

## Balancing legume-cereal proportions in cover crop mixtures to minimize N<sub>2</sub>O emissions

Guillermo Guardia<sup>a,b,c,\*</sup>, Diego Abalos<sup>c</sup>, Emanuele Ribatti<sup>d</sup>, Federico Ardeni<sup>d</sup>, Federico Capra<sup>d</sup>, Giacomo Mortella<sup>d</sup>, Vincenzo Tabaglio<sup>d</sup>, Miguel Ángel Ibáñez<sup>e</sup>, Ji Chen<sup>f</sup>, Andrea Fiorini<sup>d</sup>

<sup>a</sup> Departamento de Química y Tecnología de Alimentos, ETSI Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Madrid, Spain

<sup>b</sup> Centro de Estudios e Investigación para la Gestión de Riesgos Agrarios y Medioambientales (CEIGRAM), Madrid, Spain

<sup>c</sup> Department of Agroecology, Pioneer Center LandCRAFT, Aarhus University, Tjele, Denmark

<sup>d</sup> Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Piacenza, Italy

<sup>e</sup> Departamento de Economía Agraria, ETSI Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Madrid, Spain

<sup>f</sup> State Key Laboratory of Loess and Quaternary Geology, Institute of Earth Environment, Chinese Academy of Sciences, Xi'an, China

### ARTICLE INFO

Handling Editor: D. Said-Pullicino

#### Keywords:

Green manure  
Greenhouse gas emissions  
Nitrogen cycling  
Pulses  
Intercropping  
Denitrification

### ABSTRACT

Legume-cereal cover crop mixtures offer a promising approach to reduce nitrate leaching and enhancing soil fertility. However, the impacts of these mixtures on N<sub>2</sub>O emissions during both the cover cropping and post-incorporation phases, as well as the relative contribution of roots and shoots to N<sub>2</sub>O emission, remain uncertain. To address these knowledge gaps, we conducted a two-phase greenhouse experiment. In the first phase, cover crops were grown encompassing six treatments: control (no cover crop), pure vetch (*Vicia villosa* Roth), pure rye (*Secale cereale* L.), and mixtures with 33 %, 50 % and 66 % of the pure rye sowing rate paired with 66 %, 50 % and 33 % of the pure vetch sowing rate, respectively. In the second phase, focusing on the post-incorporation effects, the same treatments were arranged in mesocosms with both roots and shoots, and in mesocosms with roots only. During the first phase, the proportion of fine/very fine roots and root length density were negatively correlated with mineral N content and N<sub>2</sub>O emissions. Mixing rye with vetch increased total dry biomass and N yield for all mixtures compared to rye alone. In mixtures, the proportion of fine roots, root length density, and the root C:N ratio decreased compared to rye. Most of the N<sub>2</sub>O emissions occurred after cover crop incorporation, with roots contributing more (average 57 %) than shoots (average 31 %). Total N<sub>2</sub>O emissions increased with increasing proportion of vetch, but the mixture with 33 % vetch and 66 % rye maintained N<sub>2</sub>O emissions as low as rye monoculture. Our study indicates that adjusting the seed proportion in legume-cereal mixtures serves as an effective tool to balance the benefits of pure legume (increased total biomass, and C and N yields) and pure cereal (decreased N<sub>2</sub>O emissions and soil mineral N pool) cover cropping.

### 1. Introduction

Cover cropping is a cornerstone of both current and future agricultural sustainability and regenerative practices, supporting diversified rotations (Quintarelli et al., 2022; Khangura et al., 2023; Yang et al., 2024). This practice offers valuable ecosystem services, by reducing nitrogen (N) leaching and phosphorus loading to water bodies (Liu et al., 2021; Thapa et al., 2018) and increasing soil organic carbon (Hu et al., 2023). Additionally, cover crops positively impact erosion control,

weeds suppression, microbial diversity, soil quality, and climate change adaptation while mitigating net global warming potential (Ardeni et al., 2023; García-González et al., 2018; Guardia et al., 2019; Kaye and Quemada, 2017; Van Eerd et al., 2023). However, a major drawback of cover cropping is a potential stimulation of direct soil nitrous oxide (N<sub>2</sub>O) emissions (Basche et al., 2014; Rivière et al., 2022), a potent greenhouse gas contributing to global warming and stratospheric ozone depletion (Ussiri and Lal, 2013).

Understanding the effect of cover cropping on N<sub>2</sub>O emissions is

\* Corresponding author at: Departamento de Química y Tecnología de Alimentos, ETSI Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Madrid, Spain.

E-mail address: [guillermo.guardia@upm.es](mailto:guillermo.guardia@upm.es) (G. Guardia).

<https://doi.org/10.1016/j.geoderma.2025.117195>

Received 15 October 2024; Received in revised form 23 January 2025; Accepted 27 January 2025

Available online 8 February 2025

0016-7061/© 2025 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

complex and variable (Basche et al., 2014; Muhammad et al., 2019), as it depends on environmental (climatic and soil) factors, cover crop duration, cropping phase within the annual rotation (i.e., cover crop growth or post-termination), and especially cover crop species. Legume and non-legume cover crops have distinct impacts, with the latter generally linked to lower N<sub>2</sub>O emissions due to their higher mineral N-acquisition capacity and potential N immobilization from C-rich residues (Abalos et al., 2022a; Fiorini et al., 2020). Yet, the addition of labile C from non-legume residues or rhizodeposition may stimulate denitrification and lead to N<sub>2</sub>O peaks, particularly after soil rewetting (García-Gutiérrez et al., 2024).

Mixtures of legume and non-legume cover crops may strike a balance between the benefits of legumes (e.g., reduced synthetic N fertilizer needs, improved subsequent crop yields) and non-legume species (e.g., lower nitrate (NO<sub>3</sub><sup>-</sup>) leaching) (Meyer et al., 2022; Muhammad et al., 2019; Poffenbarger et al., 2015; White et al., 2017). Although mixtures have shown positive outcomes in biomass yields, soil quality and microbial diversity compared to pure stands (Drost et al., 2020; Elhakeem et al., 2021; Restovich et al., 2022), information on their effects on N<sub>2</sub>O emissions remains limited (Muhammad et al., 2019; Van Eerd et al., 2023). Identifying optimal legume-cereal proportions is crucial, with grassland studies suggesting specific ratios for maximum biomass production (Kirwan et al., 2007), especially for legume-non-legume mixtures (Suter et al., 2015). For example, 66 %–33 % legume-cereal proportion can lead to the best agronomic performance (yield and protein) in mixtures (Osman and Nersoyan (1986). Mixtures with a 75 %–25 % cereal-legume proportion can improve biological N fixation of pure legumes while avoiding potential increases in soil mineral N availability susceptible to be lost from the agroecosystem (Rodríguez et al., 2020). Nevertheless, the potential to fine-tune the sowing rates of cover crop legume-cereal mixtures to regulate N cycling and N<sub>2</sub>O emissions has not been explored yet.

To understand the effect of cover cropping on N<sub>2</sub>O fluxes, it is essential to analyze both the growing phase (often neutral or positive for N<sub>2</sub>O mitigation compared to bare soil) and the post-termination phase (associated to N<sub>2</sub>O increases) (Basche et al., 2014; Abalos et al., 2022b). Particularly, understanding the contributions of cover crop roots and shoots to the emission of N<sub>2</sub>O after termination is crucial, as it can provide valuable insights to determine the best cover crop residue management practices (i.e., incorporation, removal, or leaving on the soil surface) for N<sub>2</sub>O mitigation. For instance, in the case of legume aboveground residues, its removal was found to decrease N<sub>2</sub>O emissions by 60 % in the subsequent manure-amended maize crop (Saha et al., 2021). Shoot residues are expected to decay faster than roots due to their lower C:N ratio and lignin content, while root residues result in lower rates of mineralization, potentially decreasing the availability of mineral N substrates involved in N<sub>2</sub>O emissions (Kim et al., 2020; Vanlauwe et al., 1996; Whittaker et al., 2023).

Plant traits have been used to predict plant-soil interactions in natural ecosystems (Bardgett et al., 2014; Faucon et al., 2017), and now they are increasingly used in agricultural systems to understand N dynamics (Bardgett, 2018; Abalos et al., 2019). For example, traits like high specific leaf area (SLA) and low leaf dry matter content (LDMC), typical of fast-growing species, have been associated with decreased N losses, including N<sub>2</sub>O emissions and NO<sub>3</sub><sup>-</sup> leaching (Abalos et al., 2018). Although these concepts have started to be applied in the selection of cover crop species to reduce N losses (Fernandez-Pulido et al., 2023), their predictive capacity for cover crop mixtures remains unexplored. Furthermore, cover crop traits can predict legacy effects after soil incorporation. Fast-growing species produce high quality, labile residues that may trigger N<sub>2</sub>O emissions, while slow-growing species produce litter rich in structural compounds that are more resistant to breakdown (Reich, 2014), decreasing the soil mineral N pool available for N<sub>2</sub>O production. Root exudation of C is another critical trait that can trigger denitrification, promoting N<sub>2</sub>O and dinitrogen (N<sub>2</sub>) emissions from native soil N (Song et al., 2024). A better understanding of the

relationships between cover crop traits in legume-cereal mixtures and N<sub>2</sub>O emissions, both during the cover crop phase and after incorporation, can provide useful knowledge into N transformations in agroecosystems.

A two-phase mesocosm experiment was set up under greenhouse conditions to investigate the effect of different legume-cereal proportions on N<sub>2</sub>O emissions during the growing and post-termination phases. The second experimental phase (post-termination) involved parallel sets of mesocosms with and without shoot residues to quantify the specific contribution of aboveground and belowground cover crop fractions to N<sub>2</sub>O fluxes during cover crop decomposition. We hypothesized that i) increasing cereal proportion (to the detriment of that of legume) would be negatively related to N<sub>2</sub>O emissions during cover crop growth (due to the enhanced N acquisition) and post-incorporation (due to N immobilization); ii) shoots would contribute to a higher extent than roots to post-incorporation N<sub>2</sub>O emissions due to higher chemical quality; and iii) an even proportion of cereal and legumes would be effective to maintain low N<sub>2</sub>O emissions while keeping mineral N contents at levels than can support the growth of a follow-on crop.

## 2. Materials and methods

### 2.1. Experimental design

A two-phase mesocosm experiment was conducted using two cover crop species, sown as monocultures and in mixtures. The first phase focused on the cover crop growth, and the second on the post-incorporation period. The selected species were hairy vetch (*Vicia villosa* Roth) and rye (*Secale cereale* L.), and treatments were arranged as follows: 100 % vetch, 100 % rye, 50 % vetch + 50 % rye, 33 % vetch + 66 % rye, and 66 % vetch + 33 % rye. A bare soil treatment without cover crops was included as a control. The experimental design was a complete randomized block design with four blocks and four replicates per treatment. Two identical sets of mesocosms were prepared (set A and set B), resulting in 48 mesocosms in total (6 treatments × 4 replicates × 2 sets). The experimental timeline included: i) sowing of both sets, ii) 2-months plant growth (cover cropping phase), iii) aboveground and belowground biomass harvest and plant trait analyses (only on set A; see details below), iv) cover crop biomass incorporation into the soil (only on set B, see details below), and v) 3-month post-incorporation phase.

Soil (0–30 cm layer) was collected from a bare field near Cremona, Po Valley, Northern Italy (45°12'24.1" N, 9°53'23.3" E; 52 m above sea level). The soil, classified as fine loamy, mixed, semiactive, mesic, Oxyaquic Haplustalfs, was conventionally tilled each year and cultivated with field crops. After collection, air-dried soil was sieved to 1 cm and homogenized. Initial physico-chemical characteristics were: clay, 15 %; sand, 61 %; silt, 24 % (sandy loam texture); pH-KCl, 5.06; organic matter, 14.3 g kg<sup>-1</sup>; total N, 0.10 %; available P, 98 mg kg<sup>-1</sup>; exchangeable K, 464 mg kg<sup>-1</sup> electric conductivity, 137.6 μs cm<sup>-1</sup>. The soil collection site is characterized by a temperate climate (Cfa as Köppen classification), with an average annual temperature of 13.2 °C and annual rainfall of 839 mm, based on a 30-year average.

Cylindrical PVC mesocosms (8 L; 40 cm height × 16 cm diameter) were used. A PVC base with drilled holes was set 5 cm above the bottom of each mesocosm to allow water drainage. One 3-cm layer of clay pebbles and one layer of nonwoven fabric layer were added to the bottom of the mesocosms to prevent soil loss. Mesocosms were filled with 6 kg of air-dried, homogenized soil, equivalent to a 30 cm soil column. The bulk density targeted during soil packing was 1.00 g cm<sup>-3</sup>. Before sowing, the soil was gradually rehydrated by capillary rise up to 70 % water-holding capacity (WHC) and maintained at this level throughout the whole experiment by weighing mesocosms three times a week.

Seeding rates (110 kg ha<sup>-1</sup> for rye, 80 kg ha<sup>-1</sup> for vetch) were based on local practices. The seeding rate for the mixtures followed a substitutive design. Mesocosms were placed in an outside growth tunnel

during both experimental phases. Weeds were removed by hand to maintain the original species composition inside each mesocosm. At the end of the cover cropping phase (63 days after sowing), mesocosms of set A were used for aboveground and belowground biomass sampling and plant trait analyses (destructive sampling), while soil and plants of set B – which were identical twins of set A mesocosms – were used for the post-incorporation phase.

At the start of the post-incorporation phase, the aboveground cover crop biomass was harvested, trimmed to 1 cm-length pieces and homogenized. Meanwhile, the soil was removed from the mesocosms and homogenized. Then, two new sets of mesocosms were prepared (only roots, R, and roots and shoots, R + S) to investigate the relative contribution of roots and shoots to  $N_2O$  emissions. Sets R and R + S consisted of cylindrical PVC mesocosms of 4 L (20 cm height × 16 cm diameter). The mesocosms of set R (n = 24) were filled with only half (by weight) of the previously homogenized soil. Concurrently, mesocosms of set R + S (n = 24) were filled with the other half of the homogenized soil together with half (by weight) of the collected aboveground biomass. Once prepared, sets R and R + S were placed in a growth chamber in dark conditions at 23° C for 80 days, until the end of the experiment.

## 2.2. Nitrous oxide sampling and quantification

Nitrous oxide fluxes were measured using the close chamber method (Smith et al., 1995) throughout the experiment. At the beginning of the measurement period, 30 cm height cylindrical PVC static chambers (16 cm diameter) were used. During cover crop growth, higher chambers (50 cm height) were used to contain the entire plant biomass, and during the post-incorporation phase the smaller chambers were used again. An internal battery-operated fan was installed in each chamber to maintain air mixing during measurements. During plant growth, starting from 11 days after sowing until plant biomass harvest and incorporation,  $N_2O$  emissions were measured twice a week on set A. During the post-incorporation phase, in both sets R and R + S,  $N_2O$  emissions were measured daily during the first two weeks, twice a week during the following three weeks, and once a week thereafter. In total, 35  $N_2O$  samplings were conducted (15 during cover cropping and 20 during post-incorporation). Air temperature and pressure were measured with a digital thermometer at each  $N_2O$  measurement. Nitrous oxide emissions were measured with a 1412i photo-acoustic infrared gas analyser (LumaSense Technologies A/S) at three different times after closing the chamber: right after closing ( $t_0$ ), 8 min after closing ( $t_8$ ) and 16 min after closing ( $t_{16}$ ). A soda-lime filter was also connected to the gas analyzer to reduce  $CO_2$  interference during measurements. The  $N_2O$  fluxes from chamber concentration versus time data, were estimated using the extended Hutchinson and Mosier flux calculation method, implemented with the HMR package (Pedersen et al., 2010) in the R statistical software. When the HMR statistical criteria indicated a good fit, the non-linear model was utilized; otherwise, the linear model was applied. Cumulative  $N_2O$ -N emissions in each phase were calculated by linear interpolation of daily fluxes. In the post-incorporation period, cumulative  $N_2O$  emissions from shoots were calculated by subtracting those of R mesocosms (soil + roots) from the corresponding cumulative  $N_2O$  fluxes in R + S mesocosms (soil + roots + shoots).

## 2.3. Soil sampling and analysis

Soil samples were collected five times during the experiment. During the cover cropping phase, two soil samplings were performed on each mesocosm of set A at 37 and 63 days after sowing (DAS). In the post-incorporation phase, three soil samplings were performed on each mesocosm of sets R and R + S at 77, 94 and 143 DAS. Samples were collected with a 1 cm diameter soil auger and the remaining holes in the pot were filled with inert sand to reduce alterations in gaseous diffusion. Soil  $NO_3^-$  and ammonium ( $NH_4^+$ ) concentrations were analyzed on 5 g of homogeneously mixed soil extracted with 20 mL of potassium sulphate

( $K_2SO_4$ , 0.5 M) and pipetted into 96-well quartz microplates. Nitrate-N and  $NH_4^+$ -N were then assessed with dual-wavelength UV spectroscopy (275, 220 nm) on acidified (HCl 1 M) samples. The total water-soluble N and dissolved organic C (DOC) were analyzed for each sampling at 63, 77 and 143 DAS after an extraction with  $K_2SO_4$  0.05 M, followed by an immediate filtration (0.45  $\mu$ m cellulose acetate filter). Both DOC and total water-soluble N concentrations were measured using a TOC-TN analyzer (TOC-VCSN Shimadzu). Dissolved organic N (DON) was calculated by subtracting the  $NH_4^+$  and  $NO_3^-$  concentrations from the total water-soluble N.

## 2.4. Aboveground and belowground biomass analyses

Three representative plants of each cover crop species within each mesocosm were subsampled and analyzed for aboveground traits following the procedure described by Cornelissen et al. (2003). Plant height and phenological stage were measured, and six representative fully expanded leaves of each cover crop species were scanned. The images were acquired at 600dpi with the scanner Expression 12,000 XL. The leaf area, length and width, were measured by using the ImageJ software (Schneider et al., 2012). The specific leaf area (SLA,  $cm^2 g^{-1}$ ) was calculated as the ratio between the leaf area and leaf dry weight, while the leaf dry matter content (LDMC,  $mg g^{-1}$ ) was calculated as the ratio of dry to fresh weight. The total root biomass in each mesocosm of set A was collected by wet-sieving and washed with deionized water. Shoot and root biomass were oven-dried at 60 °C until constant weight. The shoot and root dry weights were recorded and the relative aboveground biomass weight of each species growing in each mesocosm was calculated. Cover crop biomass overyielding and cover crop N uptake overyielding (overN) for vetch and rye mixtures were calculated by comparing the actual yields in the mixtures (total biomass DW and N yield, respectively), and the expected yield based on the average yields of the pure stands (rye and vetch monocultures) multiplied by the relative proportion of each cover crop species in the mixture (Wendling et al., 2017). Both overyielding and overN were expressed as percentages of the expected yield.

A representative root subsample per mesocosm was scanned and the images were acquired at 600 dpi with the scanner Epson Expression 12,000 XL, equipped with a double light source to prevent roots overlapping (Fiorini et al., 2018). Root Length Density (RLD,  $cm cm^{-3}$ ) and root diameter were measured by using the software WinRHIZO Pro 2021. Subsequently, the root dry weight (RDW,  $mg cm^{-3}$ ) was gravimetrically determined by oven-drying the roots at 60 °C until constant weight. The diameter class length ( $mm cm^{-3}$ ) was calculated for very fine (0.0–0.075 mm), fine (0.075–0.2 mm), medium (0.2–0.5 mm), coarse (0.5–1 mm), and very coarse (> 1 mm) diameters for the whole soil profile, as adapted from Reinhardt and Miller (1990). A subsample of 1 g of each shoots and roots sample was then weighed and analyzed by the Dumas combustion method with an elemental analyzer (VarioMax C; N, Elementar, Germany) to obtain C and N concentrations.

## 2.5. Statistical analyses

Analyses of variance (ANOVAs) were performed for  $N_2O$ , soil and plant variables, previously checking the normal distribution (Shapiro-Wilk) and homogeneity of variances (Levene) and log-transforming the data when necessary. One (i.e., cover crop plant treatment) and two-way (i.e., cover crop plant treatment and presence/absence of shoots) ANOVAs were conducted during the cover cropping and post incorporation phases, respectively. The ‘block’ factor was included in the linear general model according to the experimental design. Means were separated by the LSD test at  $P < 0.05$ . Linear correlation analyses (Pearson) were also performed for each period. The community weighted mean value for each mesocosm was calculated for all the shoot traits (ANOVA and correlation analyses). All these analyses were conducted with Statgraphics 19 – X64.

Three structural equation models (SEM) were performed with R (*lavaan* package) for the cover cropping phase and the post incorporation phase (separately for R and R + S mesocosms). Only the data for cover crop mesocosms was used in the SEM analyses (i.e., control mesocosms were excluded) since the variances-covariances matrix is calculated excluding by default the missing data. Some latent variables were included in the models to explain the relationships between observed variables (e.g., “Root traits” in the SEM model of the cover cropping phase was positively related to very fine and fine roots, root length density, root dry weight, root C and root C:N ratio). The fitting of the SEMs models were evaluated using the chi-square, root mean square error of approximation (RMSEA), comparative fit index (CFI) and standardized root mean square residual (SRMR) (Kline, 2016). We established hypothetical causal models based on *a priori* knowledge of plant-soil interactions and N<sub>2</sub>O emissions (Abalos et al., 2018).

### 3. Results

#### 3.1. Cover cropping phase

##### 3.1.1. Soil parameters

In the first soil sampling event (37 DAS) no significant differences in NH<sub>4</sub><sup>+</sup> concentrations were observed (Fig. 1a), while at 63 DAS, NH<sub>4</sub><sup>+</sup> concentrations were higher in pure vetch (and the mixtures with 50 % and 66 % vetch) than in rye monoculture and control ( $P < 0.05$ ). At 63 DAS, NH<sub>4</sub><sup>+</sup> concentrations ranged from 2.6 (control) to 4.6 mg N kg<sup>-1</sup> soil (100 % vetch), and were higher than those at 37 DAS. Nitrate concentrations were generally higher (by 24 % on average) at 37 DAS than at 63 DAS (Fig. 1b), showing a consistent pattern among treatments at both sampling dates. At 37 DAS, NO<sub>3</sub><sup>-</sup> values decreased with an increasing proportion of rye. At 63 DAS, NO<sub>3</sub><sup>-</sup> were on average nearly 7-fold higher in the control than in cover crop treatments ( $P < 0.05$ ), with no significant differences between them. In the same sampling event, DON concentrations did not differ between treatments. Dissolved organic C concentrations generally decreased with a decreasing proportion of rye (Fig. 1d), with rye monoculture reaching the highest concentrations (207 mg C kg soil<sup>-1</sup>).

##### 3.1.2. Shoot and root traits

Total aboveground biomass was significantly lower in pure rye than

in the other cover crop treatments (Table 1). Similar results were obtained for C and N yields in the aboveground biomass, with a tendency for increasing N yield with a higher proportion of vetch. The dry matter content was lowest for pure vetch and highest for the 33 % vetch and 66 % rye mixture (Table S1). The biomass C:N ratio and the leaf length/width ratio were both highest for pure rye and lowest for pure vetch. The mesocosms with 33 % vetch and 66 % rye showed lower values for both variables than the other mixtures. The highest and lowest values of leaf length and leaf width were obtained in the pure stands, while the mixtures showed intermediate values. No significant differences between treatments were obtained for LDMC or SLA. All legume-cereal mixtures resulted in overyielding for dry biomass (23 %–40 %) and N uptake in the aboveground biomass (28 %–61 %) (Fig. 2). No significant differences were found between the different mixture-based treatments. The contribution of rye to overyielding was often higher than that of vetch, except in the 33 % vetch and 66 % rye mixture (Fig. S1).

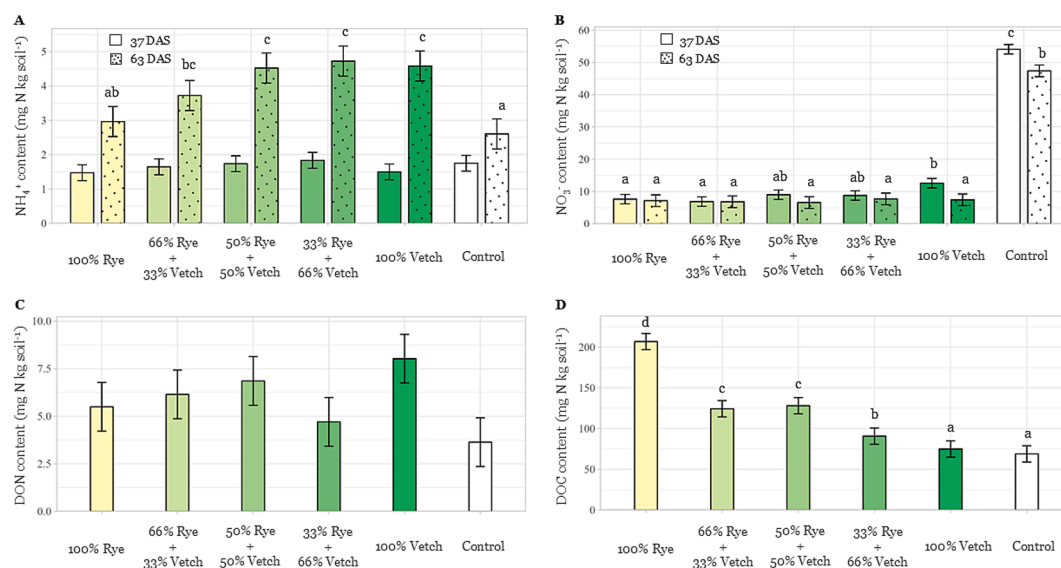
Mineral N contents (i.e., NH<sub>4</sub><sup>+</sup> at 63 DAS and NO<sub>3</sub><sup>-</sup> at 37 DAS) were negatively correlated with shoot C:N ratio ( $r = -0.39$  and  $r = -0.41$ , respectively,  $P < 0.05$ ) and leaf length:width ratio ( $r = -0.38$  and  $r = -0.56$ , respectively,  $P < 0.05$ ). Soil DOC was positively correlated with C:N ratio ( $r = 0.85$ ), leaf length:width ratio ( $r = 0.79$ ) and negatively with SLA ( $r = -0.56$ ).

A higher abundance of rye in the mixtures increased the length of very fine and fine roots in the diameter classes, with no statistical

**Table 1**

Aboveground dry biomass, N and C yields in the different cover crop treatments (see Section 2.1). Means followed by different lowercase letters indicate significant differences within each variable, by applying LSD test at 95 % probability level. S.E. indicates the standard error of the ANOVA.

Treatment	Total dry biomass (kg m <sup>-2</sup> )	N yield (g m <sup>-2</sup> )	C yield (g m <sup>-2</sup> )
100 % Rye	1.07 a	13.6 a	435 a
33 % Vetch 66 % Rye	1.83b	52.2b	754b
50 % Vetch 50 % Rye	1.91b	54.1 bc	785b
66 % Vetch 33 % Rye	1.92b	65.6 cd	789b
100 % Vetch	1.84b	70.9 d	749b
P value	0.000	0.000	0.000
S.E.	0.07	3.94	30



**Fig. 1.** Soil (A) NH<sub>4</sub><sup>+</sup>, (B) NO<sub>3</sub><sup>-</sup>, (C) dissolved organic N (DON) and (D) dissolved organic C (DOC) concentrations at 37 and 63 days after showing (DAS) in the different treatments (see section 2.1) during the cover cropping phase. Vertical bars denote the standard errors of the ANOVA ( $n = 4$ ). Treatments with different lowercase letters within each sampling date are statistically different by applying the LSD test at 95 % probability level. The variables NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup> and DOC were log-transformed before the analysis of variance to meet the normal distribution and homogeneity of variances.



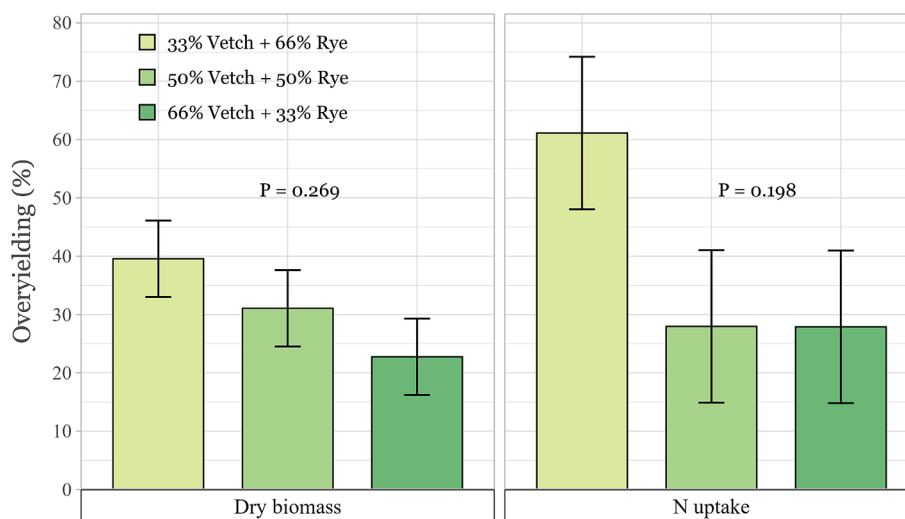


Fig. 2. Cover crop biomass overyielding and cover crop N uptake overyielding for the different cover crop mixtures (see section 2.1). Vertical bars denote the standard errors of the ANOVA ( $n = 4$ ). Treatments with different lowercase letters are statistically different by applying the LSD test at 95 % probability level.

differences between pure rye and the mixture with 66 % rye and 33 % vetch (Table 2). A similar tendency was found for medium or coarse roots, although in this case only the mixture with 66 % rye differed statistically from pure vetch. Monoculture vetch also showed a lower diameter class length of very coarse roots than the rest of treatments (except 66 % vetch + 33 % rye). Rye monoculture resulted in the highest values of RLD, which decreased with increasing proportion of vetch. Both root C:N ratio and RDW showed a similar trend as RLD, but without statistical differences between 100 % and 66 % rye. Soil  $\text{NO}_3^-$  content at 37 DAS was negatively correlated with very fine and fine roots ( $r = -0.65$  and  $r = -0.57$ , respectively) as well as with RLD ( $r = -0.57$ ), RDW ( $r = -0.63$ ) and root C:N ratio ( $r = -0.66$ ). Strong negative correlations were also found between biomass N yield and RLD ( $r = -0.81$ ), RDW ( $r = -0.74$ ) and root C ( $r = -0.75$ ), and also between aboveground biomass yield and RLD ( $r = -0.56$ ) and DOC ( $r = -0.62$ ). On the contrary, DOC concentrations positively correlated with RLD ( $r = 0.90$ ), RDW ( $r = 0.83$ ) and root C ( $r = 0.84$ ).

### 3.2. $\text{N}_2\text{O}$ emissions

On average, the cover cropping phase contributed 17 % (ranging from 8 % to 29 %) to the total cumulative emissions over the two phases

(Fig. 3a). Cumulative emissions were highest in the mixture with 66 % vetch and 33 % rye ( $38.5 \text{ mg N/m}^{-2}(-|-)$ ) and lowest in pure rye and control ( $25.8$  and  $15.8 \text{ mg N/m}^{-2}(-|-)$ , respectively). Nitrous oxide emissions were negatively correlated with soil  $\text{NH}_4^+$  concentrations at 37 DAS ( $r = -0.40$ ).

### 3.3. Post-incorporation phase

#### 3.3.1. Soil parameters

The average  $\text{NH}_4^+$  content in the post-incorporation phase ranged from 3.4 (control) to  $4.3 \text{ mg N kg soil}^{-1}$  (Fig. 4a), being significantly higher in all cover crop mixtures than in pure stands and the control. The incorporation of the aboveground biomass (R + S) significantly increased soil  $\text{NH}_4^+$  concentrations (by average 13 %) compared to treatments where only roots were left (R) (Fig. S2a). Average  $\text{NO}_3^-$  concentrations were lowest in pure rye, but with an increasing tendency from 77 to 143 DAS, as also observed for the vetch-rye mixtures. In the rest of treatments,  $\text{NO}_3^-$  concentrations were highest in 100 % vetch (reaching  $60.3 \text{ mg N kg soil}^{-1}$ ), followed by 66 % vetch + 33 % rye and the control, and then by mixtures with 50 % and 33 % vetch (Fig. 4b). Similarly to  $\text{NH}_4^+$ , average  $\text{NO}_3^-$  concentrations were 35 % higher in R + S than in R mesocosms, even though this was not observed for the 100 %

Table 2

Root traits in the different CC treatments (see section 2.1). Means followed by different lowercase letters indicate significant differences within each variable, by applying LSD test at 95% probability level. S.E. indicates the standard error of the ANOVA.

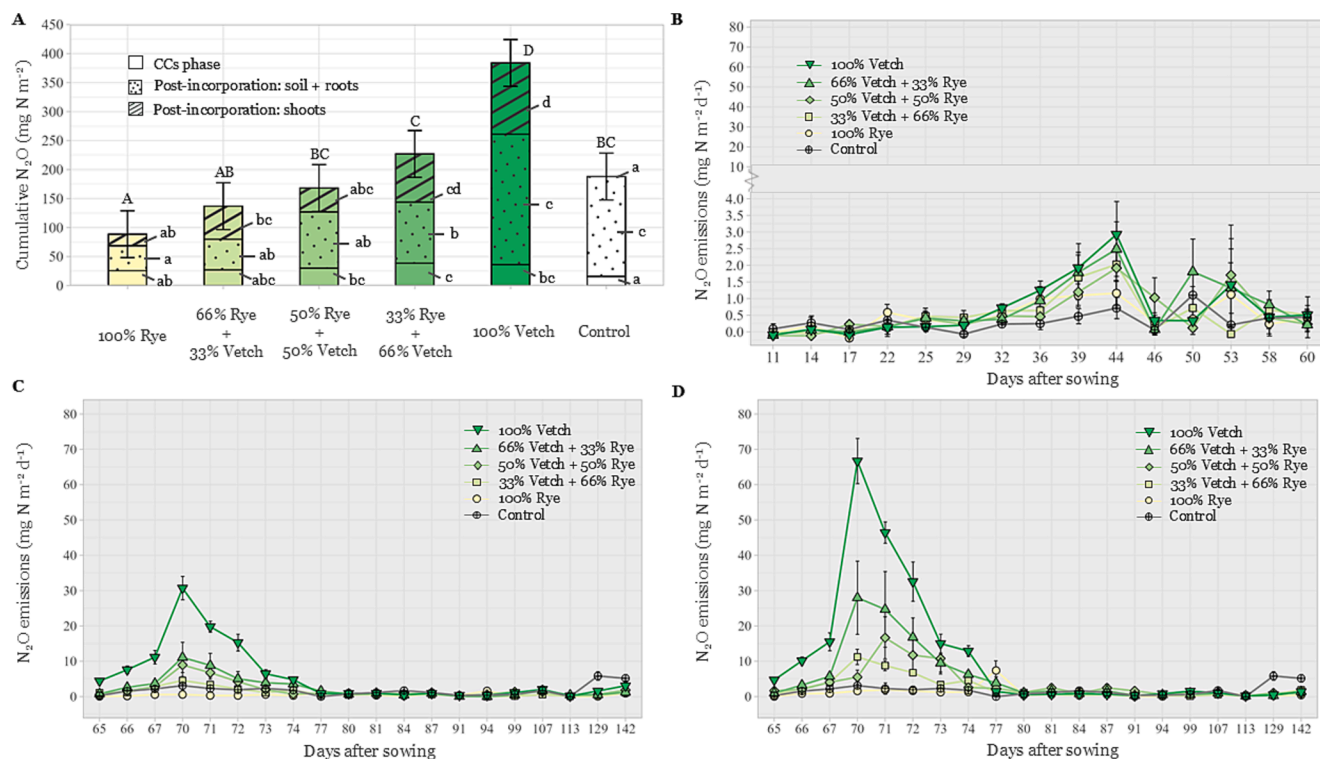
Treatment	Very fine root DCL <sup>a</sup> ( $\text{m cm}^{-3}$ )	Fine root DCL <sup>a*</sup> ( $\text{m cm}^{-3}$ )	Medium/coarse root DCL ( $\text{m cm}^{-3}$ )	Very coarse root DCL ( $\text{m cm}^{-3}$ )	RLD <sup>b</sup> ( $\text{cm cm}^{-3}$ )	RDW <sup>c</sup> ( $\text{mg cm}^{-3}$ )	Root C ( $\text{kg m}^{-2}$ )	Root N ( $\text{g m}^{-2}$ )	Root C:N ( $\text{kg kg}^{-1}$ )
100 % Rye	53.9c	228.9 d	61.8 bc	2.7b	52.4 d	1.97 d	0.29 d	5.8 ab	50.6 d
33 % Vetch 66 % Rye	50.5c	219.4 cd	64.2c	2.4b	42.6c	1.75 cd	0.24c	4.9 a	49.6 d
50 % Vetch 50 % Rye	30.9b	156.6c	59.2 bc	2.5b	37.6c	1.64c	0.26 cd	6.4b	40.2c
66 % Vetch 33 % Rye	23.5b	71.3b	35.9 a	1.8 ab	20.0b	1.05b	0.15b	5.3 ab	29.1b
100 % Vetch	1.6 a	14.6 a	48.3 ab	0.9 a	9.9 a	0.57 a	0.08 a	6.5b	13.0 a
P value	0.001	0.000	0.008	0.032	0.000	0.000	0.000	0.151	0.000
S.E.	6.3	21.7	4.9	0.4	2.0	0.09	0.01	0.5	1.8

\*log-transformed variable.

<sup>a</sup> Diameter class length.

<sup>b</sup> Root length density.

<sup>c</sup> Root dry weight.



**Fig. 3.** (A) Cumulative  $N_2O$  emissions during the cover cropping and post-incorporation phases, and daily  $N_2O$  emissions during (B) the CC phase, (C) post-incorporation phase in R mesocosms (see section 2.1), and (D) post-incorporation phase in R + S mesocosms (see section 2.1). Vertical bars denote the standard errors ( $n = 4$ ) of the ANOVA (A) and the experimental standard errors of the mean (B-D). In (A), treatments with different lowercase letters within each phase are statistically different by applying the LSD test at 95 % probability level. Similarly, different uppercase letters denote significant differences between treatments for total  $N_2O$  emissions. Cumulative  $N_2O$  emissions during post-incorporation phase were log-transformed before the analysis of variance to meet the normal distribution and homogeneity of variances.

rye treatment ( $P < 0.05$ , Fig. S2b).

Soil DOC concentrations showed a slight decreasing trend from 77 to 143 DAS (Fig. 4c), except for pure vetch. No differences in DOC were found between treatments. Incorporation of shoots together with roots generally increased DOC concentrations in both DOC sampling events (and by 9 % on average,  $P < 0.05$ , Fig. S2c). Soil DON concentrations were highest in 100 % vetch ( $34.8 \text{ mg N kg soil}^{-1}$ ) and lowest in 100 % rye ( $6.0 \text{ mg N kg soil}^{-1}$ ), with no differences between cover crop mixtures and the control. The incorporation of shoots (R + S) significantly increased soil DON concentrations with respect to treatments where only the roots were left (Fig. S2d), although the differences between R and R + S mesocosms were attenuated with the increasing presence of rye.

### 3.4. $N_2O$ emissions

Nitrous oxide peaked at the beginning of the post-incorporation phase, reaching the maximum values in vetch monoculture (i.e.,  $30.7$  and  $66.7 \text{ mg N/m}^{-2}(-|) \text{ d}^{-1}$  in R and R + S mesocosms, respectively) (Fig. 3c, d). The contribution of roots during the post-incorporation phase to total  $N_2O$  emissions ranged from 39 % (33 % vetch + 66 % rye) to 92 % (control), averaging 57 % (Fig. 2a). Cumulative  $N_2O$  emissions from mesocosms with only roots were highest in 100 % vetch and the control, followed by the 66 % vetch + 33 % rye treatment, and minimum in 100 % rye; the rest of mixtures had intermediate values. During this post-incorporation period, the contribution of cover crop aboveground biomass residues (shoots) to total  $N_2O$  emissions (excluding control) ranged from 22 % (100 % rye) to 42 % (33 % vetch + 66 % rye), averaging 31 % (Fig. 2a). Cumulative emissions from shoots decreased with a decreasing proportion of vetch, thus being highest and lowest in pure vetch and pure rye, respectively. A similar

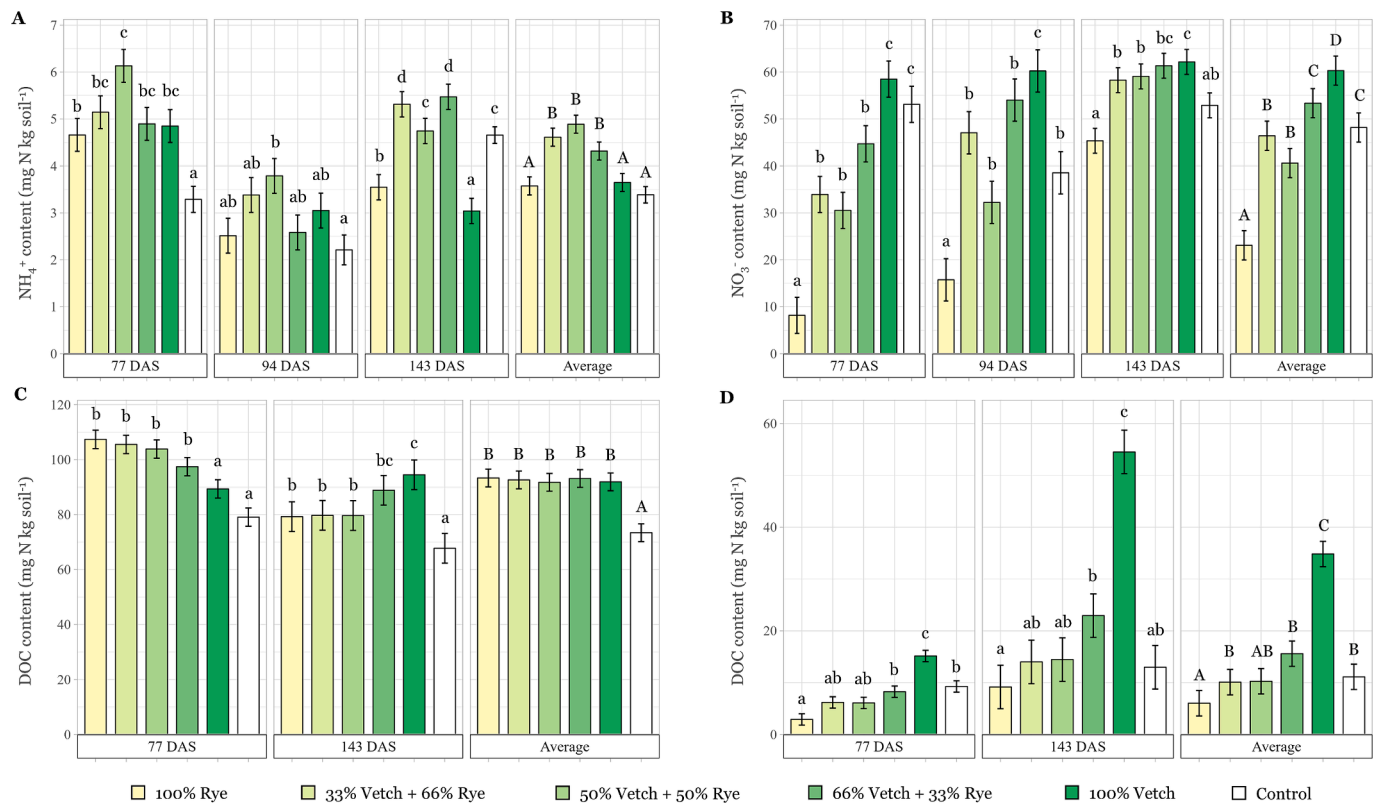
trend was obtained for total cumulative emissions over the two phases, with vetch monoculture resulting in the highest fluxes, followed by the 66 % vetch and 33 % rye combination, and with pure rye leading to the lowest cumulative emissions (Fig. 2a). During the post-incorporation phase, highly positive and significant correlations were found between  $N_2O$  emissions with soil  $NO_3^-$  ( $r = 0.80$ ) and DON ( $r = 0.80$ ).

Considering only the cover crop treatments, strong negative relationships were found between both  $N_2O$  emissions during the post-incorporation phase and total  $N_2O$  emissions with dry matter content in the aboveground biomass ( $-0.85 < r < -0.90$ ) and most root traits, particularly the diameter class length of very fine roots ( $-0.69 = r = -0.72$ ) and fine roots ( $-0.64 = r = -0.82$ ), RLD ( $-0.72 = r = -0.86$ ), RDW ( $-0.75 = r = -0.87$ ) and root C content ( $-0.69 = r = -0.87$ ). Correlations with medium to very coarse roots were also negative but weaker ( $-0.23 = r = -0.57$ , data not shown), while positive relationships were found between root N content and  $N_2O$  emissions from roots and soil ( $r = 0.49$ ) and between total  $N_2O$  emissions and CC aboveground biomass ( $r = 0.46$ ).

## 4. Discussion

### 4.1. Cover cropping phase: $N_2O$ emissions and cover crop biomass

Our results show that the cover cropping phase had a relatively minor influence on total  $N_2O$  emissions in comparison with the post-incorporation phase, which is consistent with García-Gutiérrez et al. (2024). Cover crops are expected to reduce  $N_2O$  emissions during their growth period due to the partial removal of soil residual mineral N (Fernandez-Pulido et al., 2023). This was indeed observed in our experiment, with a highly effective decrease of soil  $NO_3^-$  content in all cover crop-based treatments compared to the control mesocosms. Such



**Fig. 4.** Soil (A)  $\text{NH}_4^+$ , (B)  $\text{NO}_3^-$ , (C) dissolved organic C (DOC) and (D) dissolved organic N (DON) concentrations in mesocosms containing both cover crop roots and shoots (R + S) at 77, 94 (only for mineral N) and 143 days after showing (DAS) in the different treatments (see section 2.1) during the post-incorporation phase. Vertical bars denote the standard errors of the ANOVA ( $n = 4$ ). Treatments with different lowercase letters within each sampling date are statistically different by applying the LSD test at 95 % probability level. The variables  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and DON were log-transformed before the analysis of variance to meet the normal distribution and homogeneity of variances.

an effect could be particularly relevant for the abatement of this reactive N pool in the soil and potential N leaching losses (Thapa et al., 2018). In this sense, cover crop mixtures had a similar  $\text{NO}_3^-$  reduction as pure rye, probably due to the regulation of biological N-fixation in legumes according to soil fertility (De Notaris et al., 2021) and the higher N uptake in mixtures in legume-cereal mixtures due to complementarity effects (Abalos et al., 2021).

The SEM revealed a clear association between root traits and biomass variables with  $\text{N}_2\text{O}$  emissions during the cover cropping phase (Fig. 5). The strongest relationships were the direct negative effects of root dry weight, very fine and fine roots, root length density, root C, and root C:N ratio, on  $\text{N}_2\text{O}$  fluxes. Previous studies have also identified root length density and the abundance of fine/very fine roots as key root traits for plant N acquisition (Griffiths et al., 2022; Liu et al., 2022). Therefore, these relationships support the notion that vigorous root development and N acquisition are critical for  $\text{N}_2\text{O}$  mitigation (Abalos et al., 2018), despite the unexpected lack of a positive correlation between soil mineral N and  $\text{N}_2\text{O}$  emissions. Since mineral N can be lost or immobilized through several and complex pathways distinct from  $\text{N}_2\text{O}$  emissions, this lack of correlation does not allow us to conclude that soil  $\text{NO}_3^-$  uptake by the plant did not influence  $\text{N}_2\text{O}$  emissions. Root traits and biomass variables indirectly stimulated  $\text{N}_2\text{O}$  emissions through an increase of DOC concentrations in our experiment. Root dry weight, root length density and root C were positively associated with DOC, suggesting that the stimulatory effect was driven by root C exudation, as these traits determine the amount of contact surface between roots and soil and the amount of root-derived C inputs to soil (Freschet et al., 2021). Since labile organic C is a substrate for heterotrophic denitrifiers (Butterbach-Bahl et al., 2013), rhizodeposition probably stimulated denitrification.

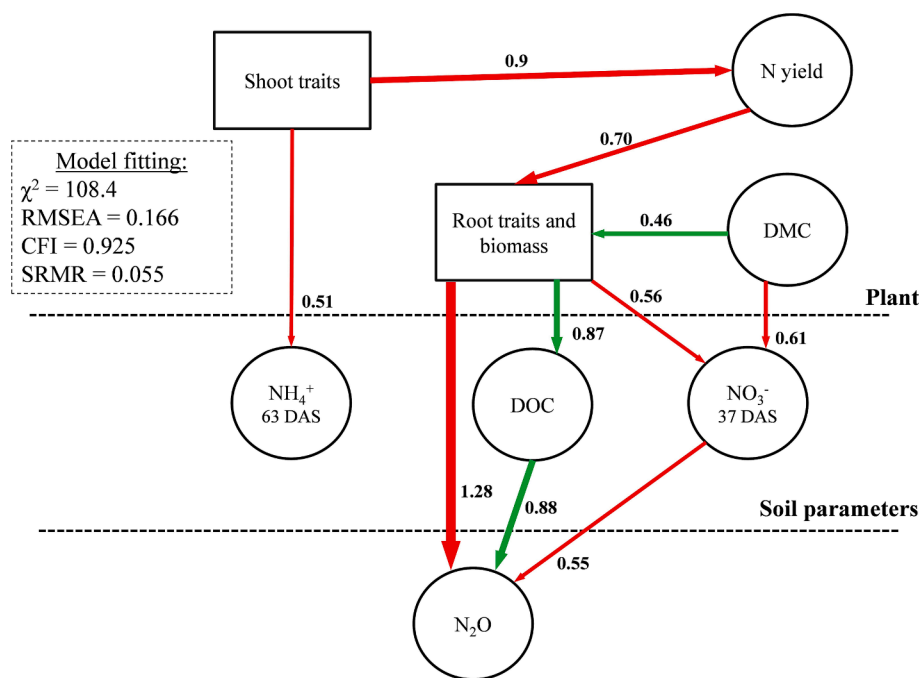
Legume-cereal mixtures promoted overyielding (both in terms of dry

matter biomass and N yield), which supported our initial hypothesis and suggests an effective N transfer from vetch to rye plants (Liu et al., 2023). Increasing rye abundance had a detrimental effect on above-ground biomass but a stimulatory effect on root biomass. Mixing vetch with rye proved effective in mitigating biomass penalties, with important consequences not only for C and N cycling but also for  $\text{N}_2\text{O}$  emission during the critical post-incorporation phase.

The low dry matter biomass in cereal monoculture significantly decreased soil C and N input. While this might have positive implications for  $\text{N}_2\text{O}$  emissions after cover crop termination (if residues are incorporated), it could have negative implications for their potential to enhance SOC (Peng et al., 2023), as observed in the present study with regards to DOC contents during the cover crop growing phase. In this sense, mixing rye with vetch increased both the C and N yields, thus suggesting a pivotal role in improving the C footprint and ecosystem services (Guardia et al., 2019; Quintarelli et al., 2022). Furthermore, legume-non-legume mixtures can balance the main SOC formation pathways (i.e., via particulate organic matter and mineral-associated organic matter), with positive impacts on short- and long-term SOC stabilization and stocks (Zhang et al., 2022).

#### 4.2. $\text{N}_2\text{O}$ emissions after cover crop incorporation

Our results reveal an important finding for cover cropping:  $\text{N}_2\text{O}$  fluxes after termination are not affected by the management of cover crop shoots (removal versus incorporation). This finding can be explained by the major influence of the belowground system (roots and soil with the legacy effect from cover crops and previous cropping phases). As we initially hypothesized, emissions from cover crop shoots decreased with an increasing proportion of rye, reaching the lowest



**Fig. 5.** Structural equation model (SEM) of the cover cropping phase (including only cover crop-based treatments and excluding control), showing the relationships between shoot traits, root traits, N yield, dry matter content (DMC) in the aboveground biomass, soil  $\text{NH}_4^+$  (at 63 days after sowing, DAS) and  $\text{NO}_3^-$  (at 37 DAS) concentrations, dissolved organic carbon (DOC) and  $\text{N}_2\text{O}$  emissions. Only significant relationships are shown. Green and red arrows denote positive and negative relationships, respectively. Numbers next to the arrows represent the path coefficients. The variables  $\text{N}_2\text{O}$  and DOC were transformed before the analysis. ‘shoot traits’ is a latent variable which positively correlates with leaf length/width ratio, leaf length and C:N ratio (path coefficients 0.94–0.98). ‘Root traits and biomass’ is a latent variable which positively correlates with very fine and fine roots, root length density, root dry weight, root C and root C:N ratio (path coefficients 0.91–0.99). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

levels in pure rye stands. The incorporation of residues with high C:N ratio (>30 in rye monoculture) can result in net N immobilization (Redin et al., 2014), thus reducing the availability of mineral N substrates for microbial processes required for  $\text{N}_2\text{O}$  release (Abalos et al., 2022a). The later revision of Abalos et al. (2022b) argued that maturity class of residues is a stronger predictor of the effect of cover crop residues on  $\text{N}_2\text{O}$  fluxes, by integrating C:N ratio with C and oxygen ( $\text{O}_2$ ) availability. This was supported in our study by the strong and negative correlation between  $\text{N}_2\text{O}$  emissions and dry matter content of the aboveground biomass.

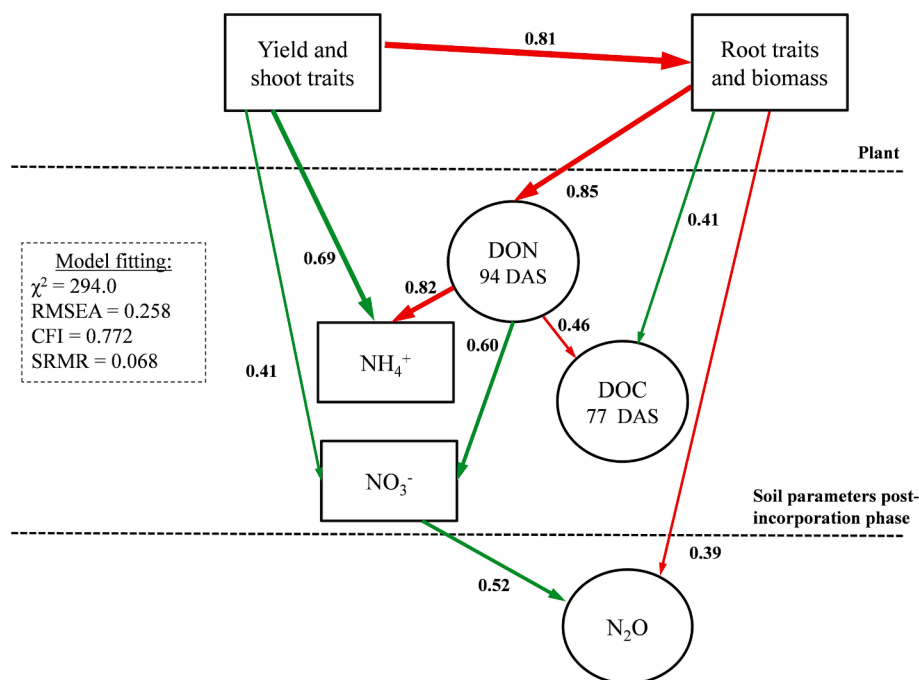
The SEM of mesocosms including both belowground and aboveground biomass confirmed the direct effect (but weaker than during the cover cropping phase) of root biomass and traits (such as very fine and fine roots, RLD, root C or root C:N ratio) in decreasing  $\text{N}_2\text{O}$  emissions post-incorporation (Fig. 6), as well as the competitive relationship between shoot and root development. The decomposition of aboveground residues represented a significant input of soil  $\text{NH}_4^+$  during the post-incorporation phase, although the relationships with  $\text{N}_2\text{O}$  emissions were weaker and complex, possibly as a result of the intense nitrifying activity, supported by low  $\text{NH}_4^+$  but high  $\text{NO}_3^-$  concentrations in the soil (Fig. 4). The end product of nitrification ( $\text{NO}_3^-$ ) stimulated  $\text{N}_2\text{O}$  emissions (Fig. 6), while DON derived from plant residues had an indirect role on  $\text{N}_2\text{O}$  emissions by fueling the reactive  $\text{NO}_3^-$  pool. The importance of DON for  $\text{N}_2\text{O}$  emissions is often overlooked, despite strong positive relationships previously reported between soil DON contents and gross N fluxes (Cookson et al., 2007), including  $\text{N}_2\text{O}$  emissions (Cui et al., 2023). Schimel and Bennett (2004) suggested that DON plays a critical role in regulating the active N-cycling microbial communities, thus driving short-term N supply and potential mineralization and nitrification rates. Indeed, the conversion rate of organic matter to soluble C and N is often a key factor restricting gross N transformation rates in agricultural soils (Cookson and Murphy, 2004). The negative relationship between DON and  $\text{NH}_4^+$  in our mesocosms experiment possibly reveals a

detrimental effect of the C-rich rye residues on organic N mineralization (Capra et al., 2023; Li et al., 2020).

Our SEM with data from mesocosms including both belowground and aboveground biomass suggests a pivotal influence of  $\text{NO}_3^-$  (i.e., the end product of nitrification) on  $\text{N}_2\text{O}$  emissions induced by shoots during the post-incorporation phase. The decay of shoots was indicated by the positive relationship between shoot N content and leaf width (associated to legumes) with  $\text{NH}_4^+$  (nitrification substrate) and  $\text{NO}_3^-$  (nitrification product) concentrations during the post-incorporation phase (Fig. 6). The lack of significant relationship between  $\text{NH}_4^+$  and  $\text{N}_2\text{O}$ , in contrast with that between  $\text{N}_2\text{O}$  and  $\text{NO}_3^-$ , could possibly reveal a fast nitrification process. Root decomposition would provide labile organic C substrates for denitrifiers (positive relationship between root traits/biomass and DOC, Fig. 6) thus possibly enhancing denitrification activity under low  $\text{O}_2$  conditions and without  $\text{NO}_3^-$  limitation (Rummel et al., 2021). This may contribute to explain the higher  $\text{N}_2\text{O}$  emissions during the post-incorporation phase compared to those during cover cropping (Fig. 3a), in addition to the lack of living plants to compete for the inorganic N released from cover crop residues.

In contrast to our second hypothesis, the belowground biomass of cover crops was found to be a major contributor to total  $\text{N}_2\text{O}$  fluxes, particularly in pure vetch (Fig. 3a). These results suggest that the key drivers of  $\text{N}_2\text{O}$  emissions from roots were the decomposition of N-rich vetch roots (De Notaris et al., 2020; Poffenbarger et al., 2015) and the previous changes in the soil environment due to rhizodeposition (Abdalla et al., 2019). The N legacy effect and the enhanced  $\text{NH}_4^+$  uptake potential from rye plants during the cover crop growing phase could have also contributed to the low  $\text{N}_2\text{O}$  fluxes in pure cereal mesocosms. This is supported by the lower soil  $\text{NH}_4^+$  content at the end of the cover cropping phase (63 DAS) with increasing rye proportion (Fig. 1a). However, we hypothesized that this effect could have had a limited impact on  $\text{N}_2\text{O}$  emissions after incorporation, due to i) the lower N yield of the rye stands compared to those of vetch (Table 1); ii) the lack of





**Fig. 6.** Structural equation model (SEM) in “R + S” pots during post-incorporation phase (including only cover crop-based treatments and excluding control), showing the relationships between root traits, soil dissolved organic C (DOC), dissolved organic N (DON),  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations (during cover cropping and post incorporation) and  $\text{N}_2\text{O}$  emissions. Only significant relationships are shown. Green and red arrows denote positive and negative relationships, respectively. Numbers next to the arrows represent the path coefficients. All variables were transformed before the analysis. ‘Root traits and biomass’ is a latent variable which positively correlates with very fine and fine roots, root length density, root dry weight, root C and root C:N ratio (path coefficients 0.91–0.98). ‘Yield and shoot traits’ is a latent variable which positively correlates with N yield and leaf width (path coefficients 0.84–1.00) and negatively with C:N ratio and leaf length:width ratio (path coefficients 0.97). ‘ $\text{NH}_4^+$ ’ and ‘ $\text{NO}_3^-$ ’ are latent variables which positively correlate with the corresponding mineral N concentrations during the post-incorporation phase (path coefficients 0.59–1.00). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

significant relationship between  $\text{NH}_4^+$  concentrations and  $\text{N}_2\text{O}$  in only-root mesocosms at post-incorporation (Fig. S3); and iii) the lack of significant differences between cover crop treatments at 63 DAS in soil  $\text{NO}_3^-$  concentrations (Fig. 1b), which was the key variable related to  $\text{N}_2\text{O}$  emissions in only-root mesocosms (Fig. S3). On the contrary, we hypothesized that the root residue quality – and particularly the C:N ratio – would had the strongest impact on  $\text{N}_2\text{O}$  fluxes from only-root mesocosms. This ratio increased with the increasing rye proportion, thus driving the predominance of net mineralization (vetch) or immobilization (rye) of root residue-N (Nicolardot et al., 2001). Negative relationships between root dry weight and some traits (i.e., very fine and fine roots, root length density, root C and root C:N ratio) with mineral N in the mesocosms with roots only (Fig. S3) could be attributed to net N immobilization caused by legume and particularly non-legume residues (Li et al., 2020). The trend in cumulative  $\text{N}_2\text{O}$  emissions from the mesocosms with roots only (Fig. 3a) mirrored that observed for soil  $\text{NO}_3^-$  content at 77 DAS (Fig. 4b), which was closest to the main  $\text{N}_2\text{O}$  peak during the post-incorporation phase.

The predominance of belowground (soil + roots) over aboveground-derived  $\text{N}_2\text{O}$  emissions was an unexpected result due to the lower biomass and C:N ratio of root compared to shoots (Table 2, S1). These results were not in agreement with those of Saha et al. (2021), who reported an abatement of c. 60 %  $\text{N}_2\text{O}$  emissions during the post-incorporation phase when aboveground residues were removed. However, the presence of plants in that trial (i.e., maize as cash crop) and manure amendment may modify shoot decomposition and trigger a priming effect (Daly et al., 2024), causing the comparison to be biased. In any case, the uncertainties regarding the calculations of cumulative  $\text{N}_2\text{O}$  fluxes from shoots (see section 2.2), including the potential negative interaction between roots and shoots, suggests that further research is needed to elucidate the specific contribution of roots (distinguishing

from endogenous soil N from previous phases) and that of aboveground residues after cover crop incorporation.

#### 4.3. Implications and limitations

Our reported effects on N cycling and  $\text{N}_2\text{O}$  emissions based on mesocosm experiments should be confirmed under open field conditions, also assessing the concurrent effects of roots and shoots on carbon sequestration in the medium/long term. In this sense, it is also recommended to further explore the potential benefits of other cover crop species in the mixtures, such as brassicas (*Sinapis* spp., *Raphanus sativus* L.) or plantain (*Plantago lanceolata* L.), since both families can disrupt or modify N-cycling microbial processes communities by releasing secondary metabolites with biological nitrification inhibition activity (Fernandez-Pulido et al., 2023; Judson et al., 2019).

Our study unveils the critical timing of  $\text{N}_2\text{O}$  emissions from cover crop usage, emphasizing the dominance of emissions after termination and the significant contribution of roots over aboveground residues. Even if farmers remove aboveground cover crop residues for alternative purposes like biorefining, biogas, or biofuel,  $\text{N}_2\text{O}$  emissions remain substantial. Interestingly, our findings show that while total  $\text{N}_2\text{O}$  emissions increased with a higher vetch proportion, a mixture of 33 % vetch and 66 % rye maintained  $\text{N}_2\text{O}$  emissions as low as pure rye. This optimal ratio not only minimized  $\text{N}_2\text{O}$  emissions but also enhanced N yield for forage purposes and contributes to SOC stocks. Accordingly, this seems to be an optimum cereal-legume proportion to balance the agronomic and environmental aspects of cover crops. One of the potential constraints for upscaling our results is the maintenance of the legume-cereal proportions under field conditions. Soil fertility largely influences the outcome of aboveground biomass production of different species in a cover crop mixture, regardless of the seeding proportion. For example,

grasses and legumes tend to dominate in soils with high and low N availability, respectively (Baraibar et al., 2020).

Our study highlights the importance of specific root traits as robust predictors of cover crop effects on N<sub>2</sub>O emissions during both the cover cropping and post-incorporation phases. These results offer a framework for selecting species and designing mixtures to reduce N<sub>2</sub>O emissions. Combining this knowledge with a deep understanding of the relationships between root traits and SOC can provide a conceptual roadmap to maximize climate change mitigation in agroecosystems.

During the cover cropping phase, our multivariate analysis revealed contrasting effects of root traits on N<sub>2</sub>O emissions: a direct mitigating effect and an indirect emission-increasing effect by raising soil DOC concentrations. These complexities may contribute to explain the high variability which has been previously reported in the literature. The interplay of residual NO<sub>3</sub><sup>-</sup> and labile organic C inputs, along with the oxygen availability and anaerobicity (as drivers of denitrification), can tip the balance between mitigation and emission. Further research is needed to fully elucidate these relationships.

### CRedit authorship contribution statement

**Guillermo Guardia:** Writing – original draft, Data curation. **Diego Abalos:** Writing – review & editing, Supervision, Data curation, Conceptualization. **Emanuele Ribatti:** Writing – review & editing, Methodology, Investigation. **Federico Ardeni:** Writing – review & editing, Methodology, Investigation. **Federico Capra:** Writing – review & editing, Methodology, Investigation. **Giacomo Mortella:** Writing – review & editing, Methodology, Investigation. **Vincenzo Tabaglio:** Writing – review & editing, Methodology, Investigation. **Miguel Ángel Ibáñez:** Software, Formal analysis, Data curation. **Ji Chen:** Writing – review & editing, Methodology, Investigation. **Andrea Fiorini:** Writing – review & editing, Visualization, Supervision, Investigation, Funding acquisition, Data curation, Conceptualization.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgements

This work is part of the ReFarmS project (LS9 -202227EXNM - CUP: J53D23009550008), which has received funding from the Ministry of University and Research through the PRIN 2022 grant, as part of the PNRR for Mission 4, Component 2, Investment 1.1. Notice 104/2022 “financed by the European Union’s Next Generation EU initiative. DA thanks financial support by the Danish Council for Independent Research via the projects No. 9041-00324B and No. 1051-00060B.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2025.117195>.

### Data availability

Data will be made available on request.

### References

Abalos, D., van Groenigen, J.W., De Deyn, G.B., 2018. What plant functional traits can reduce nitrous oxide emissions from intensively managed grasslands? *Glob. Change Biol.* 24, e248–e258. <https://doi.org/10.1111/gcb.13827>.  
 Abalos, D., van Groenigen, J.W., Philippot, L., Lubbers, I.M., De Deyn, G.B., 2019. Plant trait-based approaches to improve nitrogen cycling in agroecosystems. *J. Appl. Ecol.* 56, 2454–2466. <https://doi.org/10.1111/1365-2664.13489>.

Abalos, D., De Deyn, G.B., Philippot, L., Oram, N.J., Oudová, B., Pantelis, I., Clark, C., Fiorini, A., Bru, D., Mariscal-Sancho, I., van Groenigen, J.W., 2021. Manipulating plant community composition to steer efficient N-cycling in intensively managed grasslands. *J. Appl. Ecol.* 58, 167–180. <https://doi.org/10.1111/1365-2664.13788>.  
 Abalos, D., Recous, S., Butterbach-Bahl, K., De Notaris, C., Rittl, T.F., Topp, C.F.E., Petersen, S.O., Hansen, S., Bleken, M.A., Rees, R.M., Olesen, J.E., 2022a. A review and meta-analysis of mitigation measures for nitrous oxide emissions from crop residues. *Sci. Total Environ.* 828, 154388. <https://doi.org/10.1016/j.scitotenv.2022.154388>.  
 Abalos, D., Rittl, T.F., Recous, S., Thiébeau, P., Topp, C.F., van Groenigen, K.J., Butterbach-Bahl, K., Thorman, R.E., Smith, K.E., Ahuja, I., Olesen, J.E., Bleken, M.A., Rees, R.M., Hansen, S., 2022b. Predicting field N<sub>2</sub>O emissions from crop residues based on their biochemical composition: a meta-analytical approach. *Sci. Total Environ.* 812, 152532. <https://doi.org/10.1016/j.scitotenv.2021.152532>.  
 Abdalla, M., Hastings, A., Cheng, K., Yue, Q., Chadwick, D., Espenberg, M., Truu, J., Rees, R.M., Smith, P., 2019. A critical review of the impacts of cover crops on nitrogen leaching, net greenhouse gas balance and crop productivity. *Glob. Change Biol.* 25, 2530–2543. <https://doi.org/10.1111/gcb.14644>.  
 Ardeni, F., Capra, F., Lommi, M., Fiorini, A., Tabaglio, V., 2023. Long-term C and N sequestration under no-till is governed by biomass production of cover crops rather than differences in grass vs. legume biomass quality. *Soil Till. Res.* 228, 105630. <https://doi.org/10.1016/j.still.2022.105630>.  
 Baraibar, B., Murrell, E.G., Bradley, B.A., Barbercheck, M.E., Mortensen, D.A., Kaye, J.P., White, C.M., 2020. Cover crop mixture expression is influenced by nitrogen availability and growing degree days. *PLoS One* 15, e0235868. <https://doi.org/10.1371/journal.pone.0235868>.  
 Bardgett, R.D., Mommer, L., De Vries, F.T., 2014. Going underground: root traits as drivers of ecosystem processes. *Trends Ecol. Evol.* 29, 692–699. <https://doi.org/10.1016/j.tree.2014.10.006>.  
 Bardgett, R.D. 2018. Plant trait-based approaches for interrogating belowground function. In *Biology and Environment: Proceedings of the Royal Irish Academy* 117b, pp. 1–13. Royal Irish Academy.  
 Basche, A.D., Miguez, F.E., Kaspar, T.C., Castellano, M.J., 2014. Do cover crops increase or decrease nitrous oxide emissions? A meta-analysis. *J. Soil Water Conserv.* 69, 471–482. <https://doi.org/10.2489/jswc.69.6.471>.  
 Butterbach-Bahl, K., Baggs, E.M., Dannenmann, M., Kiese, R., Zechmeister-Boltenstern, S., 2013. Nitrous oxide emissions from soils: How well do we understand the processes and their controls? *Philos. Trans. R. Soc. B Biol. Sci.* 368, 20130122. <https://doi.org/10.1098/rstb.2013.0122>.  
 Capra, F., Abalos, D., Maris, S.C., Ardeni, F., Lommi, M., Tabaglio, V., Fiorini, A., 2023. Towards efficient N cycling in intensive maize: role of cover crops and application methods of digestate liquid fraction. *GCB Bioenergy* 15, 867–885. <https://doi.org/10.1111/gcbb.13036>.  
 Cookson, W.R., Murphy, D.V., 2004. Quantifying the contribution of dissolved organic matter to soil nitrogen cycling using 15N isotopic pool dilution. *Soil Biol. Biochem.* 36, 2097–2100. <https://doi.org/10.1016/j.soilbio.2004.05.013>.  
 Cookson, W.R., Osman, M., Marschner, P., Abaye, D.A., Clark, I., Murphy, D.V., Stockdale, E.A., Watson, C.A., 2007. Controls on soil nitrogen cycling and microbial community composition across land use and incubation temperature. *Soil Biol. Biochem.* 39, 744–756. <https://doi.org/10.1016/j.soilbio.2006.09.022>.  
 Cornelissen, J.H.C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D.E., Reich, P.B., Ter Steege, H., Morgan, H.D., Van Der Heijden, M.G.A., Pausas, J.G., Poorter, H., 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Aust. J. Bot.* 51, 335–380. <https://doi.org/10.1071/BT02124>.  
 Cui, P., Chen, Z., Fan, F., Yin, C., Song, A., Li, T., Zhang, H., Liang, Y., 2023. Soil texture is an easily overlooked factor affecting the temperature sensitivity of N<sub>2</sub>O emissions. *Sci. Total Environ.* 862, 160648. <https://doi.org/10.1016/j.scitotenv.2022.160648>.  
 Daly, E.J., Hernandez-Ramirez, G., Congreves, K.A., Clough, T., Voigt, C., Harris, E., Ruser, R., 2024. Soil organic nitrogen priming to nitrous oxide: a synthesis. *Soil Biol. Biochem.* 189, 109254. <https://doi.org/10.1016/j.soilbio.2023.109254>.  
 De Notaris, C., Olesen, J.E., Sørensen, P., Rasmussen, J., 2020. Input and mineralization of carbon and nitrogen in soil from legume-based cover crops. *Nutr. Cycl. Agroecosys.* 116, 1–18. <https://doi.org/10.1007/s10705-019-10026-z>.  
 De Notaris, C., Mortensen, E.Ø., Sørensen, P., Olesen, J.E., Rasmussen, J., 2021. Cover crop mixtures including legumes can self-regulate to optimize N<sub>2</sub> fixation while reducing nitrate leaching. *Agric. Ecosyst. Environ.* 309, 107287. <https://doi.org/10.1016/j.agee.2020.107287>.  
 Drost, S.M., Rutgers, M., Wouterse, M., De Boer, W., Bodelier, P.L., 2020. Decomposition of mixtures of cover crop residues increases microbial functional diversity. *Geoderma* 361, 114060. <https://doi.org/10.1016/j.geoderma.2019.114060>.  
 Elhakeem, A., Bastiaans, L., Houben, S., Couwenberg, T., Makowski, D., van der Werf, W., 2021. Do cover crop mixtures give higher and more stable yields than pure stands? *Field Crops Res.* 270, 108217. <https://doi.org/10.1016/j.fcr.2021.108217>.  
 Faucon, M.P., Houben, D., Lambers, H., 2017. Plant functional traits: soil and ecosystem services. *Trends Plant Sci.* 22, 385–394. <https://doi.org/10.1016/j.tplants.2017.01.005>.  
 Fernandez-Pulido, C.R., Rasmussen, J., Eriksen, J., Abalos, D., 2023. Cover crops for nitrogen loss reductions: functional groups, species identity and traits. *Plant Soil* 1–14. <https://doi.org/10.1007/s11104-023-05895-x>.  
 Fiorini, A., Boselli, R., Amaducci, S., Tabaglio, V., 2018. Effects of no-till on root architecture and root-soil interactions in a three-year crop rotation. *Eur. J. Agron.* 99, 156–166. <https://doi.org/10.1016/j.eja.2018.07.009>.  
 Fiorini, A., Maris, S.C., Abalos, D., Amaducci, S., Tabaglio, V., 2020. Combining no-till with rye (*Secale cereale* L.) cover crop mitigates nitrous oxide emissions without

- decreasing yield. *Soil Till. Res.* 196, 104442. <https://doi.org/10.1016/j.still.2019.104442>.
- Freschet, G.T., Roumet, C., Comas, L.H., et al., 2021. Root traits as drivers of plant and ecosystem functioning: current understanding, pitfalls and future research needs. *New Phytol.* 232, 1123–1158. <https://doi.org/10.1111/nph.17072>.
- García-González, I., Hontoria, C., Gabriel, J.L., Alonso-Ayuso, M., Quemada, M., 2018. Cover crops to mitigate soil degradation and enhance soil functionality in irrigated land. *Geoderma* 322, 81–88. <https://doi.org/10.1016/j.geoderma.2018.02.024>.
- García-Gutiérrez, S., García-Marco, S., Jiménez-Horcadada, R., Montoya, M., Vallejo, A., Guardia, G., 2024. Maize residue input rather than cover cropping influenced N<sub>2</sub>O emissions and soil–crop N dynamics during the intercrop and cash crop periods. *Agric. Ecosyst. Environ.* 363, 108873. <https://doi.org/10.1016/j.agee.2023.108877>.
- Griffiths, M., Delory, B.M., Jawahir, V., Wong, K.M., Bagnall, G.C., Dowd, T.G., Nusinow, D.A., Miller, A.J., Topp, C.N., 2022. Optimisation of root traits to provide enhanced ecosystem services in agricultural systems: a focus on cover crops. *Plant Cell Environ.* 45, 751–770. <https://doi.org/10.1111/pce.14247>.
- Guardia, G., Aguilera, E., Vallejo, A., Sanz-Cobena, A., Alonso-Ayuso, M., Quemada, M., 2019. Effective climate change mitigation through cover cropping and integrated fertilization: a global warming potential assessment from a 10-year field experiment. *J. Clean. Prod.* 241, 118307. <https://doi.org/10.1016/j.jclepro.2019.118307>.
- Hu, Q., Thomas, B.W., Powlson, D., Hu, Y., Zhang, Y., Jun, X., Shi, X., Zhang, Y., 2023. Soil organic carbon fractions in response to soil, environmental and agronomic factors under cover cropping systems: A global meta-analysis. *Agric. Ecosyst. Environ.* 355, 108591. <https://doi.org/10.1016/j.agee.2023.108591>.
- Judson, H.G., Fraser, P.M., Peterson, M.E., 2019. Nitrification inhibition by urine from cattle consuming *Plantago lanceolata*. *J. New Zeal. Grasslands* 81, 111–116. <https://doi.org/10.33584/jnzg.2019.81.413>.
- Kaye, J.P., Quemada, M., 2017. Using cover crops to mitigate and adapt to climate change. A review. *Agron. Sustain. Dev.* 37 (1), 4. <https://doi.org/10.1007/s13593-016-0410-x>.
- Khangura, R., Ferris, D., Wagg, C., Bowyer, J., 2023. Regenerative agriculture—A literature review on the practices and mechanisms used to improve soil health. *Sustainability* 15, 2338. <https://doi.org/10.3390/su15032338>.
- Kim, K., Guber, A., Rivers, M., Kravchenko, A., 2020. Contribution of decomposing plant roots to N<sub>2</sub>O emissions by water absorption. *Geoderma* 375, 114506. <https://doi.org/10.1016/j.geoderma.2020.114506>.
- Kirwan, L., Lüscher, A., Sebastià, M.T., et al., 2007. Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. *J. Ecol.* 95, 530–539. <https://doi.org/10.1111/j.1365-2745.2007.01225.x>.
- Kline, R.B., 2016. *Principles and Practice of Structural Equation Modeling*, 4th ed. Guilford Publications.
- Li, F., Sørensen, P., Li, X., Olesen, J.E., 2020. Carbon and nitrogen mineralization differ between incorporated shoots and roots of legume versus non-legume based cover crops. *Plant Soil* 446, 243–257. <https://doi.org/10.1007/s11104-019-04358-6>.
- Liu, H., Colombi, T., Jäck, O., Westerbergh, A., Weih, M., 2022. Linking wheat nitrogen use to root traits: shallow and thin embryonic roots enhance uptake but reduce conversion efficiency of nitrogen. *Field Crops Res.* 285, 108603. <https://doi.org/10.1016/j.fcr.2022.108603>.
- Liu, J., Elliott, J.A., Wilson, H.F., Macrae, M.L., Baulch, H.M., Lobb, D.A., 2021. Phosphorus runoff from Canadian agricultural land: a cross-region synthesis of edge-of-field results. *Agric. Water Manag.* 255, 107030. <https://doi.org/10.3390/w13202929>.
- Liu, H., Struik, P.C., Zhang, Y., Jing, J., Stomph, T.J., 2023. Forage quality in cereal/legume intercropping: a meta-analysis. *Field Crops Res.* 304, 109174. <https://doi.org/10.1016/j.fcr.2023.109174>.
- Meyer, N., Berge, J.E., Justes, E., Constantin, J., 2022. Influence of cover crop on water and nitrogen balances and cash crop yield in a temperate climate: a modelling approach using the STICS soil-crop model. *Eur. J. Agron.* 132, 126416. <https://doi.org/10.1016/j.eja.2021.126416>.
- Muhammad, I., Sainju, U.M., Zhao, F., Khan, A., Ghimire, R., Fu, X., Wang, J., 2019. Regulation of soil CO<sub>2</sub> and N<sub>2</sub>O emissions by cover crops: a meta-analysis. *Soil Tillage Res.* 192, 103–112. <https://doi.org/10.1016/j.still.2019.04.020>.
- Nicolardot, B., Recous, S., Mary, B., 2001. Simulation of C and N mineralisation during crop residue decomposition: a simple dynamic model based on the C: N ratio of the residues. *Plant Soil* 228, 83–103. <https://doi.org/10.1023/A:1004813801728>.
- Osman, A.E., Nersoyan, N.J.E.A., 1986. Effect of the proportion of species on the yield and quality of forage mixtures, and on the yield of barley in the following year. *Exp. Agric.* 22, 345–351. <https://doi.org/10.1017/S0014479700014599>.
- Pedersen, A.R., Petersen, S.O., Schelde, K., 2010. A comprehensive approach to soil-atmosphere trace-gas flux estimation with static chambers. *Eur. J. Soil Sci.* 61, 888–902. <https://doi.org/10.1111/j.1365-2389.2010.01291.x>.
- Peng, Y., Rieke, E.L., Chahal, I., et al., 2023. Maximizing soil organic carbon stocks under cover cropping: insights from long-term agricultural experiments in North America. *Agric. Ecosyst. Environ.* 356, 108599. <https://doi.org/10.1016/j.agee.2023.108599>.
- Poffenbarger, H.J., Mirsky, S.B., Weil, R.R., Kramer, M., Spargo, J.T., Cavigelli, M.A., 2015. Legume proportion, poultry litter, and tillage effects on cover crop decomposition. *Agron. J.* 107, 2083–2096. <https://doi.org/10.2134/agronj15.0065>.
- Quintarelli, V., Radicetti, E., Allevato, E., Stazi, S.R., Haider, G., Abideen, Z., Bibi, S., Jamal, A., Mancinelli, R., 2022. Cover crops for sustainable cropping systems: a review. *Agriculture* 12, 2076. <https://doi.org/10.3390/agriculture12122076>.
- Redin, M., Recous, S., Aita, C., Dietrich, G., Caitan, A., Hytalo, W., Schmatz, R., Jos, S., 2014. How the chemical composition and heterogeneity of crop residue mixtures decomposing at the soil surface affects C and N mineralization. *Soil Biol. Biochem.* 78, 65–75. <https://doi.org/10.1016/j.soilbio.2014.07.014>.
- Reich, P.B., 2014. The world-wide ‘fast-slow’ plant economics spectrum: a traits manifesto. *J. Ecol.* 102, 275–301. <https://doi.org/10.1111/1365-2745.12211>.
- Reinhardt, D.R., Miller, R.M., 1990. Size classes of root diameter and mycorrhizal fungal colonization in two temperate grassland communities. *New Phytol.* 116, 129–136. <https://doi.org/10.1111/J.1469-8137.1990.TB00518.X>.
- Restovich, S.B., Andriulo, A.E., Portela, S.I., 2022. Cover crop mixtures increase ecosystem multifunctionality in summer crop rotations with low N fertilization. *Agron. Sustain. Dev.* 42, 19. <https://doi.org/10.1007/s13593-021-00750-8>.
- Rivière, C., Bèthinger, A., Berge, J.E., 2022. The effects of cover crops on multiple environmental sustainability indicators—a review. *Agronomy* 12, 2011. <https://doi.org/10.3390/agronomy12092011>.
- Rodriguez, C., Carlsson, G., Englund, J.E., Flöhr, A., Pelzer, E., Jeuffroy, M.H., Makowski, D., Jensen, E.S., 2020. Grain legume-cereal intercropping enhances the use of soil-derived and biologically fixed nitrogen in temperate agroecosystems. A meta-analysis. *Eur. J. Agron.* 118, 126077. <https://doi.org/10.1016/j.eja.2020.126077>.
- Rummel, P.S., Well, R., Pausch, J., Pfeiffer, B., Dittert, K., 2021. Carbon availability and nitrogen mineralization control denitrification rates and product stoichiometry during initial maize litter decomposition. *Appl. Sci.* 11, 5309. <https://doi.org/10.3390/app11115309>.
- Saha, D., Kaye, J.P., Bhowmik, A., Bruns, M.A., Wallace, J.M., Kemanian, A.R., 2021. Organic fertility inputs synergistically increase denitrification-derived nitrous oxide emissions in agroecosystems. *Ecol. Appl.* 31, e02403. <https://doi.org/10.1002/eap.2403>.
- Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. *Ecology* 85, 591–602. <https://doi.org/10.1890/03-8002>.
- Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9, 671–675. <https://doi.org/10.1038/nmeth.2089>.
- Smith, K.A., Clayton, H., McTaggart, I.P., Thomson, P.E., Arah, J.R.M., Scott, A., 1995. The measurement of nitrous oxide emissions from soil by using chambers. *Philos. Trans. R. Soc. Lond. Ser. A Phys. Eng. Sci.* 351, 327–338. <https://doi.org/10.1098/rsta.1995.0037>.
- Song, X., Parker, J., Jones, S.K., Zhang, L., Bingham, I., Rees, R.M., Ju, X., 2024. Labile carbon from artificial roots alters the patterns of N<sub>2</sub>O and N<sub>2</sub> production in agricultural soils. *Environ. Sci. Technol.* 58, 3302–3310. <https://doi.org/10.1021/acs.est.3c10833>.
- Suter, M., Connolly, J., Finn, J.A., Loges, R., Kirwan, L., Sebastià, M.T., Lüscher, A., 2015. Nitrogen yield advantage from grass–legume mixtures is robust over a wide range of legume proportions and environmental conditions. *Glob. Change Biol.* 21, 2424–2438. <https://doi.org/10.1111/gcb.12880>.
- Thapa, R., Mirsky, S.B., Tully, K.L., 2018. Cover crops reduce nitrate leaching in agroecosystems: a global meta-analysis. *J. Environ. Qual.* 47, 1400–1411. <https://doi.org/10.2134/jeq2018.03.0107>.
- Ussiri, D., Lal, R., 2013. *Soil Emission of Nitrous Oxide and its Mitigation*. Springer Science & Business Media.
- Van Eerd, L.L., Chahal, I., Peng, Y., Awrey, J.C., 2023. Influence of cover crops at the four spheres: a review of ecosystem services, potential barriers, and future directions for North America. *Sci. Total Environ.* 858, 159990. <https://doi.org/10.1016/j.scitotenv.2022.159990>.
- Vanlauwe, B., Nwoko, O.C., Sanginga, N., Merckx, R., 1996. Impact of residue quality on the C and N mineralization of leaf and root residues of three agroforestry species. *Plant Soil* 183, 221–231. <https://doi.org/10.1007/BF00011437>.
- Wendling, M., Büchi, L., Amossé, C., Jeangros, B., Walter, A., Charles, R., 2017. Specific interactions leading to transgressive overyielding in cover crop mixtures. *Agric. Ecosyst. Environ.* 241, 88–99. <https://doi.org/10.1016/J.AGEE.2017.03.003>.
- White, C.M., Dupont, S.T., Hautau, M., Hartman, D., Finney, D.M., Bradley, B., LaChance, J.C., Kaye, J.P., 2017. Managing the trade off between nitrogen supply and retention with cover crop mixtures. *Agric. Ecosyst. Environ.* 237, 121–133. <https://doi.org/10.1016/j.agee.2016.12.016>.
- Whittaker, J., Nyiraneza, J., Zebarth, B.J., Burton, D.L., 2023. Potato and soil 15N recoveries from different labelled forage root and shoot. *Nutr. Cycl. Agroecosys.* 125, 187–204. <https://doi.org/10.1007/s10705-022-10245-x>.
- Yang, X., Xiong, J., Du, T., Ju, X., Gan, Y., Li, S., Xia, L., Shen, Y., Pacenka, S., Steenhuis, T.S., Siddique, K.H.M., Kang, S., Butterbach-Bahl, K., 2024. Diversifying crop rotation increases food production, reduces net greenhouse gas emissions and improves soil health. *Nat. Commun.* 15, 198. <https://doi.org/10.1038/s41467-023-44464-9>.
- Zhang, Z., Kaye, J.P., Bradley, B.A., Amsili, J.P., Suseela, V., 2022. Cover crop functional types differentially alter the content and composition of soil organic carbon in particulate and mineral-associated fractions. *Glob. Change Biol.* 28, 5831–5848. <https://doi.org/10.1111/gcb.16296>.