harris *et al.*, 2015)	GHG emissions	cropland	woody	poplar, willow	long-term	Global	↑
(Gelfand *et al.*, 2013)	GHG emissions	cropland	woody	poplar	long-term	USA	=
(Drewer *et al.*, 2012)	GHG emissions	cropland	woody	willow	long-term (4-6y)	USA	↑
(Gauder *et al.*, 2012)	GHG emissions	cropland	woody	willow	long-term (7y)	Germany	↑
(Zona *et al.*, 2013b)	GHG emissions	cropland	woody	poplar	short-term (0-1y)	Belgium	↓
(Sabbatini *et al.*, 2015)	GHG emissions	cropland	woody	poplar	short-term (0-2y)	Italy	↓
(Zona *et al.*, 2013a)	GHG emissions	cropland	woody	poplar	short-term (0-2y)	Belgium	↓
(Abou Jaoudé *et al.*, 2010)	GHG emissions	cropland	woody	poplar	short-term	Italy	↓
(Harris *et al.*, 2015)	GHG emissions	grassland	herbaceous	switchgrass/ miscanthus	long-term	Global	↑
(Roth *et al.*, 2013)	GHG emissions	grassland	herbaceous	miscanthus	long-term (14y)	Ireland	↑
(Donnelly *et al.*, 2011)	GHG emissions	grassland	herbaceous	miscanthus	long-term (4-15y)	Ireland	↑
(Roth *et al.*, 2013)	GHG emissions	grassland	herbaceous	miscanthus	short-term (1y)	Ireland	↓
(Harris *et al.*, 2015)	GHG emissions	grassland	woody	poplar, willow	long-term	Global	↓
(Palmer *et al.*, 2014)	GHG emissions	grassland	woody	poplar, willow	long-term	USA	↓
(Palmer *et al.*, 2014)	GHG emissions	grassland	woody	poplar, willow	short-term	USA	↓
(Nikièma *et al.*, 2012)	GHG emissions	grassland	woody	poplar, willow	short-term (1y)	USA	↓
(Holland *et al.*, 2015)	Groundwater N regulation	cropland	herbaceous	herbaceous crops	long-term	global	↑
(Milner *et al.*, 2015)	Groundwater N regulation	cropland	herbaceous	miscanthus	long-term	global	↑
(Powers *et al.*, 2011)	Groundwater N regulation	cropland	herbaceous	switchgrass	long-term	USA	↑
(van der Hilst *et al.*, 2012)	Groundwater N regulation	cropland	herbaceous	miscanthus	long-term	Netherla nd	↑
(McIsaac *et al.*, 2010)	Groundwater N regulation	cropland	herbaceous	switchgrass/ miscanthus	long-term (3-6y)	USA	↑
(Lesur *et al.*, 2014)	Groundwater N regulation	cropland	herbaceous	miscanthus	short-term (0-3y)	France	↓
(Mayer *et al.*, 2007)	Groundwater N regulation	cropland	herbaceous buffer	herbaceous crops	long-term	global	↑

(Zhou *et al.*, 2010)	Groundwater N regulation	cropland	herbaceous buffer	herbaceous crops	short-term (0-3y)	USA	↑
(van Beek *et al.*, 2007)	Groundwater N regulation	cropland	herbaceous buffer (3.5m)	herbaceous crops	long-term (4-5 y)	Netherland	↑
(Gopalakrishnan *et al.*, 2012)	Groundwater N regulation	cropland	herbaceous buffer (50m)	switchgrass/ miscanthus	long-term	USA	↑
(Noij *et al.*, 2012)	Groundwater N regulation	cropland	herbaceous buffer (5m)	herbaceous crops	long-term	Netherland	↑
(Balestrini *et al.*, 2011)	Groundwater N regulation	cropland	herbaceous/woody buffer	mixed buffer	long-term	Italy	↑
(Christen & Dalgaard, 2013)	Groundwater N regulation	cropland	woody	poplar, willow	long-term	Europe	↑
(Holland *et al.*, 2015)	Groundwater N regulation	cropland	woody	SRC crops	long-term	global	↑
(Milner *et al.*, 2015)	Groundwater N regulation	cropland	woody	SRC crops	long-term	global	↑
(Aronsson *et al.*, 2000)	Groundwater N regulation	cropland	woody	willow	long-term (9 y)	Sweden	=
(Gielen *et al.*, 2005)	Groundwater N regulation	cropland	woody	willow	short-term (3y)	UK	↓
(Mayer *et al.*, 2007)	Groundwater N regulation	cropland	woody buffer	SRC crops	long-term	global	↑
(Gumiero *et al.*, 2011)	Groundwater N regulation	cropland	woody buffer (10m)	willow	long-term	Italy	↑
(Haycock & Pinay, 1993)	Groundwater N regulation	cropland	woody buffer (20m)	poplar	long-term	UK	↑
(Young & Briggs, 2005)	Groundwater N regulation	cropland	woody buffer (3.5m)	willow	short-term	USA	↑
(Holland *et al.*, 2015)	Groundwater N regulation	grassland	herbaceous	herbaceous crops	long-term	global	↑
(Milner *et al.*, 2015)	Groundwater N regulation	grassland	herbaceous	miscanthus	long-term	global	↑
(Donnelly *et al.*, 2011)	Groundwater N regulation	grassland	herbaceous	miscanthus	long-term (4-15y)	Ireland	↑
(Donnelly *et al.*, 2011)	Groundwater N regulation	grassland	herbaceous	miscanthus	short-term (0-3y)	Ireland	↓
(Milner *et al.*, 2015)	Groundwater N regulation	grassland	herbaceous	miscanthus	short-term (0-3y)	UK	=
(Noij *et al.*, 2012)	Groundwater N regulation	grassland	herbaceous buffer (5m)	herbaceous crops	long-term	Netherland	↑
(Holland *et al.*, 2015)	Groundwater N regulation	grassland	woody	SRC crops	long-term	global	↑
(Milner *et al.*, 2015)	Groundwater N regulation	grassland	woody	SRC crops	long-term	global	↑
(Nikièma *et al.*, 2012)	Groundwater N regulation	grassland	woody	poplar, willow	short-term (1y)	USA	↓

(Asbjornsen *et al.*, 2012)	Nutrient runoff and soil erosion regulation	cropland	herbaceous	herbaceous crops	long-term	USA	↑
(Kort *et al.*, 1998)	Nutrient runoff and soil erosion regulation	cropland	herbaceous	herbaceous crops	long-term	Global	↑
(Milner *et al.*, 2015)	Nutrient runoff and soil erosion regulation	cropland	herbaceous	miscanthus	long-term	global	↑
(Parish *et al.*, 2012)	Nutrient runoff and soil erosion regulation	cropland	herbaceous	switchgrass	long-term	USA	↑
(Powers *et al.*, 2011)	Nutrient runoff and soil erosion regulation	cropland	herbaceous	switchgrass	long-term	USA	↑
(Sarkar & Miller, 2014)	Nutrient runoff and soil erosion regulation	cropland	herbaceous	switchgrass	long-term	USA	↑
(van der Hilst *et al.*, 2012)	Nutrient runoff and soil erosion regulation	cropland	herbaceous	miscanthus	long-term	Netherla nds	↑
(Sarkar & Miller, 2014)	Nutrient runoff and soil erosion regulation	cropland	herbaceous	switchgrass	short-term	USA	↑
(Mayer *et al.*, 2007)	Nutrient runoff and soil erosion regulation	cropland	herbaceous buffer	herbaceous crops	long-term	global	↑
(Sheppard *et al.*, 2006)	Nutrient runoff and soil erosion regulation	cropland	herbaceous buffer	herbaceous crops	long-term	Canada	↑
(Stutter *et al.*, 2009)	Nutrient runoff and soil erosion regulation	cropland	herbaceous buffer	herbaceous crops	short-term	UK	↓
(Eghball *et al.*, 2000)	Nutrient runoff and soil erosion regulation	cropland	herbaceous buffer (0.75m)	switchgrass	long-term (6y)	USA	↑
(Blanco-Canqui *et al.*, 2006)	Nutrient runoff and soil erosion regulation	cropland	herbaceous buffer (0.7m)	switchgrass	long-term	Mexico	↑
(Meehan *et al.*, 2013)	Nutrient runoff and soil erosion regulation	cropland	herbaceous buffer (100m)	herbaceous crops	long-term	USA	↑
(Sanderson *et al.*, 2001)	Nutrient runoff and soil erosion regulation	cropland	herbaceous buffer (16m)	Switchgrass	short-term	USA	↑
(Rachman *et al.*, 2008)	Nutrient runoff and soil erosion regulation	cropland	herbaceous buffer (1m)	switchgrass	long-term	USA	↑
(Dabney *et al.*, 2009)	Nutrient runoff and soil erosion regulation	cropland	herbaceous buffer (1m)	miscanthus	long-term (0-13 y)	USA	↑
(Dabney *et al.*, 2012)	Nutrient runoff and soil erosion regulation	cropland	herbaceous buffer (1m)	switchgrass	long-term (1-8 y)	USA	↑
(Lee *et al.*, 1998)	Nutrient runoff and soil erosion regulation	cropland	herbaceous buffer (3,6m)	switchgrass	long-term	USA	↑
(Ssegane *et al.*, 2015)	Nutrient runoff and soil erosion regulation	cropland	herbaceous buffer (30m)	switchgrass	long-term	USA	↑

(Borin *et al.*, 2005)	Nutrient runoff and soil erosion regulation	cropland	herbaceous buffer (4-5 m)	herbaceous crops	long-term	Italy	↑
(Lee *et al.*, 2003)	Nutrient runoff and soil erosion regulation	cropland	herbaceous buffer (7m)	switchgrass	short-term (3y)	USA	↑
(Christen & Dalgaard, 2013)	Nutrient runoff and soil erosion regulation	cropland	woody	poplar, willow	long-term	Europe	↑
(Kort *et al.*, 1998)	Nutrient runoff and soil erosion regulation	cropland	woody	SRC crops	long-term	global	↑
(Milner *et al.*, 2015)	Nutrient runoff and soil erosion regulation	cropland	woody	SRC crops	long-term	global	↑
(Mayer *et al.*, 2007)	Nutrient runoff and soil erosion regulation	cropland	woody buffer	SRC crops	long-term	global	↑
(Schultz *et al.*, 2004)	Nutrient runoff and soil erosion regulation	cropland	woody buffer	SRC crops	long-term	USA	↑
(Zaimes *et al.*, 2004)	Nutrient runoff and soil erosion regulation	cropland	woody buffer	SRC crops	long-term	USA	↑
(Ssegane *et al.*, 2015)	Nutrient runoff and soil erosion regulation	cropland	woody buffer (30m)	willow	long-term	USA	↑
(Milner *et al.*, 2015)	Nutrient runoff and soil erosion regulation	grassland	herbaceous	miscanthus	long-term	global	↑
(Donnelly *et al.*, 2011)	Nutrient runoff and soil erosion regulation	grassland	herbaceous	miscanthus	long-term (4-15y)	Ireland	↑
(Milner *et al.*, 2015)	Nutrient runoff and soil erosion regulation	grassland	woody	SRC crops	long-term	global	↑
(Zaimes *et al.*, 2004)	Nutrient runoff and soil erosion regulation	grassland	woody buffer	SRC crops	long-term	USA	↑
(Zaimes *et al.*, 2004)	Soil health and belowground biodiversity	cropland	herbaceous	herbaceous crops	long-term	USA	↑
(Felten & Emmerling, 2011)	Soil health and belowground biodiversity	cropland	herbaceous	miscanthus	long-term	Germany	↑
(Liang *et al.*, 2012)	Soil health and belowground biodiversity	cropland	herbaceous	switchgrass	long-term	USA	↑
(Liang *et al.*, 2013)	Soil health and belowground biodiversity	cropland	herbaceous	switchgrass	long-term	USA	=
(Mao *et al.*, 2011)	Soil health and belowground biodiversity	cropland	herbaceous	switchgrass/ miscanthus	short-term (0-2y)	USA	↑
(Chauvat *et al.*, 2014)	Soil health and belowground biodiversity	cropland	herbaceous	switchgrass/ miscanthus	short-term (1-3y)	France	↑
(Kallenbach & Grandy, 2015)	Soil health and belowground biodiversity	cropland	herbaceous	switchgrass	short-term (1.5-3y)	USA	↑

(Jesus *et al.*, 2010)	Soil health and belowground biodiversity	cropland	herbaceous	switchgrass	short-term (2 y)	USA	↑
(Hargreaves & Hofmockel, 2013)	Soil health and belowground biodiversity	cropland	herbaceous	switchgrass	short-term (3y)	USA	↑
(Stutter & Richards, 2012)	Soil health and belowground biodiversity	cropland	herbaceous buffer	switchgrass	long-term	UK	↑
(Udawatta *et al.*, 2008)	Soil health and belowground biodiversity	cropland	herbaceous buffer (4.5m)	herbaceous crops	long-term	USA	↑
(Paudel *et al.*, 2011)	Soil health and belowground biodiversity	cropland	woody buffer	poplar	long-term	USA	↑
(Udawatta *et al.*, 2008)	Soil health and belowground biodiversity	cropland	woody buffer (4.5m)	SRC crops	long-term	USA	↑
(Donnelly *et al.*, 2011)	Soil health and belowground biodiversity	grassland	herbaceous	miscanthus	short-term (0-3y)	Ireland	=
(Kallenbach & Grandy, 2015)	Soil health and belowground biodiversity	grassland	herbaceous	switchgrass	short-term (1.5-3y)	USA	↓
(Paudel *et al.*, 2011)	Soil health and belowground biodiversity	grassland	woody buffer	poplar	long-term	USA	↓
(Asbjornsen *et al.*, 2012)	Aboveground biodiversity and pest regulation	cropland	herbaceous	herbaceous crops	long-term	USA	↑
(Dauber *et al.*, 2010)	Aboveground biodiversity and pest regulation	cropland	herbaceous	miscanthus, switchgrass	long-term	Global	↑
(Haughton *et al.*, 2009)	Aboveground biodiversity and pest regulation	cropland	herbaceous	miscanthus	long-term	UK	↑
(Holland *et al.*, 2015)	Aboveground biodiversity and pest regulation	cropland	herbaceous	herbaceous crops	long-term	global	↑
(Immerzeel *et al.*, 2014)	Aboveground biodiversity and pest regulation	cropland	herbaceous	herbaceous crops	long-term	global	↑/↓
(Milner *et al.*, 2015)	Aboveground biodiversity and pest regulation	cropland	herbaceous	miscanthus	long-term	global	↑
(Robertson *et al.*, 2012)	Aboveground biodiversity and pest regulation	cropland	herbaceous	switchgrass	long-term	USA	↑
(Semere & Slater, 2007)	Aboveground biodiversity and pest regulation	cropland	herbaceous	miscanthus	long-term	UK	↑
(van der Hilst *et al.*, 2012)	Aboveground biodiversity and pest regulation	cropland	herbaceous	miscanthus	long-term	Netherland	↓
(Werling *et al.*, 2013)	Aboveground biodiversity and pest regulation	cropland	herbaceous	switchgrass	long-term	USA	↑
(Meehan *et al.*, 2012)	Aboveground biodiversity and pest regulation	cropland	herbaceous	herbaceous crops	short-term	USA	↑

(Zangerl *et al.*, 2013)	Aboveground biodiversity and pest regulation	cropland	herbaceous	switchgrass/ miscanthus	short-term	USA	↑
(Bourke *et al.*, 2014)	Aboveground biodiversity and pest regulation	cropland	herbaceous	miscanthus	short-term (2-3y)	Ireland	=
(Meehan *et al.*, 2013)	Aboveground biodiversity and pest regulation	cropland	herbaceous buffer (100m)	herbaceous crops	long-term	USA	↑
(Christen & Dalgaard, 2013)	Aboveground biodiversity and pest regulation	cropland	woody	poplar, willow	long-term	Europe	↑
(Dauber *et al.*, 2010)	Aboveground biodiversity and pest regulation	cropland	woody	poplar, willow	long-term	Global	=
(Haughton *et al.*, 2009)	Aboveground biodiversity and pest regulation	cropland	woody	willow	long-term	UK	↑
(Holland *et al.*, 2015)	Aboveground biodiversity and pest regulation	cropland	woody	SRC crops	long-term	global	↑
(Immerzeel *et al.*, 2014)	Aboveground biodiversity and pest regulation	cropland	woody	SRC crops	long-term	global	↑/↓
(Milner *et al.*, 2015)	Aboveground biodiversity and pest regulation	cropland	woody	SRC crops	long-term	global	↑
(Rowe *et al.*, 2010)	Aboveground biodiversity and pest regulation	cropland	woody	willow	long-term	UK	↑
(Rowe *et al.*, 2013)	Aboveground biodiversity and pest regulation	cropland	woody	willow	long-term	UK	↑
(Campbell *et al.*, 2012)	Aboveground biodiversity and pest regulation	cropland	woody	willow	short-term (1-3y)	USA	↑
(Dauber *et al.*, 2010)	Aboveground biodiversity and pest regulation	grassland	herbaceous	switchgrass/ miscanthus	long-term	Global	=
(Holland *et al.*, 2015)	Aboveground biodiversity and pest regulation	grassland	herbaceous	herbaceous crops	long-term	global	=
(Immerzeel *et al.*, 2014)	Aboveground biodiversity and pest regulation	grassland	herbaceous	herbaceous crops	long-term	global	↑/↓
(Milner *et al.*, 2015)	Aboveground biodiversity and pest regulation	grassland	herbaceous	miscanthus	long-term	global	=
(Donnelly *et al.*, 2011)	Aboveground biodiversity and pest regulation	grassland	herbaceous	miscanthus	long-term (4-15y)	Ireland	=
(Donnelly *et al.*, 2011)	Aboveground biodiversity and pest regulation	grassland	herbaceous	miscanthus	short-term (0-3y)	Ireland	=
(Bourke *et al.*, 2014)	Aboveground biodiversity and pest regulation	grassland	herbaceous	miscanthus	short-term (2-3y)	Ireland	=
(Dauber *et al.*, 2010)	Aboveground biodiversity and pest regulation	grassland	woody	poplar, willow	long-term	Global	↑

(Holland *et al.*, 2015)	Aboveground biodiversity and pest regulation	grassland	woody	SRC crops	long-term	global	↑
(Immerzeel *et al.*, 2014)	Aboveground biodiversity and pest regulation	grassland	woody	SRC crops	long-term	global	↑/↓
(Milner *et al.*, 2015)	Aboveground biodiversity and pest regulation	grassland	woody	SRC crops	long-term	global	=
(Young-Mathews *et al.*, 2010)	Aboveground biodiversity and pest regulation	grassland	woody buffer	SRC crops	long-term	USA	=
(Lewandowski *et al.*, 2003)	Biomass and energy provisioning	cropland	herbaceous	switchgrass/ miscanthus	long-term	USA/ Europe	↑
(Lewandowski & Heinz, 2003)	Biomass and energy provisioning	cropland	herbaceous	miscanthus	long-term	Europe	↑
(Monti *et al.*, 2012)	Biomass and energy provisioning	cropland	herbaceous	switchgrass	long-term	global	↑
(Parish *et al.*, 2012)	Biomass and energy provisioning	cropland	herbaceous	switchgrass	long-term	USA	↑
(Wilson *et al.*, 2013)	Biomass and energy provisioning	cropland	herbaceous	switchgrass	long-term	USA	↑
(Asbjornsen *et al.*, 2012)	Biomass and energy provisioning	cropland	herbaceous	herbaceous crops	long-term	USA	↑
(Guretzky *et al.*, 2010)	Biomass and energy provisioning	cropland	herbaceous	switchgrass	long-term	USA	↑
(Heaton *et al.*, 2004)	Biomass and energy provisioning	cropland	herbaceous	switchgrass/ miscanthus	long-term	global	↑
(Lasorella *et al.*, 2011)	Biomass and energy provisioning	cropland	herbaceous	switchgrass/ miscanthus	long-term	Europe	↑
(Zeri *et al.*, 2011)	Biomass and energy provisioning	cropland	herbaceous	switchgrass/ miscanthus	short-term (0-3y)	USA	↑
(Meehan *et al.*, 2013)	Biomass and energy provisioning	cropland	herbaceous buffer (100m)	herbaceous crops	long-term	USA	↑
(Silveira *et al.*, 2012)	Biomass and energy provisioning	cropland	herbaceous buffer (3m)	switchgrass	short-term	USA	=
(Kelly *et al.*, 2007)	Biomass and energy provisioning	cropland	herbaceous buffer (5m)	switchgrass/ miscanthus	short-term (1-3y)	USA	↑
(Sabbatini *et al.*, 2015)	Biomass and energy provisioning	cropland	woody	poplar	short-term (0-2y)	Italy	↑
(Christen & Dalgaard, 2013)	Biomass and energy provisioning	cropland	woody buffer	poplar, willow	long-term	Europe	↑
(Fortier *et al.*, 2010b)	Biomass and energy provisioning	grassland	woody buffer	poplar	long-term (6y)	Canada	↑
(Fortier *et al.*, 2013b)	Biomass and energy provisioning	grassland	woody buffer	poplar	long-term (9y)	Canada	↑

Chapter 3

Impacts of willow and miscanthus bioenergy buffers on biogeochemical N removal processes along the soil-groundwater continuum

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Impacts of willow and miscanthus bioenergy buffers on biogeochemical N removal processes along the soil-groundwater continuum

Abstract

In this paper the below- and above-ground biomass production in bioenergy buffers and biogeochemical N removal processes along the soil-groundwater continuum were assessed. In a sandy loam soil with shallow groundwater, bioenergy buffers of miscanthus and willow (5 and 10 m wide) were planted along a ditch of an agricultural field (AF) located in the Po valley (Italy). Mineral N forms and dissolved organic C (DOC) were monitored monthly over an 18 month period in groundwater before and after the bioenergy buffers. Soil samples were measured for inorganic N, DOC, microbial biomass C (MBC) and N (MBN), and potential nitrate reductase activity (NAR). The results indicated that bioenergy buffers are able to efficiently remove from groundwater the incoming NO3-N (62%-5 m and 80%-10 m). NO_3-N removal rate was higher when nitrate input from AF increased due to N fertilization. Willow performed better than miscanthus in terms of biomass production (17 Mg DM ha^{-1} y^{-1}), fine root biomass (5.3 Mg ha^{-1}) and N removal via harvesting (73 kg N ha^{-1}). The negative nonlinear relationship found between NO_3-N and DOC along the soil-groundwater continuum from AF to bioenergy buffers indicates that DOC:NO_3-N ratio is an important controlling factor for promoting denitrification in bioenergy buffers. Bioenergy buffers promoted soil microbial functioning as they stimulated plant–microbial linkages by increasing the easily available C sources for microorganisms (as DOC). First, willow and miscanthus promoted high rates of biological removal of nitrate (NAR) along the soil profile. Second, rhizosphere processes activated the soil microbial community leading to significant increases in MBC and microbial N immobilization. Herbaceous and woody bioenergy crops have been confirmed as providing good environmental performances when cultivated as bioenergy buffers by mitigating the disservices of agricultural activities such as groundwater N pollution.

Keywords: bioenergy buffers, miscanthus, willow, biomass production, groundwater quality, nitrate removal, dissolved organic C, fine root biomass, soil microbial biomass, ecological stoichiometry

3.1 Introduction

In the last decade it has become increasingly important to identify which proportion of the landscape should be occupied by bioenergy cropping systems (Gelfand *et al.*, 2013; Manning *et al.*, 2015). The key question is which land use strategy can be implemented to avoid land use conflicts while maximizing yields and ecosystem services provision (Fritsche *et al.*, 2010; Payne, 2010; Dale *et al.*, 2011a; Popp *et al.*, 2011; Anderson-Teixeira *et al.*, 2012). To solve the so called “food, energy and environment trilemma” (Tilman *et al.*, 2009), several scenarios in which food and bioenergy cropping systems are spatially mixed within farmlands have been recently proposed (Asbjornsen *et al.*, 2012; Gopalakrishnan *et al.*, 2012; Christen & Dalgaard, 2013; Manning *et al.*, 2015).

Positive impacts on the regulation of climate, water and biodiversity ecosystem services have been reviewed during the transition of cropland to the production of bioenergy feedstock with perennial herbaceous and woody crops (Holland *et al.*, 2015; Milner *et al.*, 2015). The application of spatial multicriteria analysis revealed that a careful allocation of perennial cropping systems into the landscape would foster multiple ecosystem services and mitigate ecosystem disservices from current annual food cropping systems (Powers *et al.*, 2011; Parish *et al.*, 2012; Meehan *et al.*, 2013). Nevertheless, it emerged that the links of bioenergy crops with the provision of ecosystem services are strictly dependent on the spatial allocation of the crops relative to the adjacent land uses as revealed for pest regulation and pollination (Meehan *et al.*, 2012; Werling *et al.*, 2013; Bourke *et al.*, 2014) and for water quality regulation (Meehan *et al.*, 2013).

Within this framework, an excellent case study area in which to explore the possibility to optimize land use for food, energy, and ecosystem services is the agricultural landscape of Po valley (northern Italy). In the last decades, this area experienced an intensification of the conventional farming systems with the result that several areas suffer from problems of nitrate contamination of surface and groundwater (Capri *et al.*, 2009). At the EU level, buffer strips have become a widely adopted measure to mitigate such problems of non-point source agricultural pollution. The efficiency in removing NO_3-N from groundwater is widely reported in literature for riparian areas (Sabater *et al.*, 2003; Hickey & Doran, 2004; Mayer *et al.*, 2007) and for filter strips (van Beek *et al.*, 2007; Zhou *et al.*, 2010). For this reason, buffer strips were made mandatory among member states in order to fulfill the obligations to maintain and improve Good Ecological Status under the EU Water Framework Directive (EC 2000/60). In Italy, 5m wide buffer strips are mandatory along watercourses where water quality status is scarce or bad (Italian Ministerial Decree DM 27417 of 22nd December 2011). Within the 2014-2020 Rural Development Programmes (RDP) of the Emilia-Romagna and Lombardy regions in Italy two voluntary measures that provide money to farmer to install and maintain herbaceous buffers or woodland buffer strips have been introduced. Nevertheless, some operating spaces are left by these RDP measures for including bioenergy crops in buffer strips. For this reason, the water quality issue seems to offer an opportunity to redesign bioenergy landscapes with buffers for biomass production.

In this manuscript, bioenergy buffers have been proposed as an alternative land use scenario for bioenergy production within the intensively managed agricultural landscape of the Po valley. Bioenergy buffers, in our view, are perennial landscape elements consisting of linear narrow bands placed along watercourses, and cultivated with perennial herbaceous or woody bioenergy crops. Although extensive knowledge on the ecological functioning of buffer strips with natural vegetation is available for the case study area (Balestrini *et al.*, 2008, 2011), several research questions on bioenergy buffers relative to their productive performances still have to be explored, as do their role in providing ecosystem services and sustaining soil functioning (such as mitigation of groundwater N pollution and soil microbial C- and N cycling). To date, the only literature available on the effectiveness of bioenergy buffers in removing N are modelling studies (Gopalakrishnan *et al.*, 2012; Meehan *et al.*, 2013; Ssegane *et al.*, 2015). Furthermore, there have been no specific studies for bioenergy crops on the role of dissolved organic C (DOC) and belowground biomass as indicators for the activation of the soil microbial community and its implications on N removal processes from soil (e.g. denitrification and microbial N immobilization). To be adopted under different climatic and pedological conditions, there needs to more evidence on the biogeochemical processes involved in N removal in the plant-soil-groundwater system under bioenergy buffers. Within the case study area, an experimental field trial of bioenergy buffers with miscanthus and willow was set up in a sandy loam soil with shallow groundwater. The main objectives of the experiment were: 1) to evaluate bioenergy buffers effectiveness (BSE) in removing N from shallow groundwater; 2) to identify the main biogeochemical processes and key factors governing N removal along the soil-groundwater continuum; and 3) to quantify root fine biomass, biomass production and plant N removal in bioenergy buffers.

3.2 Materials and Methods

3.2.1 Site description and experimental design of bioenergy buffers

The experiment was located in a typical farm in the north-west of Italy (Figure 2.1a) (45° 3'37.87"N, 9°47'30.19"E altitude 43 m a.s.l.), where the climate is continental with an average annual rainfall of 980 mm and rainfall peaks in autumn and spring. The average temperatures during the experiment were 5.5°C, 15.5°C, 15°C, 24.4 °C, respectively for winter, autumn, spring and summer. The field was flat, rectangular and bordered at one side by a ditch (Figure 3.1b). It was 200 m wide with a 180 m long 2% slope downward to a 3 m wide ditch. The water level in the ditch fluctuated from 0.2 to 0.9 m below soil surface (-bss). The field was characterized by a deep sandy aquifer interrupted by a silty clay aquitard (Figure 3.1c). The local groundwater system showed a prevalent SW-NE direction, and it was perpendicular to the ditch. The agricultural field was cultivated following a common crop rotation for the area: maize (2013), soybean (2014) and tomato (2015). Maize was fertilized with KNO_3 (170 kg N ha^{-1}). Soybean was irrigated twice in June 2014 (total 60 mm of water) but not fertilized. In May 2015 there was a pre-planting fertilization (70, 110 and 170 kg ha^{-1}, respectively for N, P and K) and after planting the tomatoes there was a biweekly fertirrigation from June to August (18 events; total 210 mm water and 50, 40 and 100 kg ha^{-1} respectively of N, P and K). According to the USDA Soil Taxonomy (Soil Survey Staff, 2014) the soil is Udifluventic Haplustept, the texture is sandy loam and the content of soil organic C and total N is low. The main soil physical and chemical characteristics of the soil profile are reported in Table S3.1.

The bioenergy buffers were installed in April 2013, with two buffer widths: the mandatory 5m width (as requested by Italian Ministerial Decree DM 27417 of 22th December, 2011) and 10m width. No pest management, irrigation and fertilization was applied. Soil was ploughed at 30 cm depth before the experiment started. The experiment was organized following a randomized block design (RBD) with three replicates (Figure 3.1b). Bioenergy buffers consisted of miscanthus (*Miscanthus x giganteus* L.) and willow (*Salix matsudana* Koidz (hybrid)). The plots hosting the control treatment (hereinafter referred to as "spontaneous species") were not planted in order to enable natural revegetation. The control treatment has been considered as an unsown field margin strip (De Cauwer *et al.*, 2007) Spontaneous species recorded were (%): *Echinochloa crus-galli* (L.) Beauv. (30%), *Sorghum halepense* (L.) Pers. (30%), *Amaranthus retroflexus* L. (10%), *Convolvulus arvensis* L. (10%), *Cynodon dactylon* (L.) Pers. (10%) and other species (10%). Willow bioenergy buffers were planted by stem transplantation (up to 40 cm depth). Plant density was 13.000 plants ha^{-1} (0.6 × 1.5 m spacing). The failure of the transplants was nearly zero after establishment. Miscanthus buffers were planted with rhizomes (0.1 m depth) with a density of 4 rhizomes m^2 (0.36 × 0.7 m spacing). Emergence rates for rhizomes in May 2013 ranged from 15% to 20% due to a severe waterlogging event. New rhizomes were planted in June 2013 in order to reduce patchiness (in February 2015 patchiness reached values <5%).

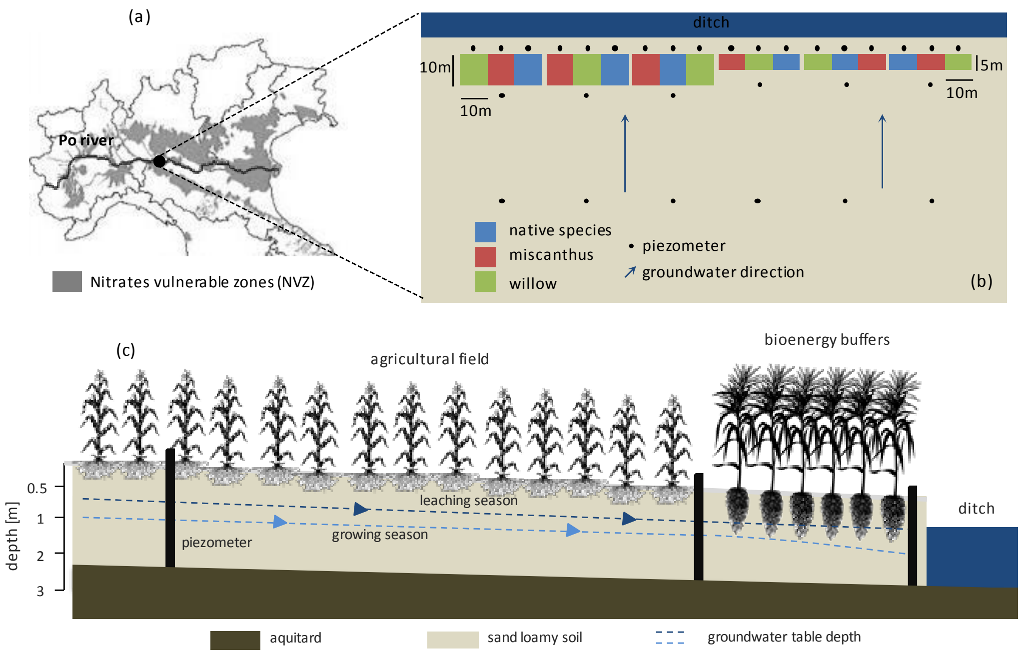

Figure 3.1 Localization of the field trial in NW Italy (a) and distribution of the Nitrate Vulnerable Zones (source: ISPRA - Institute for Environmental Protection and Research), field experimental design for bioenergy buffers (b) and vertical-cross section of the field trial representing the shallow groundwater system (c).

3.2.2 Groundwater, soil, root and aboveground biomass measurements

Before bioenergy buffer establishment a whole soil profile was opened to describe the soil horizons (Table S3.1) and a geological survey was carried out in order to characterize the local aquifer system. Some preliminary piezometers were installed at 2 and 5 m depth at random intervals to get information on groundwater hydraulic head and the groundwater table dynamics. This was done in order to spatially design the RBD experimental design. After having fully characterized the aquifer, the experimental site was equipped in May 2014 with piezometers installed along a series of perpendicular transects from the agricultural field to the ditch (Figure 3.1b). Each of the transects consisted of three sampling piezometers. Two piezometers were installed within the agricultural field upgradient of each group of experimental blocks and one was installed immediately downgradient of each buffer plot in order to study the effects of bioenergy buffers on groundwater N coming from the agricultural field (AF). The PVC piezometers were installed at a depth of 1.5–2 m. Piezometers were 2.5 m long, 5 cm diameter PVC pipe, and were screened at 1 to 2 m -bss. Piezometers were installed by driving into the soil twice a steel corer with an inner removable PVC pipe (5 cm diameter, 1 m long) using a hydraulic jackhammer and extracted using a tripod ratchet. The final piezometer was then manually inserted into the soil. This procedure was also used to obtain soil samples.

Groundwater samples were collected from May 2014 until August 2015 approximately with a monthly sampling frequency during the 2014 and 2015 growing seasons. Hereinafter the monitoring season are divided as follow: 2014 growing season (T1: May 30, 2014, T2: 28 June, 2014, T3: August 1, 2014, T4: September 10, 2014), 2014 leaching season (T5: December 18, 2014, T6: February 4, 2015) and 2015 growing season (T7: April 24, 2015, T8: May 6, 2015, T9: June 2, 2015, T10: July 15, 2015, T11: August 1, 2015). Groundwater table depth was measured using a sounding probe during each sampling event. Differences in groundwater table depth in total heads along the piezometer transects were used to determine dominant flow paths of groundwater. Before sampling the wells were pumped empty and allowed to settle again. Dissolved O_2 (ppm), groundwater total dissolved solids (ppm), conductivity (µs cm^{-1}), pH and water temperature (°C) were measured within each piezometer by inserting a specific multiparameter probe (HI 98196, Hanna Instruments). Groundwater was sampled with a slow pumping technique, 0.5-1 L was collected from each piezometer and samples were kept refrigerated during the transport to the laboratory. Samples were then immediately filtered (0.45µm cellulose acetate) and kept at 4°C until analysis. Samples were analysed for NO_3-N, NH_4-N, NO_2-N, TDN (Total Dissolved N), DOC (Dissolved Organic C), TDP (Total dissolved P), PO_4-P and chlorides (Cl^-). The sum of NO_3-N, NH_4-N and NO_2-N forms the dissolved inorganic N (DIN) and the difference between TDN and DIN is the dissolved organic N (DON).

NO_3-N was analysed with dual wavelength UV spectroscopy (275nm, 220nm) on acidified (HCL 1M) samples and pipetted into 96-well quartz microplates. NH_4-N, NO_2-N and PO_4-P were measured through colorimetric reactions based on a 96-well microplate format and read with a microplate reader (Biotek Synergy 2, Winooski, VT, USA). NH_4-N was measured with Berthelot reaction (Rhine *et al.*, 1998), NO_2-N with Griess reaction (Griess Reagent Kit G-7921, Molecular Probes) and PO_4-P with the green malachite method (D'Angelo *et al.*, 2001). TDN and DOC were measured using a TOC–TN analyser (TOC-VCSN Shimadzu). TDP was measured by an inductively coupled plasma atomic emission-spectrometry. Chlorides were analysed by ion chromatography using a Dionex DX-120 equipped with an AS22A column and Na_2CO_3+$NaHCO_3$ as eluent. Chlorides were used as a conservative tracer in groundwater to separate between dilution and N removal (Altman & Parizek, 1995). TDP and PO_4-P in most of the groundwater samples were lower than the detection limit and the data were therefore not included in this manuscript. Buffer strip effectiveness (BSE) in removing N forms in shallow groundwater was calculated using the formula:

$$buffer\ strip\ effectiveness\ (BSE)_i = \left(1 - \frac{C_{i\,gw,BUFFER}}{C_{i\,gw,avg,AF}}\right) \times\ 100 \qquad \text{Eq.3.1}$$

where *i* is the N_i form for which BSE was calculated (NO_3-N, NH_4-N, NO_2-N, DIN, TDN and their respective *i* /Cl^- ratios), $C_{i\,gw,BUFFER}$ is the concentration of the *Ni* form in groundwater after buffer plots and $C_{i\,gw,avg,AF}$ is the average concentration of the *Ni* form in piezometers installed in the agricultural field (AF).

Soil was sampled four times in 10m wide buffers with the same procedure used for piezometer installation. There were two soil samplings in the first growing season after buffer establishment (July 1, 2013 and February 10, 2014), one at the end of second (February 4, 2015) and one in the third season (August 1, 2015). At each sampling time, three soil cores were taken from each plot to a depth of 60cm. For miscanthus and willow four soil cores were taken in two different sampling positions: two cores in the middle of the plant row and two in the inter-row centre.

Four random cores were taken from the spontaneous species plots and from the agricultural field. Each soil core was then divided into four sections (0-10 cm, 10-20 cm, 20-30 cm and 30-60 cm depth). The divided soil cores from each plot were immediately bulked in one composite sample in plastic bags according to the respectively depth, stored at -18°C and analysed within a month. Soil samples were analysed for extractable NO_3-N, NO_2-N, NH_4-N, DOC, TDN, microbial biomass C (MBC) and for the two microbial N removal processes in soils: microbial N immobilization (MBN) and potential nitrate reductase activity (NAR), the latter as marker for denitrification. Microbial biomass was determined by the fumigation-extraction technique in fresh soil (Vance *et al.*, 1987). The unfumigated soil extracts were used to measured DOC, TDN, extractable NO_3-N, NH_4-N and NO_2-N. As for groundwater samples, DIN and DON were calculated.

Extractable mineral N pools were measured with the same microplate-based colorimetric methods adopted for groundwater analysis. For the entire set of soil C and N pools analysed the values are reported on a stock basis (kg ha^{-1}). Soil nitrate reductase activity (NRA) was measured by soil anaerobic incubation following the modifications of the protocol of Abdelmagid & Tabatabai (1987) introduced by Chèneby *et al.* (2010). NRA were calculated as µg of NO_2-N produced per g of dry soil per day (µg NO_2-N g_{soil}^{-1} day^{-1}). See Supporting Information (Appendix 3.1) for a detailed description of the procedure adopted for NRA.

Soil cores for fine root biomass were collected during the last soil sampling (August 1, 2015). During this soil sampling, three additionally soil cores were collected for fine root biomass quantification. For miscanthus and willow, soil cores were taken in three different sampling positions following the scheme proposed by Zatta *et al.* (2014); one next to the plants, one in the middle of the plant row and one in the inter-row centre. From the spontaneous species plots three cores were taken randomly. All cores were divided into the same four sections as for soil cores (0–10, 10–20, 20–30 and 30-60cm depth). Before root extraction, soil samples were stored at -18 °C. To extract fine roots (<2mm), soil samples were immersed in oxalic acid (2 %) for 2 h, and then washed in a hydraulic sieving-centrifuge device (Chimento & Amaducci, 2015). Once cleaned, roots were recovered by hand picking from the water using a 2 mm mesh sieve, oven dried at 65 °C for 48h, and weighed.

Some samples of miscanthus included rhizomes, which were not included in the root biomass sample. The dry root weight was divided by the whole volume of soil samples and reported as Mg of fine roots per hectare (Mg ha^{-1}). After weighing, the three replicates were combined by depth for each plot and ground to 1 mm. The samples were then analyzed for N using a CN analyzer (Vario Max CN Analyzer, Elementar Americas, Inc., NJ).

Harvestable biomass from bioenergy buffers was collected in late winter periods every year for miscanthus (February 10, 2014 and February 15, 2015) and at the end of 2^{nd} growing season for willow (February 15, 2015). Aboveground biomass samples were collected cutting each row of plants along a transect in each plot. Each plant row was weighed in the field and a sub-sample was taken for fresh weight to dry matter (DM) conversion and CN analysis. Calculations for harvestable biomass (Mg DM ha^{-1}) and N exportations by harvesting (kg N ha^{-1}) were performed for each plot as a whole (by averaging the DM values of all plant rows along the buffer transect) and on a plant row basis (DM and kg N ha^{-1} plant row^{-1}).

3.2.3 Statistical analysis

All the data were analysed using the "nlme" package (Pinheiro *et al.*, 2015) of RStudio 0.99.484. For groundwater data (concentration and BSE), a mixed model of repeated measures ANOVA was used with crop type (CROP), buffer width (WIDTH), and monitoring season (SEASON) as fixed effects, whereas piezometers (PIEZ) and sampling times (TIME) were crossed within the random effects structure of the model. Significance of the fixed effects was assessed with F and P values. Model residuals were checked for normality by the Kolmogorov–Smirnov test and for homogeneity of variances by the Levene's test for each of the fixed factors. The temporal autoregressive structure (based on moving average residual) was used as covariance matrix within the mixed model. This structure obtained the lowest Akaike's Information Criteria (AIC) values than those obtained for other structure tested (autoregressive temporal structure and block-diagonal). Significant differences among levels of the fixed factors were identified at the 0.05 probability level of significance constructing specific contrast matrices based on Tukey contrasts carried out using the *multcomp* package of R software (Hothorn *et al.*, 2015).
Similar mixed models of repeated ANOVA and post-hoc analysis were applied to soil variables. Crop type (CROP), soil depths (DEPTH), and sampling seasons (SEASON) were used as fixed effects, whereas experimental blocks (BLOCK) and SEASON were defined as random effects. For belowground measurements, only CROP and DEPTH as fixed effects were studied, being root biomass sampled only once during the 2015 growing season. To assess differences in harvestable biomass and N exportation, one-way ANOVA comparisons for RBD designs were run, with CROP and BLOCK as fixed factors. For these parameters, to assess their differences among plant rows along buffer transect, one-way ANOVA comparisons were made separately for miscanthus and willow buffers, with PLANT ROW (n_{rows}= 13 for miscanthus, n_{rows} = 7 for willow) and BLOCK as main factors. For all these one-way ANOVAs, means were compared by the Tukey test (α=0.05), after confirmation that data were normally distributed and variance was homogeneous.
Additional regression analyses were then performed on soil, root and groundwater data by using *nlme* package of R software. The relationship between the concentration (mg L^{-1}) of DOC and NO_3-N in groundwater samples and soil extracts were calculated applying a nonlinear regression model ($y = a+be^{-k(x)}$) (Taylor and Townsend 2010). The relationship between groundwater nitrate input entering the buffers and buffer strips effectiveness (BSE) in removing N was calculated by a power function: y=ax^{b} (Mayer *et al.*, 2007). BSE (%) in removing NO_3-N was also plotted against buffer width. A non-linear regression model (y=ax^{b}) was used here to obtain information on the optimal buffer width necessary to obtain a given value of BSE (50%, 75%, 90% and 100%).

3.3 Results

3.3.1 N concentration patterns in groundwater

The concentrations of NO_3-N, NH_4-N, DIN and TDN in groundwater were significantly lower after the bioenergy buffers by comparison to the concentration in the agricultural field (AF) (Table 3.1). In particular, groundwater nitrate had the highest reduction compared to AF (F: 77.1 P: <0.0001). For TDN the values were F: 40.1 P: <0.0001. The only mineral N that resulted slightly increased was NO_2-N that after bioenergy buffers increased up to 0.2 mg NO_2-N L^{-1}. No significant differences (F: 1.9 P: 0.55) were found in Cl^- concentration after bioenergy buffers suggesting that no input of Cl^- occurred in the local aquifer system and thus no dilution effects were observed in groundwater before and after bioenergy buffers (Table S3.2). Cl^-/NO_3-N and Cl^-/TDN ratios increased in groundwater after bioenergy buffers (data not shown), indicating that for the entire period of monitoring all N forms were effectively removed from the shallow groundwater. On average 70% and 85% of groundwater TDN was mineral N (DIN), respectively in bioenergy buffer and AF. On average groundwater DIN in AF was formed by NO_3-N (60%), NH_4-N (29%) and NO_2-N (10%). After the bioenergy buffers, NO_3-N (47%) was still the main component of groundwater DIN, but the proportion of NO_2-N (14%) and NH_4-N (38%) increased significantly.The NO_3-N concentration in groundwater after the bioenergy buffers ranged from 0.32 mg NO_3-N L^{-1} (or 1.4 mg NO_3^- L^{-1}) to 1.27 mg NO_3-N L^{-1} (5.6 mg NO_3^- L^{-1}). TDN ranged from 1.54 mg L^{-1} to 2.77 mg L^{-1}. The mean input of NO_3-N and TDN from the AF was different when soybean (2014) and tomato (2015) were cultivated. N fertilization during the fertirrigation of tomato affected the concentration of NO_3-N in groundwater; it was on average 4.73 mg NO_3-N L^{-1} (20.9 mg NO_3^- L^{-1}) during the tomato growing season. The maximum NO_3 level of 11.3 mg N L^{-1} (50 mg NO_3^- L^{-1}) indicated in the EU Nitrate Directive (91/676/EEC) was not exceeded.

Table 3.1 Average concentrations of the N forms measured in shallow groundwater after bioenergy buffers (BS- crop) of two different widths and in the agricultural field (AF-crop). Values with different letters in superscript show statistically different means among crop types across growing seasons (Tukey's HSD test, P < 0.05) and within N forms.

SEASON		CROP	NO_3-N		NO_2-N		NH_4-N		DIN		DON		TDN	
			5 m	10m	5 m	10m	5 m	10m	5 m	10m	5 m	10m	5 m	10m
2014 growing season	BS	spontaneous spp.	0.66 ^{A}	0.59 ^{A}	0.15 ^{A}	0.20 ^{A}	0.69 ^{A}	0.66 ^{A}	1.44 ^{A}	1.51 ^{A}	1.00 ^{A}	0.33 ^{B}	2.44 ^{A}	1.84 ^{BC}
		miscanthus	0.58 ^{A}	0.49 ^{B}	0.15 ^{A}	0.21 ^{A}	0.80 ^{C}	0.67 ^{AB}	1.44 ^{A}	1.47 ^{A}	0.57 ^{B}	0.38 ^{B}	2.01 ^{B}	1.85 ^{C}
		willow	0.45 ^{A}	0.56 ^{A}	0.14 ^{A}	0.20 ^{A}	0.80 ^{C}	0.74 ^{BC}	1.57 ^{A}	1.33 ^{A}	0.54 ^{B}	0.34 ^{B}	2.11 ^{B}	1.67 ^{C}
	AF	soybean	1.49 ^{C*}		0.11 ^{B}		1.37 ^{D}		3.01 ^{B}		0.99 ^{A}		4.11 ^{D}	
2014 leaching season	BS	spontaneous spp.	0.43 ^{B}	0.42 ^{B}	0.15 ^{A}	0.20 ^{A}	0.23 ^{E}	0.23 ^{E}	0.85 ^{C}	0.84 ^{C}	1.29 ^{C}	0.80 ^{A}	2.10 ^{B}	1.65 ^{C}
		miscanthus	0.44 ^{B}	0.32 ^{B}	0.15 ^{A}	0.21 ^{A}	0.12 ^{F}	0.30 ^{E}	0.84 ^{C}	0.71 ^{C}	0.84 ^{A}	0.82 ^{A}	1.77 ^{C}	1.48 ^{E}
		willow	0.41 ^{B}	0.32 ^{B}	0.16 ^{A}	0.20 ^{A}	0.41 ^{G}	0.30 ^{E}	1.03 ^{C}	0.77 ^{C}	0.55 ^{A}	0.77 ^{A}	1.58 ^{AC}	1.54 ^{AC}
	AF	bare soil	1.90 ^{C}		0.12 ^{A}		0.53 ^{D}		2.62 ^{B}		0.83 ^{A}		3.45 ^{D}	
2015 growing season	BS	spontaneous spp.	1.27 ^{D}	1.19 ^{d}	0.15 ^{A}	0.20 ^{A}	0.54 ^{A}	0.49 ^{AG}	1.96 ^{D}	1.86 ^{D}	0.82 ^{A}	0.66 ^{A}	2.77 ^{A}	2.52 ^{A}
		miscanthus	1.14 ^{D}	0.95 ^{E}	0.15 ^{A}	0.18 ^{A}	0.57 ^{A}	0.51 ^{A}	1.86 ^{D}	1.65 ^{AD}	0.88 ^{A}	0.53 ^{AB}	2.74 ^{A}	2.18 ^{B}
		willow	1.38 ^{D}	0.90 ^{E}	0.20 ^{A}	0.13 ^{A}	0.55 ^{A}	0.54 ^{A}	2.14 ^{B}	1.58 ^{A}	0.36 ^{B}	0.50 ^{B}	2.48 ^{AB}	2.08 ^{B}
	AF	tomato	4.73 ^{F}		0.14 ^{A}		0.89 ^{C}		5.84 ^{E}		0.43 ^{B}		6.27 ^{F}	
All seasons	BS	spontaneous spp.	0.87 ^{A**}	0.85 ^{A}	0.15 ^{AB}	0.19 ^{A}	0.54 ^{A}	0.50 ^{A}	1.56 ^{A}	1.55 ^{A}	0.97 ^{A}	0.56 ^{B}	2.53 ^{A}	2.11 ^{B}
		miscanthus	0.78 ^{A}	0.70 ^{A}	0.15 ^{AB}	0.20 ^{A}	0.57 ^{A}	0.53 ^{A}	1.50 ^{A}	1.44 ^{A}	0.80 ^{A}	0.50 ^{B}	2.30 ^{AB}	1.93 ^{BC}
		willow	0.91 ^{A}	0.63 ^{B}	0.16 ^{A}	0.21 ^{A}	0.62 ^{A}	0.57 ^{A}	1.73 ^{B}	1.34 ^{A}	0.46 ^{B}	0.49 ^{B}	2.18 ^{B}	1.83 ^{C}
	AF	food crops	3.04 ^{C}		0.12 ^{B}		1.01 ^{B}		4.27 ^{C}		0.71 ^{AB}		4.87 ^{D}	

* average concentration of all the piezometers installed in AF along the perpendicular transects toward bioenergy buffers (see Figure 3.1 c-d)

**values with different letters in superscript show statistically different means among crop types (Tukey's LSD test, P < 0.05) within averaged values for all seasons

3.3.2 Buffer Strips Effectiveness (BSE) in removing N from shallow groundwater

Similar *F* and *P* values for BSE in removing N forms and their respective Cl^-/N ratios were observed among the ANOVA factors tested (Table 3.2). This similarity indicates that Cl^- concentration patterns in groundwater did not affected N removal dynamics.

Figure 3.2 shows the temporal dynamics of the BSE in removing NO_3-N (Figure 3.2a-b) and TDN (Figure 3.2c-d). No effect of crop types on BSE in removing any of the N forms analysed in shallow groundwater were found (Table 3.2). However, buffer width had a significant effect on NO_3-N and TDN removal rates. 10 m wide buffers (Figure 3.2a,c) removed significantly more nitrate (*F*: 31.7 *P*: <0.0001) and TDN (*F*: 5.2 *P*: 0.012) compared to 5 m wide buffers (Figure 3.2b,d). The results of non-linear regression model (Table S3.3) confirmed that a significant percentage of variance of BSE in removing NO_3-N was explained by buffer width. For the entire period of monitoring, NO_3-N removal rate indicates that 50, 75, 90 and 100% of BSE could potentially be reached by creating bioenergy buffers respectively 3, 9, 15 and 20 m wide (R^2: 0.18 *P*: 0.031) (Table S3.3). The highest percentages of variance of BSE explained by buffer width were found in the 2014 leaching season (R^2: 0.83 *P*: <0.001) and 2014 growing season (R^2: 0.29 *P*: 0.008).

Table 3.2 Results of the mixed model of repeated measures ANOVA used to investigate the effect of crop (C), buffer width (W) and season (S) on buffer strip effectiveness (BSE) in removing from shallow groundwater the different N forms. The table presents the *F* and *P* values of the main fixed effect terms and their interactions. All mixed models showed values of adjusted R^2 (including both fixed and random effects) higher than 0.87 (expect for NH_4 and NO_2 that were respectively 0.56 and 0.45).

N form	Crop		Width		Season		CxW		CxS		WxS		CxWxS	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
NO_3	1.8	ns	31	***	14	***	1.6	ns	1.4	ns	10	***	1.2	ns
Cl/NO_3	1.5	ns	17	***	13	***	1.1	ns	1.4	ns	7.8	***	1.1	ns
NO_2	0.8	ns	1.4	ns	0.2	ns	0.2	ns	0.1	ns	0.1	ns	0.1	ns
Cl/NO_2	0.2	ns	1.2	ns	0.6	ns	0.1	ns	0.1	ns	0.2	ns	0.1	ns
NH_4	0.6	ns	0.1	ns	7.4	***	0.4	ns	1.9	*	0.6	ns	0.7	ns
Cl/NH_4	0.7	ns	0.1	ns	7.3	***	0.5	ns	1.2	*	0.2	ns	0.8	ns
DIN	1.4	ns	1.4	ns	11	***	0.6	ns	0.6	ns	1.7	ns	0.4	ns
Cl/DIN	1.1	ns	1.2	ns	9.8	***	0.7	ns	0.2	ns	1.6	ns	0.2	ns
TDN	1.9	ns	5.4	*	7.0	***	0.1	ns	0.6	ns	3.1	**	1.8	*
Cl/TDN	1.7	ns	5.2	*	6.8	***	0.1	ns	0.5	ns	2.4	*	1.5	*

* P denotes significance at 0.05 **0.01 ***0.001

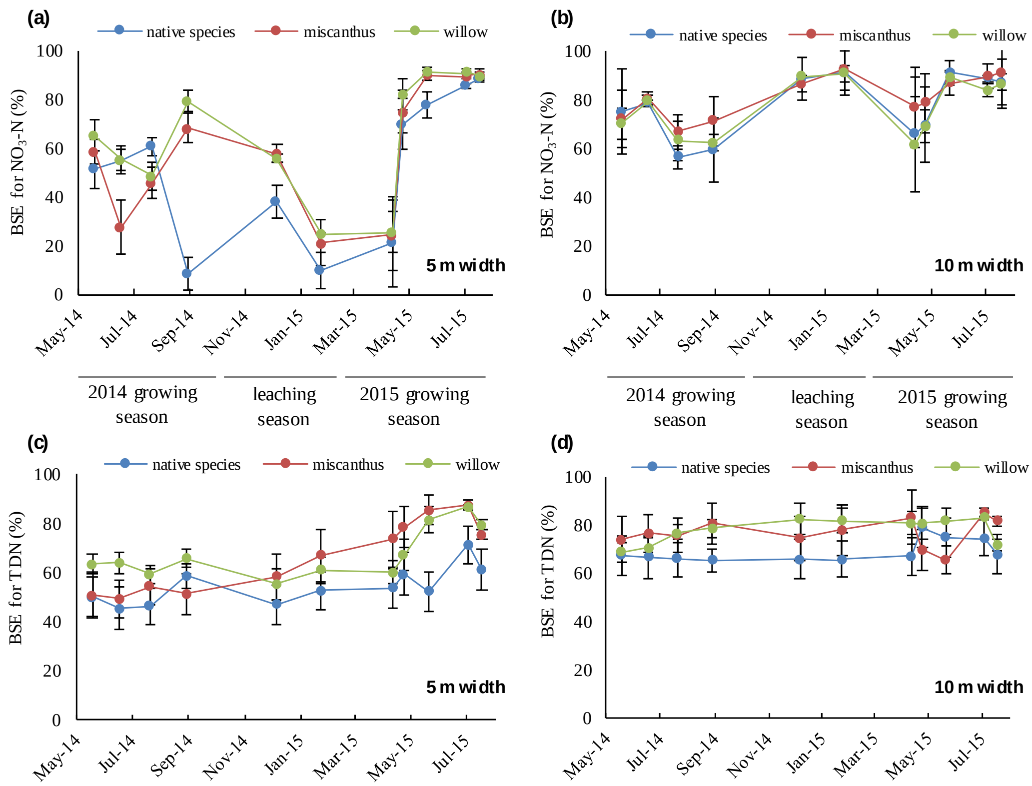

Figure 3.2 Temporal dynamics of bioenergy buffers effectiveness (BSE - %) in removing NO_3-N (a-b) and TDN (c-d) for buffers 5m wide (a-c) and 10m wide (b-d). Error bars show standard error of the mean (n = 3).

A relevant seasonal pattern of the BSE in removing nitrate was observed (Table 3.2, Table S3.3 and Figure 3.2). During the 2015 growing season, nitrate removal rates of bioenergy buffers were significantly higher than in 2014 (Table S3.3). A significant positive relationship between groundwater NO_3 input (mg NO_3^- L^{-1}) and buffer strip effectiveness in removing NO_3^- (BSE %) was found (Figure 3.3). Bioenergy buffers exponentially increase their NO_3 removal rates when they started to receive more NO_3 in May 2015 after the beginning of NPK fertirrigation of tomato in the adjacent AF. 5 m wide buffers (Figure 3.3a) were found to be more correlated with NO_3^- input than wider buffers that, on the other hand, showed to have reached their maximum buffering capacity (Figure 3.3b). As result of the influence of NO_3^- input on N removal rate, a significant interaction between buffer width and season was found for NO_3-N (*F*: 10.3 *P*: <0.0001) and TDN (*F*: 3.1 *P*: 0.023). The most significant effects of buffer width on nitrate removal were observed during the 2014 growing season (*F*: 12.45 *P*: 0.001) and in the 2014 leaching season (*F*: 16.2 *P*: <0.0001).

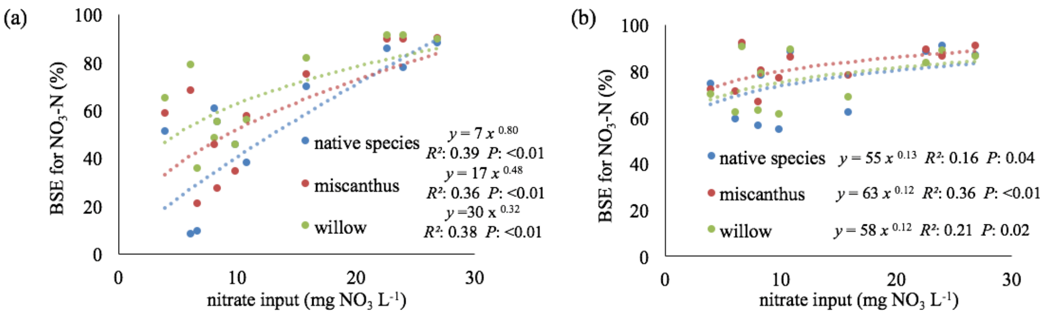

Figure 3.3 Relationship between lateral NO_3^- inputs and buffer strip effectiveness (BSE) in removing NO_3^- for bioenergy buffers 5 m wide (a) and 10 m wide (b). Data points represent mean values (n=3) of the eleven groundwater sampling events.

Based on the model $y=ax^b$, 50% and 75% of the BSE in removing NO_3 were estimated to occur during the 2015 growing season in 1 m and 4 m wide bioenergy buffers, respectively (Table S3.3). Among the other mineral N forms, NH_4-N and NO_2-N removal rates were not affected as much as NO_3-N and TDN by buffer width, season and by their interaction. NH_4-N and NO_2-N had large variances explained by the random factor in mixed model of ANOVA (data not shown). On average, NH_4-N removal rates were 44% for bioenergy buffers. Nitrite instead showed in 92% of the cases negative values of BSE indicating that release of nitrite in groundwater by bioenergy buffers prevailed over removal (Table 3.2). As consequence of the contrasting patterns revealed by NO_2-N (release) and NH_4-N (high variability among replicates), DIN resulted not significantly affected by crop type, buffer width and by the interactions of these factors (Table 3.2). DIN removal by bioenergy buffers ranged from 56% in 2014 growing season to 69% in 2015 growing season.

3.3.3 Groundwater geochemistry and hydrology

Water table fluctuated along the measuring period following the precipitations pattern (Figure S3.1). On average water table depth ranged between 0.95 m –bss in winter and autumn and 0.62 m – bss during spring and summer. Water table depth did not differ significantly in AF and bioenergy buffers (F: 0.626 P: 0.6082). Dissolved oxygen in AF resulted significantly higher (F: 5.2 P: 0.034) than under bioenergy buffers. Dissolved oxygen in AF was on average 2.74 mg L^{-1} and 2.25 mg L^{-1} in bioenergy buffers (Table S3.2). No statistical differences were found instead for dissolved O_2 among bioenergy buffers types. DOC concentration showed an increase along the transect of piezometers toward the ditch (Table S3.2). Agricultural field showed significant lower DOC levels (on average 1.71 mg DOC L^{-1}) compared to groundwater after bioenergy buffers (F: 11.2 P: 0.004). Willow showed the highest groundwater DOC values (on average 7.76 mg DOC L^{-1}), while no significant differences were found for the same parameter between spontaneous species and miscanthus.

Moreover, a significant negative nonlinear relationship was found between groundwater DOC and NO_3-N (*P:* 0.025 R^2: 0.58). Overall, groundwater after bioenergy buffers resulted more C rich and more N depleted in NO_3-N compared to groundwater coming from AF (Figure 3.4). A potential decrease in elemental DOC:NO_3-N ratio in groundwater under bioenergy buffer was found (Table S3.2). Under bioenergy buffers, starting from the 2014 leaching season until the 2015 growing season, elemental DOC:NO_3-N was below 3 in 95% of the cases. A significant inverse linear relationship between BSE (%) in removing nitrate and elemental DOC:NO_3-N was found (Figure 3.4). Elemental DOC:NO_3-N ratio was also seen to be a significant factor in determining BSE of 5 m wide buffers more than in 10 m wide buffers (Figure S3.2a). Only during the 2015 growing season was DOC:NO_3-N ratio significantly correlated with BSE (Figure S3.2b) because of the increase of N input from AF.

3.3.4 Impacts of bioenergy buffers on soil C- and N-cycling

Bioenergy buffers had a significant impact on the stock of several soil N and C pools compared to AF (Table S3.4 and Figure 3.5a-d). Considering the dissolved mineral N forms that were analysed, AF showed lower dissolved inorganic N (DIN) and NH_4-N in the soil compared to the bioenergy buffers. No effects of crop type and season were found for TDN (Table S3.4). Only in the third growing season (2015) was a significantly higher TDN stock found in the AF (*F*: 6.65 *P*: <0.0001). Under the tomato cultivation, potential leachable NO_3-N was highest (*F*: 6.05 *P*: <0.0001) at all soil depths (Figure 3.5a) and consequently TDN was increased along the soil profile. Three months after willow buffer establishment a significant increase of potential leachable NO_3-N along the soil profile was found compared to the other bioenergy buffer types (Figure 3.5a). No other significant potential leaching phenomena were found for willow in the following years compared to other bioenergy buffers. On average the proportion of NO_3-N, NH_4-N and NO_2-N in soil DIN of AF was 92%, 6%, 2% respectively. In comparison to AF, the proportions of NO_2-N (9%), and of NH_4-N (14%) in soil DIN of bioenergy buffers were significantly increased and NO_3-N was significantly reduced (77%). Soil TDN pool in bioenergy buffers consisted of a great percentage of N in a dissolved organic form (DON). DON was significantly higher in bioenergy buffers that in AF in the top soil layers (0-10, 10-20 and 20-30 cm). Three years after bioenergy buffer establishment, DOC resulted the soil C pool mostly affected (in terms of positive stocking) by the crop types (Figure 3.5c). Significant effects for crop type (*F*: 7.40 *P*: 0.006), soil depth (*F*: 5.40 *P*: 0.002), growing season (*F*: 5.97 *P*: 0.003) and their interactions were found for DOC (Table S3.4). Bioenergy buffers soils showed a significant increase of DOC stock compared to AF at all soil depths and for each of the first three growing seasons (Figure 3.5c). No differences in these parameters were found among bioenergy buffers indicating a similar trend of increase in DOC stock along the soil profile. The most significant increases in DOC under bioenergy buffers were observed in the 20-30 and the 30-60cm soil layers.

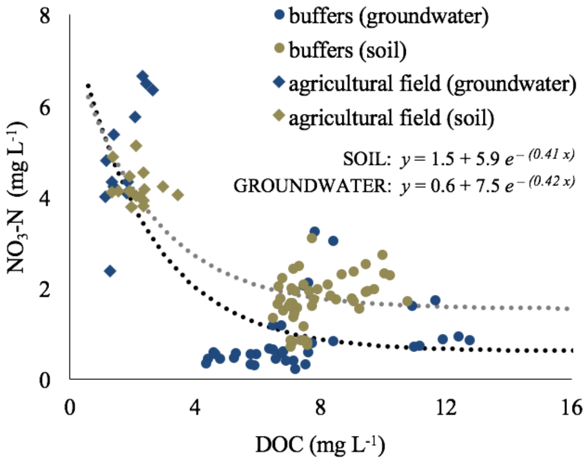

Figure 3.4 Relationship between the concentration of DOC and NO_3-N in groundwater (blue) and soil (brown). The data are grouped for bioenergy buffers (points) and agricultural field (diamonds). The soil dataset (unfumigated samples of soil microbial biomass extraction) was created using the data of the last two sampling seasons (n=64) that represent the temporal window where groundwater was monitored (samples of 10 m wide buffers, n=44, where soil samples were collected). The results of the regressions model ($y = a+be^{-k(x)}$) were significant for both dataset: groundwater (R^2: 0.58, P: 0.025) and soil (R^2: 0.74, P: 0.012).

Similarly to what observed in groundwater, a significant negative nonlinear relationship (P: 0.012 R^2: 0.74) was found in soil between the concentrations of DOC and NO_3-N (Figure 3.4). The increase of DOC in bioenergy buffers also contributed to an increased C availability for microorganisms. Figure 3.5d clearly shows how microbial biomass C (MBC) significantly increased along the soil profile in bioenergy buffers compared to AF (F: 5.92 P: 0.004). After the first period of buffers establishment, significant interactions between crop and soil depths (F: 3.91 P: 0.029) and between crop and growing seasons (F: 3.38 P: 0.013) were observed for MBC. Under bioenergy buffers the 30-60 cm soil layer showed the greatest increase in MBC stock (P 0.013) compared to AF. A significant increase in microbial biomass N (MBN) stock was also observed in bioenergy buffers compared to AF (F: 3.99 P: 0.023) (Figure 3.5b). Among bioenergy buffers, spontaneous species was seen the treatment with the highest ability to immobilize N in soil microbial biomass at different depths compared to miscanthus (P: 0.028) and willow (P: 0.003). Elemental C:N ratio of microbial biomass was found significantly higher (F: 2.11 P: 0.047) in bioenergy buffers (6.01) compared to the AF (4.45).

The rate of biological reduction of nitrate to nitrite (nitrate reductase activity - NRA) was found to be strongly affected by the crop types, soil depths and across different growing seasons (Figure 3.6 and Table S3.4). Bioenergy buffers, in particular willow, supported a soil microbial community able to remove nitrate at higher rates compared to AF since the first periods after crop establishment (Figure S3.3). On average, NRA values along the soil profile were 38.1 and 43.4 µg N-NO_2 g_{soil}^{-1} day^{-1} respectively for miscanthus and willow. These values were significantly higher (*F*: 56.50 *P*: <0.0001) than those observed for the spontaneous species (30.3 µg N-NO_2 g_{soil}^{-1} day^{-1}) and for the AF (21.5 µg N-NO_2 g_{soil}^{-1} day^{-1}).

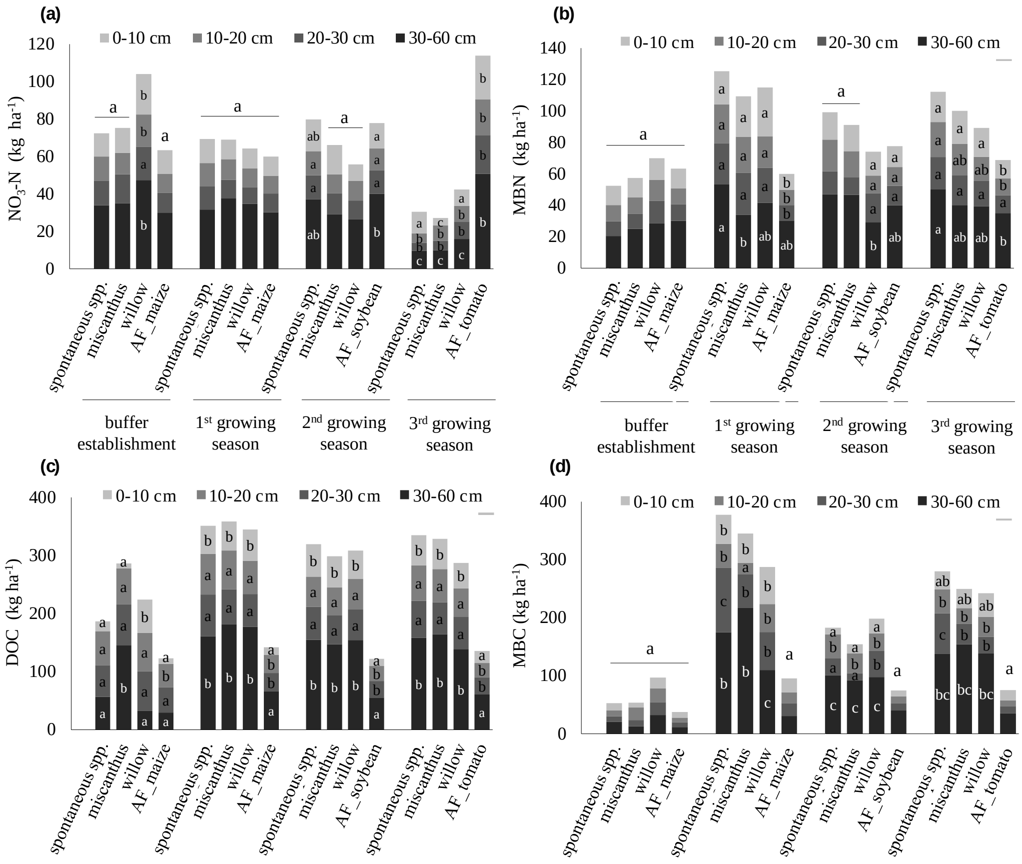

Figure 3.5 Average values of potentially leachable NO_3-N (a), microbial biomass nitrogen – MBN (b), dissolved organic C – DOC (c) and microbial biomass C – MBC (d) in bioenergy buffers and in agricultural field (AF) at different soil depths across different growing seasons. Different letters within staked columns show statistically different means among crop types (Tukey's test, P: 0.05) within the same soil depth. Horizontal lines above column(s) indicate that the letter is the same for all the soil depths.

3.3.5 Belowground and aboveground biomass production and N stocks

After three years from the establishment of bioenergy buffers, fine root biomass (<2 mm) was significantly affected by crop type, soil depths and by the interaction of both factors (Figure 3.7a). In the whole soil profile (0-60cm), willow showed the significantly highest fine root biomass (5.30 Mg ha^{-1}) compared to miscanthus (3.99 Mg ha^{-1}), while the lowest value was found for the spontaneous species (2.03 Mg ha^{-1}).

On average 59% of fine roots in willow and miscanthus were found in the top soil layer (0-30cm) and 41% in bottom soil layer (30-60 cm). In the spontaneous species the greatest proportion of fine root biomass (70%) was found in the top layer. Significant linear relationships were found between fine root biomass and soil NRA for miscanthus and willow (Figure 3.7c). The crop ranking for fine root biomass (willow>miscanthus> spontaneous species) was the same for soil NRA.

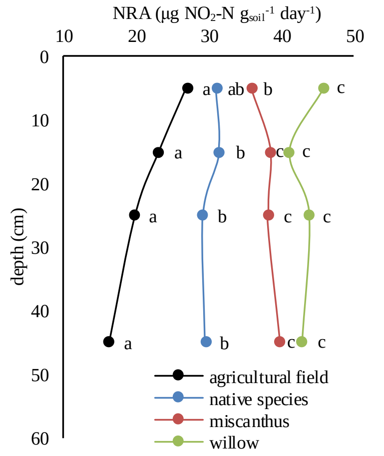

Figure 3.6 Soil nitrate reductase activity (NRA) in bioenergy buffers and in agricultural field at different soil depths. Values are reported as average values of the first three growing seasons. Different letters show statistically different means (Tukey's test, P: 0.05) within soil depths.

N root content (g kg^{-1}) did not vary significantly among crops (F: 1.67 P: 0.211) and along the soil profile (F: 0.15 P: 0.926). On average, at 0-10 cm, 10-20 cm, 20-30 cm and 30-60 cm depth root N content was respectively 5.8, 6.1, 6.1 and 5.9 kg N g_{root}^{-1}. N stock in fine roots was significantly affected by crop types, soil depths and by the interaction of both factors (Figure 3.7b). Willow showed a higher root N stock (32.40 kg N ha^{-1}) along the whole soil profile (0-60 cm) compared to miscanthus (20.79 kg N ha^{-1}). Spontaneous species instead showed the lowest root N stock (12.67 kg N ha^{-1}). Harvestable biomass in bioenergy buffers for miscanthus, after winter killing frost (February), was 3.2 ± 0.6 Mg DM ha^{-1} in the establishment year (2013) and 10.76 ± 0.51 Mg DM ha^{-1} at the second year (2014). Willow, after the first two years rotation cycle, produced significantly more than miscanthus (F: 99.55 P: <0.0001) with a harvestable biomass of 34.15 ± 1.71 Mg DM ha^{-1}. N exportations via harvesting were respectively 5.9 kg N ha^{-1} in 2013 and 16.1 kg N ha^{-1} in 2014 for miscanthus and 73.7 kg N ha^{-1} for willow in 2014.

By analyzing the biomass data of each single row, it was found an exponential decrease of the biomass yield along the buffer transect (Figure S3.4). The plant rows closer to the AF showed the highest values in harvestable biomass and N removal in comparison to the plant rows near to the ditch. Harvestable biomass for the 10 m wide willow buffers (Figure S3.4a) ranged from 47.4 Mg DM ha^{-1} in the plant rows adjacent to the AF to 26.6 Mg DM ha^{-1} in the plant rows near to the ditch. Similarly, N removal was highest in plant rows adjacent to the AF (120.6 kg N ha^{-1}) and lowest near the ditch (51.78 kg N ha^{-1}). The same effect was less evident in miscanthus and it was limited to the first two rows adjacent to the AF (Figure S3.4b).

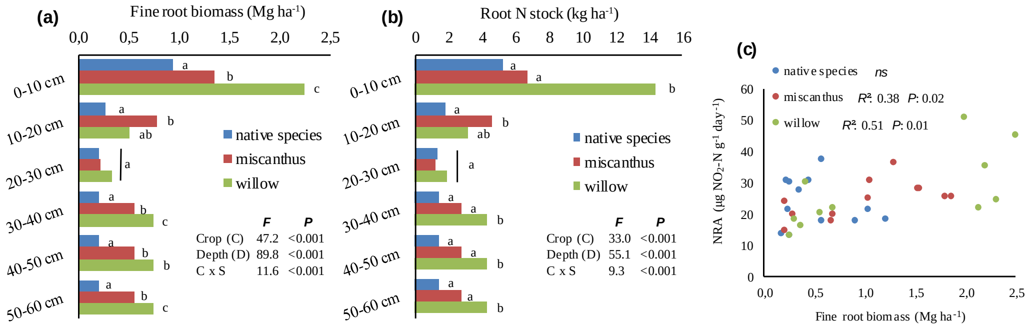

Figure 3.7 Fine root biomass (a) and root N stocks (b) in bioenergy buffers at different soil depths. Different letters show statistically different means (Tukey's test, P: 0.05) among crop types within the same soil depth interval. (c) Linear relationship between fine root biomass and soil nitrate reductase activity (NRA) in bioenergy buffers in third growing season (2015).

3.4 Discussion

3.4.1 Bioenergy buffers effectiveness (BSE) in removing N and key factors governing BSE

Our results clearly indicate bioenergy buffers effectiveness in removing NO_3-N and TDN from shallow groundwater (Figure 3.2 and Table S3.3). BSE in removing NO_3-N was 70% for miscanthus and 71% for willow, respectively (Table S3.3). These values are in accordance with the 60-70% range reported at landscape level by Ssegane *et al.* (2015) and Gopalakrishnan *et al.* (2012) for buffer strips cultivated with switchgrass, miscanthus and willow. Similar findings were reported also in riparian buffers of *Salix* spp. (Young & Briggs, 2005). No differences between herbaceous and woody crops and between bioenergy crops and spontaneous species on N removal rate were observed (Table 3.2). This indicates that vegetation types in narrow buffer strips do not remove N from subsurface water flow with significant differences (Sabater *et al.*, 2003; Mayer *et al.*, 2007). Mayer *et al.* (2007) conducted a meta-analysis over 45 published studies on nitrate removal by riparian buffers and found that the mean mass of NO_3-N removed per unit length was not statistically different between forested and herbaceous buffers. Similarly, our results confirmed that DIN was dominantly present as NO_3 and it was removed 9.38% m^{-1} by spontaneous species, 10.12 % m^{-1} by miscanthus and 9.43% m^{-1} by willow, respectively. These values are in accordance with the mean values found in 14 riparian buffers across Europe (Sabater *et al.*, 2003). Yet, it is confirmed that from the first periods after establishment bioenergy crops can remove N from groundwater as much as buffers strips with spontaneous species.

Buffer width had a significant effect on NO_3-N and TDN removal rates from shallow groundwater, with 10 m wide buffers being more effective. Nonetheless, bioenergy buffers that are as wide as national recommendations (5 m) suffice to remove more than 50% of the incoming nitrate in most cases (Table S3.3). The effect of buffer width in this study was unexpected as in literature reports have shown significant differences where buffer widths differed by more than 10-20 m (Hickey & Doran, 2004; Mayer *et al.*, 2007; Sweeney & Newbold, 2014). In addition, nitrate removal rate was seen to be even higher when nitrate input from AF increased (Figure 3.3). The results of non linear regression (Table S3.3) suggested that, in linear and straightforward hydrological conditions similar to our case study, a 3 m wide buffer, made of miscanthus or willow, can remove up to 75% of nitrate during a high N input season. This indicates that no N saturation effects occurred in our 3 year old bioenergy buffers, though clear symptoms of N saturation have been reported in situations with long-term N loadings (Aber, 1992; Hanson *et al.*, 1994; Sabater *et al.*, 2003; Hefting *et al.*, 2006).

3.4.2 Biomass production and plant N removal in bioenergy buffers

The reasons for which no evidence of N saturation was observed in this study can be found in the aboveground and belowground biomass dynamics. Biomass production and plant N uptake have been shown to be important N removal processes in forested (Hefting *et al.*, 2005) and herbaceous buffers (van Beek *et al.*, 2007; Balestrini *et al.*, 2011). In this study willow buffers performed very well in terms of biomass production in the first 2-year cycle (34.2 Mg DM ha^{-1}). This value of biomass yield is higher than the mean values reported for *Salix* spp. in Canada and the United States (Amichev *et al.*, 2014), Europe (Zegada-Lizarazu *et al.*, 2010) and in northern Italy (Rosso *et al.*, 2013). The tolerance of willow to saturated soils and oxygen shortage at deeper soil layers is widely reported (Krasny *et al.*, 1988; Jackson & Attwood, 1996; Aronsson & Perttu, 2001). Furthermore, lateral N loadings by enriched groundwater significantly affected biomass production along the buffer transect (Figure S3.4a) with the first two rows (adjacent to the AF) being the most productive (up to 48 Mg DM ha^{-1} plant row^{-1}) and the ones that contributed most to N removal via uptake and harvesting (Figure S3.4).

Miscanthus biomass production in the first two years was 3.2 and 10.8 Mg DM ha^{-1}. These values are lower than those found in field trials with similar stand age in temperate regions; from 15 to 20 Mg DM ha^{-1} (Lewandowski & Heinz, 2003; Angelini *et al.*, 2009). For miscanthus, the low yields might have been affected by the presence of shallow groundwater (Lewandowski *et al.*, 2003) and by the high soil hydraulic conductivity and sandy loam texture (Table S3.1). The latter two factors may increase the soil moisture deficit in upper soil layers for relatively long periods during the summer season; previous studies (Heaton *et al.*, 2004; Monti & Zatta, 2009; Mann *et al.*, 2012) have shown miscanthus to be highly productive where water is not limiting, but very sensitive to water shortage.

By comparison to spontaneous species, both willow and miscanthus had deeper fine root systems (Figure 3.7a) and higher root N stocks (Figure 3.7b). The ability of perennial bioenergy crops to penetrate deep rooting zones (to access nutrients more efficiently) is widely recognized (Rytter, 2001; Glover *et al.*, 2010; Ens *et al.*, 2013; Owens *et al.*, 2013; Amichev *et al.*, 2014).

The total belowground biomass found in willow can be placed at the highest ranking positions among the willow hybrids studied in Stadnyk (2010) and reviewed in Amichev *et al.* (2014). After two years from planting miscanthus had a mean belowground biomass of 4 Mg ha^{-1} between 0 and 60 cm in depth. At this depth interval this value is in line with those reported in previous studies carried out on mature stands (>3-4y) in Italy (Monti & Zatta, 2009; Chimento & Amaducci, 2015), Europe and USA (Heaton *et al.*, 2004; Amougou *et al.*, 2010; Dohleman *et al.*, 2012; Anderson-Teixeira *et al.*, 2013; Zatta *et al.*, 2014).

With regard to root biomass distribution along soil profile, it was observed that willow with 2.2 Mg ha^{-1} and miscanthus with 1.6 Mg ha^{-1} are characterized by an high contribution of fine roots (41%) to whole root biomass at deeper layers (30-60 cm). In a 6-years-old multispecies experiment (Chimento & Amaducci, 2015) found that only 0.9 Mg ha^{-1} (17%) and 2 Mg ha^{-1} (23%) of the whole root mass, was allocated respectively by willow and miscanthus at 30-60 cm depth. These results on rooting patterns clearly indicate how cultivating bioenergy crops along the field margins offers the opportunity to intercept N loads from surrounding agricultural fields at deeper soil layers compared to buffers with spontaneous species. This would ultimately increase the environmental performance of bioenergy buffers in term of plant N removal from soil. Furthermore, as root biomass was shown to be a good indicator of soil organic C sequestration (Chimento & Amaducci, 2015; Chimento *et al.*, 2016), our results suggest how bioenergy buffers have a higher potential compared to patches of adventitious plants to contribute to C storage and GHG savings in the deep soil layers.

3.4.3 Biogeochemical processes governing N removal in plant-soil-groundwater system

In addition to the role of vegetation, a series of biogeochemical processes in soil and groundwater are recognized as being important in determining N removal in bioenergy 3buffers. The patterns of dissolved O_2, pH, NO_2-N, NO_3-N and DOC in groundwater (Table 3.2, Table S3.2 and Figure 3.4) suggest that denitrification plays a predominant role in the nitrate depletion observed in bioenergy buffers. Suboxic conditions were found in groundwater after the bioenergy buffers (Table S3.2); such conditions are optimal for denitrification (Vidon & Hill, 2005). There was also a significant increase in the contribution of NO_2-N to DIN at the expense of NO_3-N which indicates that a rapid nitrate reduction occurred (Giles *et al.*, 2012; Butterbach-Bahl *et al.*, 2013).

The alkaline pH of groundwater (Table S3.2) and of soil (Table S3.1) and the average depth of the groundwater table (Figure S3.1) denote the presence of ideal conditions for soil denitrifying communities (Groffman *et al.*, 1991; Weier *et al.*, 1993; Rich & Myrold, 2004). Moreover, an increase in the stock of DOC along the soil profiles of bioenergy buffers (Figure 3.5c) might have promoted the observed enrichment of DOC in groundwater after the bioenergy buffers (Table S3.2). DOC levels in groundwater after the bioenergy buffers (>5 mg DOC L^{-1}) indicated that incoming groundwater found suitable conditions for denitrification under the bioenergy buffers (Cosandey *et al.*, 2003; Gumiero *et al.*, 2011; Senbayram *et al.*, 2012). In comparison to spontaneous species, willow and miscanthus, indeed, promoted an active zone of biological removal of nitrate along the whole soil profile because of their deep and dense root systems as revealed by the positive relation between NRA and fine root biomass (Figure 3.7c).
High soil moisture in sandy loam soils has been shown to stimulate root exudation of easily available C sources (DOC) for microorganisms, thus triggering microbial activity (Dijkstra & Cheng, 2007). On this regard, the use of DOC and the incoming nitrate respectively as donor and electron acceptor by denitrifying microbial communities plays a key role in the nitrate depletion observed in groundwater. A significant exponential negative relationship between DOC and NO_3-N was found along the groundwater-soil continuum from the AF to the bioenergy buffers (Figure 3.4). This indicate that the shift in elemental stoichiometry (DOC:NO_3-N ratio) promoted the microbial N removal by denitrification in bioenergy buffers by constraining N accrual in groundwater. The presence of a confining layer at a shallow depth (Figure 3.1c) forces most of the incoming oxic and enriched nitrate groundwater to flow through the subsurface, DOC rich, soil layer of the bioenergy buffers (Gold *et al.*, 2002). As consequence the DOC:NO_3-N ratio dropped below the range of 3-6 (Table S3.2) and triggered NO_3-N removal by denitrification (Taylor & Townsend, 2010), which is in agreement with results available in literature (Groffman *et al.*, 1992; Hedin *et al.*, 1998; Hill & Cardaci, 2000; Gold *et al.*, 2002; Cosandey *et al.*, 2003; Senbayram *et al.*, 2012).
The results discussed above indicate that the N removal processes are strictly linked to the increase of DOC in bioenergy buffers. Dissolved organic C compounds are important drivers of denitrification in riparian soils (Hill *et al.*, 2000). Easily available C for microorganisms measured as DOC has been also thought to be the main source of subsoil organic matter (Rumpel & Kögel-Knabner, 2011) and under bioenergy crops could be of relevance due to the their deep root systems (Agostini *et al.*, 2015). In fact, the observed increase of soil DOC in willow and miscanthus buffers was found to be significantly correlated to fine root biomass (R^2: 0.35 P: 0.04). Through the release of exudates of low molecular weight (the main source of DOC) the root environment (the so called rhizosphere) increases microbial activity through MB utilization of new easily available C sources (Kuzyakov, 2002; Zhu *et al.*, 2014).

The dual increase in DOC and MBC observed along the soil profile in our bioenergy buffers as compared to the AF (Figure 3.5c-d) revealed that establishment of bioenergy crops with such dense and deep-rooting systems triggered the soil microbial community. The activities of soil C, N and P-acquiring enzymes such as β-glucosidase, leucine aminopeptidase and alkaline phosphatase have been observed to significantly increase under bioenergy buffers at 0-30 cm depth (*unpublished data*). The rhizosphere priming effect promotes N mining from SOM and the mineralized N is retained by the microbial community through rapid immobilization (Kuzyakov, 2002; Dijkstra *et al.*, 2013; Kuzyakov & Xu, 2013; Blagodatskaya *et al.*, 2014; Chen *et al.*, 2014; Zhu *et al.*, 2014). Microbial biomass N, indeed, significantly increased in the top soil layers under the bioenergy buffers by comparison to the AF (Figure 3.5b). Microbial N retention was also observed in other perennial agroecosystems (Hargreaves & Hofmockel, 2013). However, elemental CN ratio of microbial biomass (MB) along the soil profile did not decrease because of MBN increase. A MB CN ratio around 6 is close to that of the SOM that would be decomposed (SOM CN of 8 at 0-60 cm) and this highlights how soil microbial biomass should not undergo adjustments of microbial element use efficiency (Mooshammer *et al.*, 2014). As the stoichiometry of the soil resource was balanced with that of the microbial biomass, soil microbes could not excrete N in excess and thus soil N is retained and N losses should have been prevented (e.g. during winter period with potential N leaching) (de Vries & Bardgett, 2012; Manzoni *et al.*, 2012). Indeed, potentially leachable nitrate did not increase significantly along the soil profile under the bioenergy buffers compared to the AF after the beginning of the second growing season (2014) (Figure 3.5a). Overall, the increase in easily available C for microorganism (DOC), MBC and MBN confirmed the results of Bengtson *et al.* (2012) and Paterson (2003) of a strong coupling of root C release, SOM cycling, and microbial N cycling.

In conclusion, herbaceous and woody bioenergy crops have been confirmed as being effective in mitigating shallow groundwater N pollution when cultivated as bioenergy buffers. Up to 50, 70 and 90% buffer strip effectiveness in removing NO_3-N could be reached by creating bioenergy buffers 3 m, 9 m and 15 m wide, respectively. The use of ecological stoichiometry (DOC:NO_3-N) revealed that denitrification plays a key role in the nitrate removal observed along the soil-groundwater continuum. Deep rooting systems of bioenergy crops promoted the activation of soil microbial processes involved in N removal from soil. Our findings also suggest that biomass production and N removal through multiple harvests further contributes to N retention in bioenergy buffers compared to unmanaged buffer strips with spontaneous species. Bioenergy crops placed along watercourses in sandy loam soils with shallow groundwater enhance ecosystem services and sustain soil functioning such as water quality regulation and soil microbial C and N cycling.

3.5 Supporting Information

Table S3.1 Main soil physical and chemical characteristics of the soil horizons

Parameter*	unit	Ap 0-40 cm	Bw 40-70 cm	2C 70-150 cm	2C 150-200 cm
SOC	%	0.8	0.4	0.3	0.1
N tot	%	0.07	0.05	0.02	0.01
C/N ratio		11.4	8	15	4
P_{olsen}	ppm	6.3	4.1	1.3	0.7
$CaCO_3$	%	10	11.5	9.3	3.3
pH		7.9	8	8.2	8.1
Texture	texture class	sandy loam	sandy loam	loamy sand	silty clay
Bulk density	$g\ cm_3^{-1}$	1.48	1.49	1.69	1.05
CEC	$cmol^{(+)}\ 100g^{-1}$	8.7	7.2	4.1	2.9
K_s***	$cm\ day^{-1}$	46	55	165	8

* all the parameters were measured according to the Italian soil analyses manual
** Cation Exchange Capacity
*** Saturated hydraulic conductivity measured with Cornell Sprinkle Infiltrometer

Table S3.2 Average concentrations of groundwater chemical species after bioenergy buffers (BS- crop) and in agricultural field (AF-crop). BS: buffers strips; AF: agricultural field.

SEASON		CROP	T °C	O_2 mg L^{-1}	pH	Cond* µs cm^{-1}	TDS** mg L^{-1}	DOC mg L^{-1}	DOC/ NO_3***	Cl mg L^{-1}
2014 season	BS	native species	$21.0^{a§}$	2.3^{a}	7.9^{a}	1162^{a}	626^{a}	7.18^{a}	3.3^{ab}	42.9^{a}
		miscanthus	20.6^{a}	2.1^{a}	7.9^{a}	1141^{a}	587^{a}	5.87^{b}	3.7^{a}	39.8^{a}
		willow	19.6^{b}	2.0^{a}	7.9^{a}	1224^{a}	639^{a}	7.49^{a}	3.9^{a}	42.0^{a}
	AF	soybean	22.2^{a}	2.5^{b}	7.8^{a}	1387^{b}	668^{a}	1.19^{c}	/	42.2^{a}
2014 leaching season	BS	native species	17.5^{b}	1.9^{ac}	8.0^{a}	1105^{a}	634^{a}	4.77^{b}	2.5^{b}	30.2^{a}
		miscanthus	17.9^{b}	1.6^{c}	8.1^{a}	1129^{a}	623^{a}	4.64^{b}	2.4^{b}	29.9^{a}
		willow	17.8^{b}	1.5^{c}	8.0^{a}	1103^{a}	615^{a}	6.88^{a}	3.0^{b}	34.2^{a}
	AF	bare soil	17.2^{b}	2.0^{a}	7.9^{a}	1134^{a}	651^{a}	1.81^{ce}	/	31.8^{a}
2015 season	BS	native species	21.7^{a}	2.6^{b}	8.1^{a}	925^{b}	471^{b}	7.02^{a}	1.4^{c}	36.8^{a}
		miscanthus	19.5^{ab}	3.2^{d}	7.9^{a}	653^{c}	321^{c}	7.11^{a}	1.5^{c}	38.7^{a}
		willow	20.1^{a}	3.2^{d}	8.2^{a}	943^{b}	465^{b}	8.91^{d}	1.9^{c}	39.6^{a}
	AF	tomato	21.2^{a}	3.6^{e}	8.5^{a}	1209^{a}	614^{a}	2.14^{e}	/	37.7^{a}

§ Values with different letters in superscript show statistically different means (Tukey's LSD test, $P < 0.05$) within chemical species.
* Conducibility
** TDS: Total Dissolved Solids
*** elemental DOC:NO_3 ratio expected to occur under bioenergy buffers. It was calculated dividing the values of DOC in groundwater after bioenergy buffers by the concentration of NO_3-N of the incoming groundwater from AF.

Table S3.3 Mean values of NO_3 removal rate as BSE (%), BSE per unit length (% m^{-1}) and mean mass of N removed per unit length (mg NO_3-N L^{-1} m^{-1}) for bioenergy buffers across the growing seasons. Results of non linear regression model of BSE plotted against buffer widths (BSE_{NO3}= a x^{width}). Buffer width (m) necessary to obtain a given value of BSE (%) as predicted by the regression model.

SEASON (N input [a])	CROP	mean BSE	mean BSE per unit length [b]	mean mass of N removed per unit length [c]	model $y=ax^b$	R^2	P	Buffer width (m) necessary to obtain a given value of BSE [d]			
		%	% m^{-1}	mg NO_3-N L^{-1} m^{-1}				**50%**	**75%**	**90%**	**100%**
All seasons	**All crops**	**67**	**9.5**	**0.327**	**33.2 $x^{0.37}$**	**0.18**	**0.031**	**3**	**9**	**15**	**20**
	Crop type										
	Native species	63	8.8	0.320	24.2 $x^{0.50}$	0.23	0.015	5	10	14	18
	Miscanthus	70	10.0	0.339	29.6 $x^{0.44}$	0.27	0.008	3	8	13	16
	Willow	71	10.3	0.330	49.4 $x^{0.19}$	0.30	0.004	1	9	23	40
	Bioenergy crops	71	10.2	0.334	38.4 $x^{0.31}$	0.17	0.034	2	9	15	21
2014 season	**All crops**	**61**	**8.7**	**0.138**	**26.4 $x^{0.42}$**	**0.29**	**0.008**	**4**	**12**	**19**	**24**
(low NO_3 input)	**Crop type**										
	Native species	56	7.8	0.126	16.3 $x^{0.61}$	0.59	<0.001	6	12	16	19
	Miscanthus	61	8.6	0.139	21.1 $x^{0.54}$	0.50	0.001	5	11	15	18
	Willow	65	9.6	0.148	48.9 $x^{0.15}$	0.14	0.016	1	18	<50	<50
	Bioenergy crops	63	9.1	0.144	32.7 $x^{0.33}$	0.30	0.004	4	12	21	28
2014 winter	**All crops**	**63**	**8.1**	**0.223**	**4.8 $x^{1.30}$**	**0.83**	<0.001	**5**	**9**	**10**	**11**
(low NO_3 input)	**Crop type**										
	Native species	57	6.9	0.220	1.1 $x^{1.91}$	0.91	<0.001	6	9	10	11
	Miscanthus	64	8.4	0.224	5.9 $x^{1.2}$	0.79	<0.001	5	9	10	11
	Willow	68	9.1	0.227	9.5 $x^{0.97}$	0.90	<0.001	5	8	10	11
	Bioenergy crops	66	8.8	0.223	7.8 $x^{1.0}$	0.89	<0.001	5	9	10	11
2015 season	**All crops**	**78**	**11.6**	**0.530**	**69.1 $x^{0.06}$**	**0.11**	**0.001**	**<1**	**4**	**>50**	**>50**
(high NO_3 input)	**Crop type**										
	Native species	75	11.2	0.521	66 $x^{0.06}$	0.11	0.023	<1	7	>50	>50
	Miscanthus	80	11.8	0.546	59 $x^{0.05}$	0.17	0.020	<1	5	15	29
	Willow	79	11.9	0.525	76.7 $x^{0.02}$	0.12	0.048	<1	2	>50	>50
	Bioenergy crops	80	11.9	0.535	68.8 $x^{0.03}$	0.14	0.032	<1	3	>50	>50

[a] for the data on NO_3-N input from AF see values in Table S3.2.

[b] mean BSE per unit length (% m^{-1}) was calculated dividing BSE (%) by buffer width (m) (Sabter *et al.*, 2003)

[c] mean mass of N removed per unit length (mg NO_3-N L^{-1} m^{-1}) is calculated as the difference in the NO_3-N groundwater concentration between AF and bioenergy buffers and divided by buffer width

[d] buffer width necessary to obtain a given value of BSE (50%,75%,90%,100%) are calculated by the predicted values from regression model (*$y=ax^b$*)

Table S3.4 Results of the mixed model of repeated measures ANOVA used to investigate the effect of crop (C), depth (D) and growing seasons (S) on the stock (kg ha^{-1}) of soil inorganic N forms, C and N pools of dissolved organic matters (DOM) and microbial biomass (MB) and the effects on potential soil nitrate reductase activity (NRA - µg NO_2-N g_{soil}^{-1} day^{-1}). The table presents the *F* and *P* values (bold P<0.05) of the main fixed effect terms and their interactions.

	Parameter	Crop (C)		Depth (D)		Season (S)		CxD		CxS		DxS		CxDxS		*adj* R^2
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	
Mineral	NO_3-N	**4.50**	**0.014**	**5.40**	**0.002**	**27.31**	**<0.001**	0.43	0.86	**6.05**	**<0.001**	**2.37**	**0.019**	0.68	0.831	0.82
	NH_4-N	**4.68**	**0.033**	0.57	0.564	0.61	0.607	**3.17**	**0.028**	0.09	0.996	0.84	0.575	0.86	0.621	0.56
	NO_2-N	0.58	0.565	0.62	0.607	**3.16**	**0.028**	**4.87**	**0.002**	0.09	0.997	0.82	0.592	0.87	0.611	0.46
	DIN	**4.18**	**0.019**	3.29	0.024	**26.47**	**<0.001**	**2.30**	**0.041**	**8.79**	**<0.001**	1.51	0.153	0.85	0.639	0.78
DOM	DON	0.94	0.394	0.72	0.541	**4.50**	**0.015**	**2.61**	**0.024**	0.65	0.626	0.43	0.856	0.54	0.882	0.78
	TDN	0.43	0.649	1.26	0.296	**13.89**	**<0.001**	1.59	0.162	**6.65**	**<0.001**	1.05	0.400	0.66	0.800	0.64
	DOC	**7.40**	**0.006**	**3.89**	**0.013**	**5.97**	**0.003**	**3.51**	**0.037**	**3.13**	**0.034**	**2.66**	**0.038**	2.40	0.060	0.78
	C:N ratio	0.14	0.864	**4.42**	**0.006**	**13.24**	**<0.001**	**2.87**	**0.015**	**8.95**	**<0.001**	0.42	0.861	1.14	0.340	0.70
MB	MBC	**5.92**	**0.004**	1.30	0.281	**3.99**	**0.022**	**3.91**	**0.029**	**3.38**	**0.013**	1.40	0.226	0.71	0.730	0.69
	MBN	**3.99**	**0.023**	0.15	0.926	**11.67**	**<0.001**	**1.91**	**0.050**	**3.55**	**0.011**	0.72	0.638	0.71	0.733	0.67
	C:N ratio	**2.11**	**0.047**	0.61	0.609	1.27	0.288	1.39	0.231	1.18	0.325	1.06	0.394	1.12	0.360	0.63
	NRA	**56.50**	**<0.001**	2.64	0.053	**14.92**	**<0.001**	**2.81**	**0.005**	**4.87**	**<0.001**	**2.21**	**0.025**	0.94	0.561	0.79

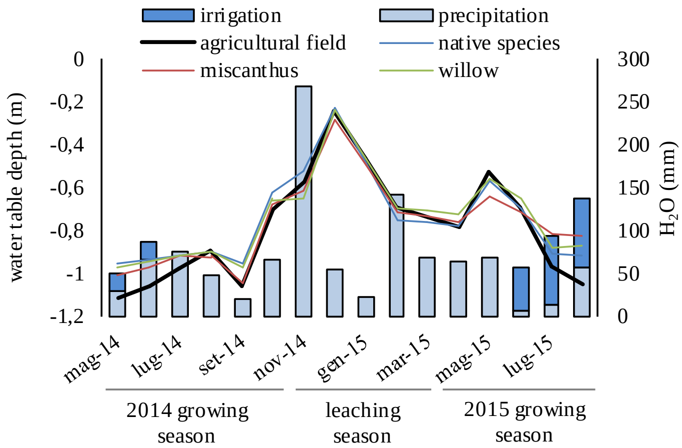

Figure S3.1 Hydrological features of the field trial

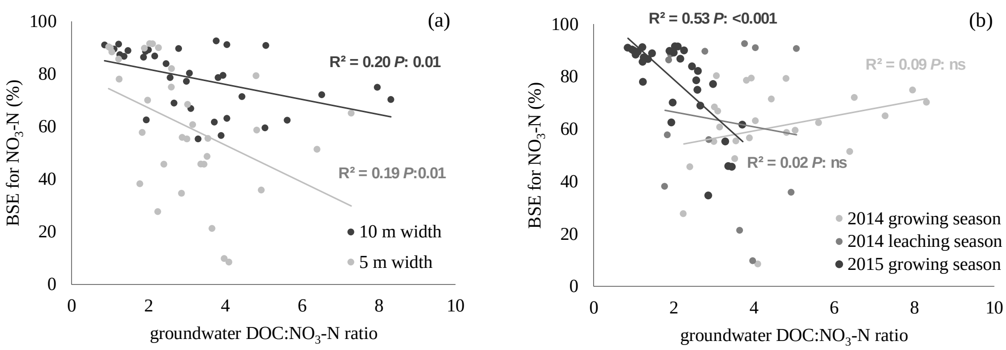

Figure S3.2 Relationship between elemental DOC:NO_3-N ratio under bioenergy buffers and buffer strip effectiveness (BSE %) in removing NO_3-N from groundwater. Data grouped by buffer width (a) and by monitoring seasons (b).

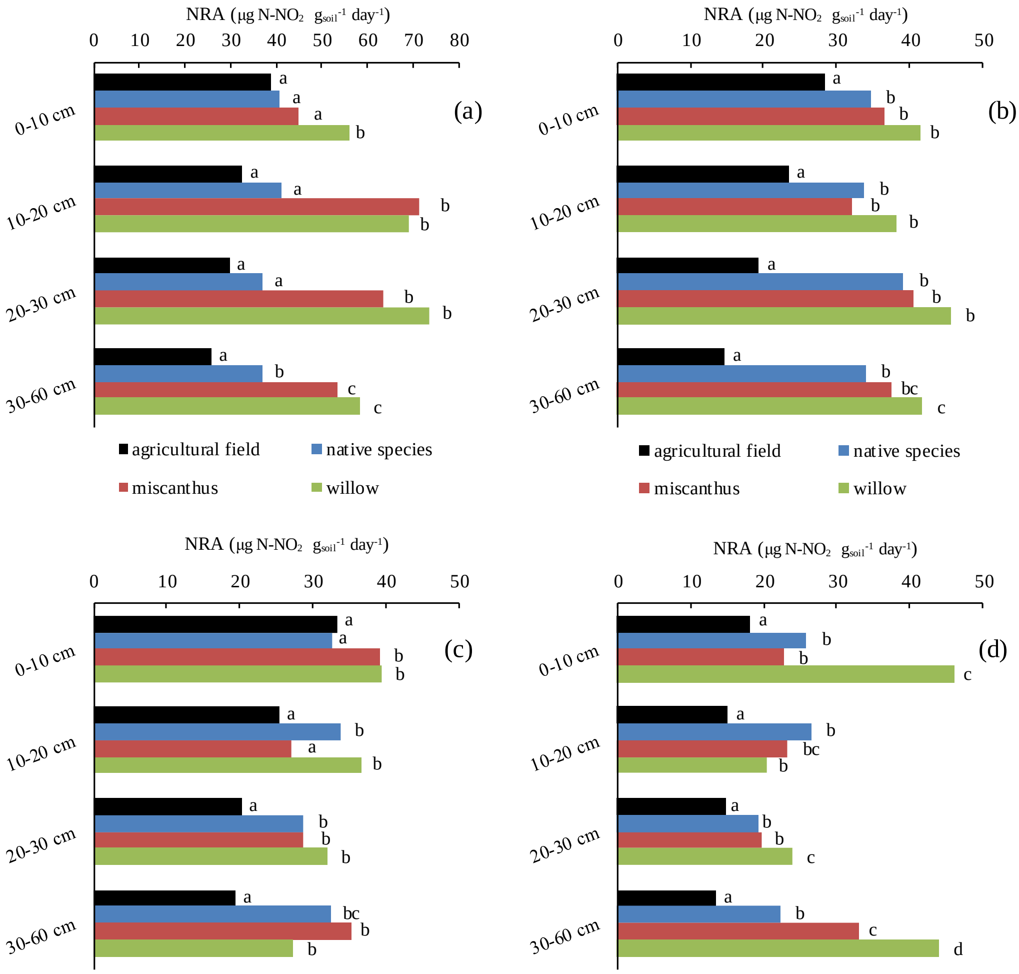

Figure S3.3 Soil NO_3 reductase activity (NRA) under bioenergy buffers and agricultural field at different soil depths across the four sampling seasons: (a) after buffers establishment (July, 2013); (b) end of 1st growing season (February, 2013); (c) end of 2nd growing season (February, 2014); (b) middle of 3rd growing season (August, 2015). Different letters show statistically different means among crop types (Tukey's test, P: 0.05) within the same soil depth and growing season.

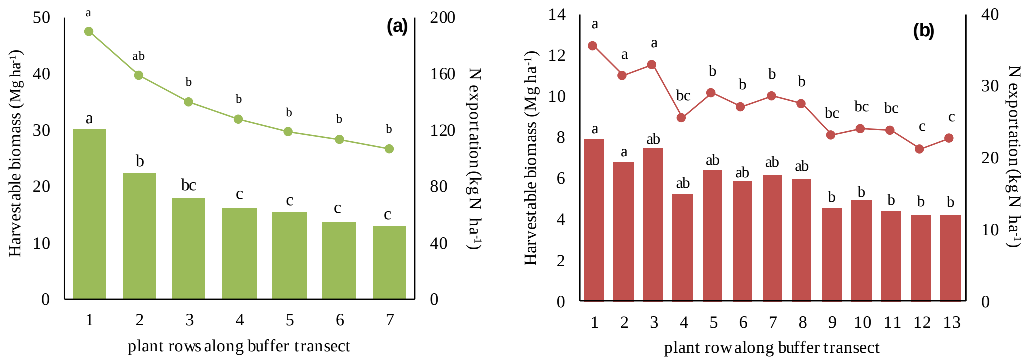

Figure S3.4 Harvestable biomass (lines) and N removal via harvesting (bars) of willow (a) and miscanthus (b) for different plant rows along the 10 m wide buffer transects. Values are reported for the harvesting carried out in late winter 2014 (end of 2nd growing season). Different letters show statistically different means among plant rows (Tukey's test, P: 0.05) within parameters and species. The lateral N loadings have to be considered coming from the left side (see also Figure 3.1d). Willow (*Salix matsudana* (hybrid)) has a stem density of 13.000 stems ha^{-1} (0.6 × 1.5 m spacing). Miscanthus (*Miscanthus x giganteous*) was planted instead with a density of 4 rhizomes m^2 (0.36 × 0.7 m spacing).

Appendix 3.1

Lab protocol adopted for potential soil Nitrate Reductase Activity (NRA)

Soil NO_3 reductase activity (NRA) was measured by soil anaerobic incubation following the modifications of the protocol of Abdelmagid & Tabatabai (1987) introduced by Chèneby *et al.* (2010). Optimal NO_3-N substrate and 2,4-dinitriphenol (DNP) inhibitor concentrations were measured to determine the maximum amount of NO_2-N produced for that specific sandy loam soil. In 2 ml eppendorf tubes, three replicates of 0.25 g for each soil sample were pre-incubated 5h with 0.5 ml deionized water to remove O_2 and incubated under waterlogged conditions for 24h at 25°C adding 0.5 ml of KNO_3 1mM as substrate (35µg DNP g_{soil}^{-1}) and 54 µl of DNP 1mM (50µg DNP g_{soil}^{-1}) to inhibit nitrite reduction. Soil mixture was extracted using a 1:2 (w/v) ratio of soil to 4M KCl extract and then centrifuged for 1min at 10.000 x g. Supernatant was pipetted into 96-well microplates and nitrite concentration was determined before and after incubation of soil samples following Griess colorimetric reaction. NRA were calculated as µg of NO_2-N produced per g of dry soil per day (µg NO_2-N g_{soil}^{-1} day^{-1}).

Chapter 4

General discussion

General discussion

The rationale for studying the implications of bioenergy cultivation on ecosystem services has changed from academic interest to necessity to design sustainable bioenergy landscapes. Tilman et al. (2009) and Manning et al. (2014) argue that if society is to realise the potential benefits of bioenergy a key requirement is that science-based principles must be introduced to ensure that the best bioenergy land use is adopted. Assessing the provision of multiple ES into the decision making process could significantly alter our conclusions about desirability of bioenergy landscapes (Howard *et al.*, 2012; Dale *et al.*, 2014; Rizzo *et al.*, 2014). The importance of incorporating ES into analysis of the implications of land use transition to perennial bioenergy crops derives from the realisation of the value of ES to society (Porter *et al.*, 2009; Gasparatos *et al.*, 2011), and the concurrent understanding that many ES are in decline (MEA, 2005a).

Increasingly, our society is seeking sustainable land use scenarios that are valid alternatives to the "food *vs*. fuel" approach (Fisher *et al.*, 2009; Gasparatos *et al.*, 2011; Bateman *et al.*, 2013). Strategic cultivation of bioenergy crops within landscape has already demonstrated to contribute to key ES. An integrated research on the relationships between ES provision and the establishment of bioenergy crops in strategic locations within landscape would be fundamental given the prevailing societal needs to produce large quantities of food and energy in agroecosystems that are supported and regulated by multiple ES (Figure 1.1 and Figure 1.2). This also holds true for this thesis, which was written as part of the HEDGE-BIOMASS project (“*Biomass production from bioenergy crops on buffer strips*”), funded by the Italian Ministry of Agricultural, Food and Forestry Policies. This thesis aimed to provide an overview of the potentials of perennial bioenergy crops to combine a sustainable supply of biomass within agricultural landscape whit multiple provision of ES. Perennial bioenergy crops cultivated as bioenergy buffers (Figure 1.4), are assessed in *Chapter 2* and *Chapter 3* for their productive and environmental performances when they replace the edges of the field of intensive food cropping systems. As described in *Chapter 1*, the main research question of this thesis is therefore: *“To what extent do the perennial bioenergy crops affect the delivery of multiple ecosystem services when cultivated as bioenergy buffers?”*

To synthetize the main findings of this thesis the main hypothesis (see section 1.5) are recalled in the following section, to indicate whether the establishment of bioenergy buffers: enhance the provision of multiple ES (H1), affect the sustainability of biomass supply chain (H2), remove efficiently N from nitrate-enriched groundwater (H3), promote biological N removal from soil (H4) and produce considerable below- and above-ground biomass (H5).

4.1 Testing hypothesis

H1 Perennial bioenergy crops could be grown as bioenergy buffers to produce bioenergy, sustain multiple ES and diversify agricultural landscapes

The systematic revision of the literature in *Chapter 2* denoted an increasing interest on the bioenergy buffers scenario, especially cropland conversion to herbaceous bioenergy buffers. Among the ES reviewed, it emerged that “climate regulation” and “biodiversity regulation” are still the ES to which is dedicated the largest interest, but is interestingly to note how the positive role of bioenergy buffers on "water quality regulation" service is becoming a current research topic. The results presented in *Chapter 2* (section 2.3 and 2.4) confirmed the hypothesis. In particular, the implementation of bioenergy buffers along field margins of former croplands has a net positive impact on multiple ES provision (Figure 2.5). Grasslands conversion to bioenergy buffers showed, instead, mostly net negative impacts on multiple ES provision. Considering both short-term (0-3y) and long-term impacts (3-15y), bioenergy buffers cultivated with miscanthus and switchgrass, compared to woody buffers with willow and poplar, have a higher net effect on the provision of multiple ES. The key findings of *Chapter 2* on the environmental impacts of herbaceous bioenergy buffers are reported in Table 4.1.

H2 Bioenergy buffers may challenge the sustainability of the biomass supply chain

Bioenergy buffers are linear landscape elements whose spatial arrangement on farmlands should be carefully designed. In *Chapter 2* (section 2.5) it is confirmed that some site-specific factors along field margins (e.g. shadowing of natural riparian areas or areas susceptible to compaction or waterlogging) may affect the success of crop establishment and buffers long-term productivity. However, on lowlands with high nutrient runoff loads, it was highlighted how the N and P trapping mechanisms observed in bioenergy buffers might, indeed, positively feedback over short-term on biomass provision. Since no fertilisation or irrigation is foreseen, the main management practice on established bioenergy buffers is biomass harvesting and collection. The systematic revision of literature on biomass supply chain of bioenergy crops conducted in *Chapter 2* (section 2.6) confirmed the hypothesis. A limited working space for the farm machinery operations has been recognized as the main disadvantages of bioenergy buffers compared to large-scale bioenergy plantations. This is due to the linear spatial arrangement of bioenergy buffers. Bioenergy buffer's width (as wide as mandatory buffer strips in many EU countries, namely 5-10 m) and the presence of obstacles and element of discontinuity within inter-field road network may strongly affect, not only buffers design within farmlands, but especially the working capacity of farm machineries (e.g. by increasing the number of passages and manoeuvring operations). These two factors may inevitably increase harvest and collection operation times, fossil fuel consumption and therefore the operating costs. Nevertheless, it is difficult to depict the overall effect of this spatial constraint on the GHG balance of bioenergy buffers. No studies addressing the contribution of perennial bioenergy crops (Table 4.1) in offsetting the GHG emissions induced by biomass harvesting and collection have been carried out to date.

Table 4.1 Key findings on the role played by herbaceous bioenergy buffers on multiple ES provision

Ecosystem services (ES) *	Value of ES to society	Role of herbaceous bioenergy buffers
Climate regulation	Sink for GHG through soil C sequestration and GHG emissions reduction	- Litter and root C inputs to soil promote the increase of the soil organic matter (SOM) content. - The absence of fertilization in buffers, plant-microbial linkages and plant N use efficiency are the key factors for reducing N_2O emissions
Groundwater N quality regulation	Filter for nutrients, pollutants to surface- and ground-waters	The deep rooting patterns of perennial grasses promote the filtering and buffering function of the N pollution from surrounding agricultural lands
Nutrient runoff and soil erosion regulation	Barrier for surface runoff in which sediments and nutrients are trapped by plant-soil system	Perennial grasses help to stabilize soils and decrease erosion and nutrient runoff through their standing vegetation and the leaf litter accumulating on soil surface
Soil health and belowground biodiversity	Soil biota supports biomass provision and the regulation of climate, water and biodiversity services	- Perennial grasses support a diverse and functional soil microbial and microfauna community. - In return, the activity and diversity of soil biota affect positively soil structure, nutrient cycling, buffering of nutrients, and the transfer of plant-derived C inputs into stable SOM pools.
Aboveground biodiversity and pest regulation	Farmlands are composed of multiple types of habitat that support many different biological species involved in pest regulation and pollination service	- Planting perennial herbaceous crops as bioenergy buffers could increase the area of perennial habitats on agricultural landscapes. - Herbaceous bioenergy buffers offer habitats and food resources for diverse communities of beneficial organisms that help control pests and pathogens, and provide pollination services in adjacent crop field.
Biomass provision and energy yield	Production of dedicated biomass for bioenergy production	Managed buffers give the possibility to farmers to produce biomass which could generate additional revenue and might contribute to securing buffer strips existence and consequently maintaining their ecological function

***** in table are listed the regulating, supporting and provisioning services as reviewed in *Chapter 2* (Figure 2.5) using the MEA framework on ES (MEA, 2003), except for "soil C sequestration" and "GHG emission regulation" which are merged into "climate regulation" service

H3 Perennial bioenergy crops, if cultivated adjacent to watercourses, may intercept and remove efficiently N from groundwater as much as buffers strips with spontaneous species

Buffers strips with natural vegetation are widely recognized to be effective at intercepting and reducing nitrogen loads entering water bodies. However, the question remained whether perennial bioenergy crops cultivated along watercourses would retain and remove N from groundwater with the same effectiveness of natural riparian buffers. In *Chapter 3* a bioenergy buffers field trial was set up at the toe of gentle slope of an intensive food cropping systems, in a sandy loam soil with nitrate-enriched shallow groundwater. The effectiveness in removing NO_3 from groundwater of miscanthus and willow buffers is compared with that one of buffers strips with spontaneous species. The results presented in *Chapter 3* (sections 3.1 and 3.2) confirmed the hypothesis. Bioenergy buffers showed to be able to efficiently intercept and remove from groundwater the incoming NO_3-N as much as buffer strips with spontaneous species (Figure 3.2). NO_3-N was removed across two growing seasons by 62% and 80% respectively in 5 and 10 m wide bioenergy buffers. The results confirmed also that NO_3-N removal rate is even higher when nitrate input increased due to N fertilization in the agricultural field (Figure 3.3), showing no symptoms of N saturation in bioenergy buffers three years after crop establishment. Moreover, the results are among the first in literature reporting the biological denitrification route under bioenergy cropping. The application of ecological stoichiometry (as DOC:NO_3 elemental ratio) revealed, indeed, that bioenergy crops promote a C-rich and NO_3-depleted environment along the soil-groundwater continuum (Figure 3.4) indicating in biological denitrification a key factor governing N removal in bioenergy buffers.

H4 Deep-rooted crops such as perennial bioenergy crops lead to significant plant microbial linkages and thus increase biological N removal from soil

The deep rooting pattern of herbaceous crops are well known. However, few data are available on the role of fine root biomass and dissolved organic C (DOC) as indicators for the activation of the soil microbial community. This is relevant because to be adopted under different climatic and pedological conditions, there needs to more evidences on the potential of bioenergy buffers to promote biological removal of N from soil. In *Chapter 3* (section 3.3.4 and 3.3.5) the results on the fine root biomass and its distribution along the soil profile of miscanthus and willow buffers confirmed the hypothesis. Compared to spontaneous species, fine root biomass in miscanthus and willow buffers showed significant relationships with dissolved organic C (DOC), microbial biomass C (MBC), and potential soil nitrate reductase activity (NRA). Bioenergy buffers lead to significant plant–microbial linkages by increasing the easily available C sources for microorganisms (as DOC). First, willow and miscanthus promoted higher rates of biological removal of nitrate (NAR) along the soil profile than spontaneous species, especially at deeper soil layers.

Second, root-derived C inputs activated the soil microbial community leading to significant increases in MBC and microbial N immobilization. As tested for groundwater in Hypothesis 3, *Chapter 3* recognized in soil DOC an important driver for biological denitrification under bioenergy buffers. These results are a very important step forward for our understanding of plant-microbial linkages, as they demonstrate for bioenergy crops a strong coupling of root C inputs, SOM cycling, and microbial N removal. Overall, deep rooted crops such as willow and miscanthus may increase the depth of the active zone of biological N removal. This ultimately indicates the opportunity with bioenergy buffers to intercept and remove subsurface N loads from surrounding agricultural fields at deeper soil layers than buffers with spontaneous species.

H5 Miscanthus and willow buffers produce a significant amount of below- and above-ground biomass if cultivated in nitrate-enriched shallow groundwater

On flat farmlands that present diffuse phenomena of water pollution, bioenergy buffers can be designed along waterways to improve Good Ecological Status of watercourses as requested under EU Water Framework Directive (EC 2000/60) (hypothesis H3). Furthermore, if the mechanisation of harvest operations is allowed, a considerable amount of biomass can be produced from perennial bioenergy crops. Biomass production and plant N uptake have been shown in literature to be important N removal processes in natural riparian buffers. This also holds true for bioenergy buffers. The results presented in *Chapter 3* (section 3.3.5) confirmed the hypothesis. Two years after crop establishment, in a sandy loam soil with a nitrate-enriched shallow groundwater, willow, more than miscanthus, showed promising values for biomass yield (17 Mg DM ha^{-1} y^{-1}), fine root biomass (5.3 Mg ha^{-1} 0-60 cm) and N removal via harvesting (73 kg N ha^{-1}). It was also found a higher contribution of fine roots (41%) to whole root biomass at deeper layers (30-60 cm) in willow (2.2 Mg ha^{-1}) and miscanthus (1.6 Mg ha^{-1}) than in spontaneous species (30% with 0.6 Mg ha^{-1}). This clearly indicates how bioenergy buffers have a high potential to contribute not only to N removal and biomass production but also to C storage and GHG savings in the deep soil layers.

4.2 Emerging principles for design of bioenergy landscape

Farmers can enhance biodiversity and ecosystem services by managing bioenergy crops to promote landscape perenniality and diversity (Werling *et al.*, 2013; Bourke *et al.*, 2014; Dauber & Bolte, 2014). If well-coordinated, these efforts could feedback to increase biodiversity and ES within farmlands and across agricultural landscapes (Power, 2010). The main findings coming from the systematic revision of literature (*Chapter 2)* and the field experimental evidences reported in *Chapter 3* could be used by policymakers to create incentives that promote landscapes that support multiple ES. Two emerging principles for design of bioenergy landscapes can be derived from this thesis.

First, to provide multiple ES at farm-scale *habitat stability and perenniality matter.* Annual food and bioenergy cropping systems impacts agroecosystem resilience and soil health. It has been shown that intensive annual cropping systems disrupt communities of soil microbes and beneficial insects through yearly tillage and use of fertilizer and pesticides, reducing the ability of these organisms to cycle nutrients, regulate GHG emissions and suppress pests (Schröter *et al.*, 2005; Zhang *et al.*, 2007; de Vries *et al.*, 2013; Tsiafouli *et al.*, 2014). Stability and perenniality could be included in agricultural landscapes by designing bioenergy buffers in strategic position within landscape (Manning *et al*., 2014). Bioenergy buffers are more stable than annual food crops because they are planted with vegetation that persists for multiple years, and even they are harvested yearly, they can favour more ES providers than annual crops (*Chapter 2* - section 2.4.5). In addition, *Chapter 3* clearly show how perennial bioenergy crops cultivated along waterways can intercept and remove subsurface N loads from surrounding intensive annual food cropping systems. The perenniality of bioenergy buffers offers also a source of dedicated biomass for energetic purposes over the long term (Golkowska *et al.*, 2016).

Second, to provide multiple ES at broader scales than farmland, *landscape perenniality and diversity matter.* Agricultural landscapes that contain a mix of annual crop and perennial habitats will support more species and greater rates of provision of multiple ES compared to landscapes dominated by few annual crops (Werling *et al*., 2013; Rowe *et al*., 2013, Meehan *et al.,* 2013, Parish *et al*., 2012). Establishing perennial bioenergy crops could increase the area of perennial habitats on landscapes and thus promoting a higher landscape diversity (Manning *et al*., 2014). Such diverse landscapes may support more types of organisms, an thus ensuring a higher functional redundancy of agroecosystems (Tscharntke *et al.*, 2007). A recent study (Haughton *et al.*, 2015) suggest that miscanthus and SRC willows, and the management associated with perennial cropping, would support significant amounts of biodiversity when compared with annual arable crops. Similarly, connecting existing natural areas with buffer strips of perennial habitat may increase the movement of pollinators, predators and wildlife across the landscape by acting as ecological corridors (Marshall & Moonen, 2002).

From this thesis, it emerged that the strategic placement of bioenergy crops has the potential to increase landscape sustainability when the pairing of location and crop type result in minimal disruption of current food production systems and provides multiple ES. This is confirmed e.g. by the field experiment described in Chapter 3, where nitrate have been successfully intercepted and removed mainly because bioenergy buffers were placed perpendicular to the subsurface water flow. *Chapter 2* shows also that bioenergy buffers could be planted along watercourses for reducing runoff into streams and hence increasing water quality. Herbaceous and woody SRC bioenergy crops could also be grow in marginal areas of the farm (not bioenergy buffers in strict sense) to provide habitat for predators of crop pests of other farmland units and to increase soil organic C of these lands. In the long term, creating these diverse landscapes could increase the productivity of food crops by supporting crop pollination and natural pest control in addition to supporting the other services that have value beyond production (Asbjornsen *et al.*, 2012; Meehan *et al.*, 2012; Pywell *et al.*, 2015). Several spatial modelling studies addressing the conversion of food crops (mainly maize and soybean) to bioenergy buffers, indicated indeed, at watershed level, that a careful of bioenergy buffers leads to increase annual energy provisioning (Meehan *et al.*, 2013) and pollination service (Meehan *et al.*, 2013) and simultaneously to decrease annual P and N load to surface water (Powers *et al.*, 2011; Meehan *et al.*, 2013; Ssegane *et al.*, 2015) and annual N_2O emissions (Gopalakrishnan *et al.*, 2012; Ssegane *et al.*, 2015).

This thesis offers only a first evidence base of the advantages of perennial bioenergy crops grown as bioenergy buffers to produce bioenergy and sustain multiple ES provision. However, it highlights a number of key trends relevant to land use transition to bioenergy buffers that optimize ES within farmlands. The results of the impact matrix of *Chapter 2* (Figure 2.5) clearly show that the net multiple ES provision of bioenergy buffers are dependent on the land use being replaced (cropland replacing cropland have a net positive effect greater than grassland conversion). Nevertheless, a general lack of understanding was identified for almost all the ES relative to the impacts of bioenergy buffers during the establishment phase (0-3 years). How long it takes after crop establishment for making bioenergy buffers an efficient C-stocking, N-removing and P-trapping land use practice are interesting research questions to be tested in future.

4.3 Challenges to overcome

Implementing a diverse landscape is challenging. Multiple farmers will need to work together to shape the landscape (Dale *et al.*, 2016). At the same time, these farmers will need to balance crop productivity, economics, market access, availability and cost of equipment when deciding where and which bioenergy crops to plant (Zegada-Lizarazu *et al.*, 2010; Christen & Dalgaard, 2013). Tradeoffs between ES and loss of income after the conversion to bioenergy buffers will challenge the ability of farmers to grow mixed food-bioenergy cropping systems that are productive and support ecosystem services. The benefits of a given bioenergy cropping system for ES will be context dependent. Holland *et al*. (2015) identified the key issues for bioenergy cropping systems that need to be addressed relating to scale of deployment, societal value and trade-offs between ES (Figure 4.1). *Chapter 2* suggest that these issues can also apply to the the case of bioenergy buffers, except for agrochemical inputs (no use of fertilizer and pesticides use is foreseen along buffers). By expressing the tradeoffs between income provisioning and other ES as benefit-cost ratios, Meehan et al. (2013) found that the benefit-cost ratios for the different ES of bioenergy buffers are correlated within landscape. This suggest that there are areas where increases in multiple ES might come at lower-than-average opportunity costs (Meehan et al., 2013). Similarly, Parish *et al.* (2012) showed that a sustainable design of bioenergy landscapes could be obtained only if spatial scenarios aiming at achieving different sustainability goals are included into decision-making process – e.g. scenarios that balance the increase of one or more ES with maximizing profit. These considerations suggest that future research will have to monetize ES using estimates e.g. for the social costs of water pollution mitigated (Bateman *et al.*, 2013; Meehan *et al.*, 2013; Orwin *et al.*, 2015). This is relevant in order to avoid that the value associated to land use transition to bioenergy buffers does not become far lower than the opportunity cost.

In addition to the issues relating to ES priorities and tradeoffs, there are other local factor that can affect the implementation of bioenergy buffers (Christen & Dalgaard, 2013): yield target, harvesting technology, local bioenergy markets, farmer personal preferences. Perennial crops may require farmers to invest in new harvesting equipment and produce delayed returns. For example, woody crops like hybrid poplars require different harvesting equipment than herbaceous crops and are harvested every 2 to 3 years, which can cause high establishment costs and receive incomes at longer intervals. In *Chapter 2* (section 2.6.1), the use of a single-pass system and a self-propelled chopper, respectively for herbaceous and woody crops, were identified as suitable options for managing biomass along bioenergy buffers. Moreover, a general lack of experience may cause some farmers to choose familiar crops for buffers (clover, ryegrass, mix of grass and wildflower species or simply let buffers grow with spontaneous species as in *Chapter 3* and Figure 1.6e) rather than perennials bioenergy crops that promote ES (Figure 1.5a-e).

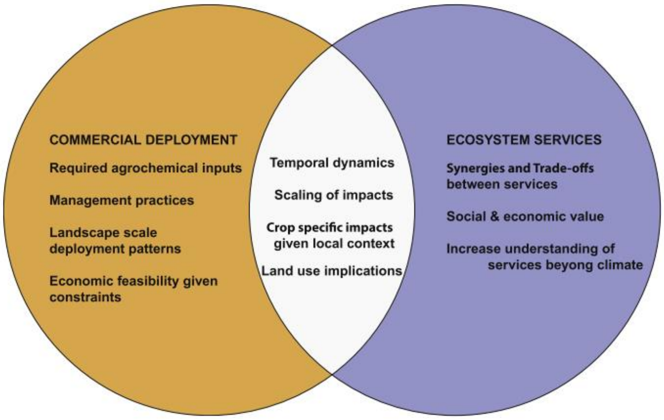

Figure 4.1 Research needs that have still to be addressed to fully understand the implications of perennial bioenergy crops on ecosystem services provision (source: Holland *et al*., 2015)

As shown in *Chapter 2* (section 2.6), another issue for bioenergy buffers may come from the limited working space for the farm machinery operations. This can be considered as one of the main logistic constraints for bioenergy buffers compared to large-scale bioenergy plantations. Moreover, the spatial fragmentation of biomass supply areas due to the presence of elements of discontinuity among and within farmlands may increase environmental costs related to biomass collection and transport operations. This ultimately can threaten the overall sustainability of biomass supply chain.

Finally, there is a need to have a more detailed analysis and possibly specification and enhancement of regulatory measures and subsidy practices for the cultivation of bioenergy buffers. This because the existing policies have still too vague references about which specific crops can be cultivated for biomass production along buffers (e.g. agri-environmental measures of Rural Development Programs). An improvement of the existing policies would be helpful in view of the barriers to implementation of bioenergy landscape design identified by Dale *et al.* (2016) such as the need to consider diverse land-management objectives from a wide array of stakeholders, up-front planning requirements, and the complexity and level of effort needed for successful stakeholder involvement.

Technical recommendations on biomass logistics management and the most suitable spatial planning instruments are research needs that have to be addressed in the future to direct bioenergy crop cultivation along buffer strips and incorporating bioenergy into sustainable landscape designs.

4.4 General conclusions

The last decade has seen the parallel emergence of policy designed to promote bioenergy as a route towards sustainable energy production, and an increasing understanding of the importance of ES for human wellbeing. A key part of the answer to the common question "How can agriculture produce bioenergy and do so in a sustainable way?" may be an increased focus on the full set of ES that a well-designed bioenergy landscape delivers. By testing hypothesis 1 through 5, this thesis provides new insights in the role of perennial bioenergy crops in the provision of multiple ES. Results of *Chapter 2* suggest that perennial bioenergy crops cultivated as bioenergy buffers on former croplands increase the provision of the full set of regulating and supporting ES described by the Millenium Ecosystems Assessment (MEA, 2003). The incorporation of such perennial landscape elements in strategic locations into agricultural landscapes dominated by annual crops can reduce GHG emissions, sequester C in soil, support soil health and offer habitats and food resources for beneficial organisms and act as filter and barrier for sediments and nutrients. Regarding the latter service, "water quality regulation", experimental evidences are provided in *Chapter 3* on the role of miscanthus and willow buffers in mitigating groundwater N pollution (hypothesis 3). The deep rooting system of these bioenergy buffers differentiates it from that one of spontaneous species in terms of biological N removal from soil (hypothesis 4). Bioenergy buffers can represent also a valuable source of dedicated biomass for energetic purposes as shown in *Chapter 3* (hypothesis 5), since no limitations in water and nutrients are found along bioenergy buffers. Suitable conditions for mobilizing biomass from buffers may come from flat agricultural landscapes. On these lands mechanization is possible especially along watercourses where buffers are mandatory under EU Water Framework Directive (EC 2000/60). Another option identified in *Chapter 2* is the use of bioenergy buffers as Ecological Focus Area (EFA). As regulated within the "greening measures" of the CAP 2014-2020, maintaining an EFA of at least 5% of the arable area of the farm e.g. with perennial landscape elements like bioenergy buffers may encourage farmers to create multifunctional bioenergy landscapes. However, the concerns emerged about the logistic constraints and the farmer ability to mobilize biomass from buffers (hypothesis 2) prevents from fully understanding of the environmental implications of an increased cultivation of bioenergy buffers. These concerns may hinder ability to inform the debate on the best subsidy practice and employ the optimum landscape design to enhance ES in response to policy that will drive the expansion of bioenergy production. In conclusion, bioenergy buffers provide a chance to shape agricultural landscapes to solve the conflict between the aim of using agricultural land to produce food and energy and the need to promote ecological sustainable intensification by maximizing multiple ES provision.

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List of publications

Wahsha M, **Ferrarini A,** Vannuzzo S, Bini C, Fontana S (2012) Evaluation of Biological Soil Quality (QBS-ar) index from Spolic Technosols in an abandoned mine area in NE Italy: 50 years of biological rehabilitation opportunity. *International Journal of Environmental Quality*, **9**, 1-9.

Ferrarini A, Fornasier F, Bini C (2014) Development of a Soil Health Index based on the ecological soil functions for organic carbon stabilization with application to alluvial soils of northeastern Italy. In: *Sustainable agroecosystems in climate change mitigation (ed.* Oelbermann M), pp. 163-184. Wageningen Academic publishers, Netherlands.

Ferrarini A, Fornasier F, Serra P, Ferrari F, Trevisan M, Amaducci S (2016) Impacts of willow and miscanthus bioenergy buffers on biogeochemical N removal processes along the soil-groundwater continuum. *GCB Bioenergy.* doi: 10.1111/gcbb.12340.

Amaducci S, Facciotto G, Bergante S, Perego A, Serra P, **Ferrarini A**, Chimento C (2016) Biomass production and energy balance of herbaceous and woody crops on marginal soils in the Po valley. *GCB Bioenergy.* doi: 10.1111/gcbb.12341.

PhD scientific activities

Review of literature

Multiple ecosystem services provision and biomass supply chain of perennial bioenergy crops (December 2013 – March 2014)

Field/lab activities

- Installation of three bioenergy buffers field trials in northern Italy (March 2013- June 2013)
- Soil, litter and groundwater sampling on the different field trials during the first three growing seasons (April 2013 – December 2015)
- Development and refinement of the lab protocols for microbial and enzymatic analyses at Department of Sustainable Crop Production of Università Cattolica del Sacro Cuore, Piacenza, Italy (March 2013 – May 2014)
- Analysis of the C:N:P stoichiometry of litter and soil samples for the following pools: total, dissolved, enzymes and microbial biomass (October 2013 – July 2015)
- Soil GHG monitoring using SASSFLUX system (January 2014 – August 2015)

Writing activities (papers not included in the thesis, submitted and to be submitted)

- Linking enzymes stoichiometry to resource and microbial biomass stoichiometry along the litter-soil continuum: new insights from leaf litter decomposition of bioenergy crops (*research paper to be submitted to Scientific Reports*)
- Soil microbial functional diversity and soil aggregate dynamics after the establishment of bioenergy buffers: implications for soil C sequestration (*research paper to be submitted to GCB Bioenergy*)
- Contribution of leaf litter decay to GHG emissions (CO_2, N_2O) under four different bioenergy crops (*research paper - under preparation*)
- Rhizodeposition and priming effect of a candidate bioenergy crops: switchgrass (*Panicum virgatum* L.) (*research paper – under preparation*)
- Soil and ecosystem services: concept and case studies (*book chapter – submitted*)

Writing of project proposal

LIFE13 ENV/IT/001192 project: "*Implementation of Bioenergy Buffer Strip networks and Innovative Sustainable Cropping Systems in Agroecosystem Planning*" (May 2013 - July 2013)

Laboratory training and working visits (PhD period abroad)

University of Goettingen, Department of Agricultural Soil Science (supervisor: Yakov Kuzyakov), Goettingen, Germany (October 2014 – April 2015)

Topic: Rhizodeposition and priming effect of a candidate bioenergy crops: switchgrass (*Panicum virgatum* L.)

Objective: Two incubation experiments were carried out to study the contribution of switchgrass rhizodeposition to soil C, N cycling and its effects on priming effect. A combination of three labelling approach has been used: ^{14}C labelling, ^{13}C shifts after C3-C4 vegetation change, ^{15}N labelling

Courses/training

- *SOMDY model training* (2 credits) Dipartimento di Scienze del Suolo, della Pianta, dell'Ambiente e delle Produzioni Animali, Universita` di Napoli "Federico II", Naples, Italy (February 2013)
- *Use of Isotope Methods in Soil Research* (3 credits), Centre for Stable Isotope Research and Analysis, Goettingen, Germany (February 2015)

PhD exams of the doctoral school (November 2012- February 2013)

- Sustainable animal production
- Sustainable crop production
- Agricultural and food policies of the EU
- Scientific communication
- English course
- Food technology and sustainability
- Nozioni giuridiche fondamentali concernenti la disciplina del sistema agroalimentare
- Dal diritto dell'agricoltura al diritto alimentare
- Diritto europeo multi-livello e disciplina agroalimentare
- Human nutrition
- Research ethics and epistemology
- Research project and research programs
- Statistics and data management
- Diritto internazionale ed europeo del commercio dei prodotti agroalimentari
- Basic management of knowledge

Attendance to international/national congress

- **Ferrarini A,** Chiazzese M, Fornasier F, Chimento C, Trevisan M, Amaducci S. Short-term effects of compost and digestate application on soil health and mechanisms of soil C stabilization. XXXI congress of Italian Society of Agricultural Chemistry (SICA), September 16-17, 2013, Naples, Italy. (*oral presentation*)
- **Ferrarini A,** Serra P, Fornasier F, Ferrari F, Trevisan M, Amaducci, S. Bioenergy buffers effectiveness in removing nitrogen in shallow groundwater. International Conference on "Perennial biomass crops for a resource-constrained world", September 7-9, 2015, Hohenheim, Germany. (*oral presentation*)

Poster presentation at international symposia, workshop and conferences

- **Ferrarini A,** Serra P, Trevisan M, Puglisi E, Amaducci S. Managing Bioenergy Production on Arable Field Margins for Multiple Ecosystem Services: Challenges and Opportunities. European Geosciences Union (EGU), April 7-12, 2013, Wien, Austria.
- Pertile G, Vasileiadis S, **Ferrarini A**, Fornasier F, Suciu N, Lamastra L, Puglisi E, Karpouzas D, Trevisan M. Soil microbial community responses to common pesticides of conventional, IPM and organic farming. 12th IUPAC International Congress of Pesticide Chemistry, September 2-4, 2013, York, UK.
- **Ferrarini A,** Serra P, Trevisan M, Amaducci S. Linking bioenergy and ecological services along field margins: the HEDGE-BIOMASS project. 22nd European Biomass Conference & Exhibition, June 23-26, 2014, Hamburg, Germany.
- Pappolla A, **Ferrarini A**, Pertile G, Puglisi E, Suciu N, Lamastra L, Vasileiadis S, Fornasier F, Karpouzas D, Trevisan M. Assessing the soil microbial toxicity of iprodione using advanced biochemical and molecular tools: Put the blame on the metabolite 3,5 dichloroaniline. 13th IUPAC International Congress of Pesticide Chemistry, August 10-14, 2014 San Francisco, California, USA.
- Suciu N, Pappolla A, **Ferrarini A**, Puglisi E, Vasileiadis S, Fornasier F, Sulowicz S, Karpouzas D, Trevisan M. Are botanical pesticides not toxic to non-target organisms: Studying the effects of azadirachtin on soil microbes using advanced culture-independent approaches. 13th IUPAC International Congress of Pesticide Chemistry, August 10-14, 2014 San Francisco, California, USA.
- Pertile G, Baguelin C, **Ferrarini A**, Fornasier F, Karas P, Papadopoulou E, Nikolaki E, Storck V, Ferrari F, Trevisan M, Tsiamis G, Sibourg O, Malandain C, Martin-Laurent F, Karpouzas D.G. Assessment of the impact of isoproturon, chlorpyrifos, and tebuconazole on soil microbial functions using a lab-to-field tiered approach. SPC XV: Symposium in Pesticide Chemistry, September 9-11, 2015, Piacenza, Italy.

- Suciu N, **Ferrarini A**, Puglisi E, Vasileiadis S, Oplos C, Fornasier F, Sułowicz S, Lucini L, Karpouzas DG, Trevisan M. Are strobilurin fungicides not toxic to non-target organisms: studying the effects of trifloxystrobin and its main soil metabolite on soil microbes using advanced culture-independent approaches. SPC XV: Symposium in Pesticide Chemistry, September 9-11, 2015, Piacenza, Italy.
- **Ferrarini A**, Amaducci S, Kuzyakov Y. Rhizodeposition and priming effect in switchgrass (*Panicum virgatum* L.) as revealed by ^{14}C pulse labelling and 3-source-partitioning approach. 5th International Symposium on Soil Organic Matter, September 20-24, 2015, Goettingen, Germany.
- **Ferrarini A**, Fornasier F, Trevisan M, Amaducci S**.** Linking enzymes stoichiometry to resource and microbial biomass stoichiometry along the litter-soil continuum: a case study from leaf litter decomposition of bioenergy crops**.** 5th International Symposium on Soil Organic Matter, September 20-24, 2015, Goettingen, Germany.

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