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The intercropping of fava bean with winter wheat enhances the crop performance and the soil biogeochemical nutrient cycling under contrasting nitrogen inputs

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Abstract

Legume–cereal intercropping supports sustainable crop intensification. However, within-season biogeochemical mechanisms underpinning yield advantages remain poorly resolved. This study monitored winter fava bean and wheat grown as sole crops and alternate-row intercrops under zero and standard N fertilization. Soil and plants were sampled at seven time points spanning establishment, growth, and postharvest. Analyses focused on crop yield, plant N and $\Delta^{13}C$, soil water and pH, nutrient and organic carbon (C) stocks, microbial biomass C and N, enzyme activities, and microbial C use efficiency (CUE). Intercropping increased land productivity under zero N, with a Land Equivalent Ratio > 1, but not under standard N. Fava bean benefited strongly from intercropping, doubling per-plant yield under zero N, while wheat yield per plant was comparable between sole and intercropping. Under zero N, intercropped wheat showed higher biomass N and increased $\Delta^{13}C$, indicating enhanced photosynthetic investment. Post-harvest, fava bean (sole and intercropped) drove higher mineral N stocks in soil than sole wheat under zero N. Soil water use differed: fava bean depleted more water than sole wheat during growth, while intercropping was intermediate. Nutrient dynamics suggested water-mediated shifts in iron, manganese, and zinc availability under intercropping. Among soil biochemical indicators, intercropping primarily affected N cycling, with ~ 26% higher L-leucine aminopeptidase activity than sole fava bean. Broader effects on enzyme activities and CUE were limited. Overall, enhanced intercropping performance arose from complementary water and nutrient use. Results support intercropping as a viable strategy to maintain yield with reduced external N in humid temperate conditions.

Introduction

Globally, crop diversification strategies are receiving increasing attention (Ehrmann and Ritz 2013). This is due to the need to enhance the ecosystem service provision of agroecosystems while intensifying crop production (Duchene et al. 2017). Legume-cereal intercropping has emerged as a very promising strategy to achieve these aims (Yu et al. 2016; Raseduzzaman and Jensen 2017; Gui et al. 2024). It consists of the contemporary cultivation of two or more plant species in close proximity, allowing a full interaction between the crops. The major advantages of the intercropping are related to the physiological complementarity of the two or more crop species, which can be exploited to boost nutrient cycling efficiency (Xue et al. 2016; Duchene et al. 2017; Yu et al. 2022; Picone et al. 2025). Optimizing the plant–soil feedback in legume-cereal intercropping systems will therefore be fundamental to maximize both the yield per hectare and the ecosystem service provision.

Previous research has focused on soil water, nitrogen (N), phosphorus (P), and organic carbon (OC) dynamics under different legume-cereal intercropping systems. The results of these indicate that soil water usage differs between the intercropping and the relative sole-cropping systems. Although some evidence suggests that a more efficient soil water use is possible (Chapagain and Riseman 2015; Durodola et al. 2025), the effects might vary based on the water availability and species-specific demands (Wang et al. 2022; Tuure et al. 2025). Generally, a more efficient water use could be possible under multi-species systems because of the diverse root architectures of the different crops (Durodola et al. 2025). Nevertheless, a more comprehensive understanding of the role of soil water in the crop interspecies interaction dynamics may suggest more targeted management options. Previous studies have also found that legumes can effectively increase the soil N and P availability profiting their cereal partners, thus leading to an enhanced crop nutrition (Jensen et al. 2020; Tang et al. 2021). In some cases, positive effects on the soil OC accrual have also been observed (Gong et al. 2019; Malviya et al. 2021; Parvin et al. 2023). However, within-season dynamics of soil nutrients and OC are intrinsically related to the crop-development stage. Comprehending how the soil elemental stocks vary during all the crop phenological stages with and without N fertilization could

allow us to make more informed decisions over the tradeoffs between crop productivity and fertilizer savings. Furthermore, the dynamics of the other macro- and micro-nutrients were generally overlooked in previous studies. This further hinders our understanding of the interspecies interactions that guide the improvement of the plant performance and the large-scale implementation of the legume-cereal intercropping.

Recently, considerable attention has been given to the soil and root microbial processes that result in the more efficient nutrient cycling (e.g., Zhao et al. 2017; Gong et al. 2019; Liu and Gong 2022). Most of the studies focused only on one or two sampling times, usually during the active plant-growth period (i.e., close to the reproductive stage). However, soil microbial dynamics are complex, and the crop phenology can have a very extensive impact on the selection of microbial functional groups (Chaparro et al. 2012; Philippot et al. 2013). During the phase of active plant growth, the influence of crop metabolism per unit of soil volume, i.e., the amount of soil colonized by the roots, on microbial functionality is anticipated to be at its peak. Conducting most of the samplings during this stage may pose a risk of misinterpreting the processes that contribute to the enhanced crop performance due to the possibility of overlooking preceding microbial functionality dynamics (Nannipieri et al. 2020).

In this study, we examined the impact of intercropping winter variants of fava bean and wheat under zero and standard doses of N fertilization on three key areas: (1) crop performance, assessed through yield, crop N acquisition, and $\Delta^{13}C$ discrimination; (2) alterations in soil water, nutrient, and C stocks; and (3) the dynamics of microbial biochemical functionality evaluated through the soil microbial biomass C and N stocks, the enzyme activities, and the C use efficiency. We posited the following hypotheses: (1) intercropping the fava bean with the winter wheat might enhance the crop performance by improving the physiological status of both crops, with the greatest enhancement occurring under the condition of no N fertilizer application; (2) the intercropping may facilitate a superior management of soil elemental resources compared to the sole-cropping systems, potentially leading to available-N levels comparable to the optimal N fertilization of the sole wheat, increased water availability, improved crop nutrient uptake, and augmented soil OC stocks; (3) the availability of C and N for soil microbial communities may be higher, resulting in an increased accrual in soil microbial biomass and a greater total resource allocation to enzyme production.

Materials and methods

Experimental field trial

A field trial was established in October 2023 near Asendorf, Germany (N 52.79327 E 9.025958). The soil is characterized as Plaggic Anthrosol (IUSS Working Group WRB 2022), affected by a long history of agricultural use (detailed description in the Supplementary Material Section 1). The climate is temperate oceanic (cfb) according to Köppen-Geiger climate classification (Beck et al. 2018) with a mean annual temperature of 10.6°C and summed annual precipitation of 827 mm (long-term mean 2015 to 2024, see Fig. S2). During the experimental period, the region experienced exceptionally high levels of precipitation, totaling 1172 mm from October 2023 to October 2024. Notably, the substantial winter precipitation resulted in waterlogging, the proliferation of fungal diseases, and the complete failure of some other winter legume crops. The experiment was conducted across two blocks utilizing a Completely Randomized Block Design (CRBD). The blocks corresponded to two levels of the N fertilization factor: the standard N fertilization (fertilized according to regional standard recommendations) and the zero N (no N fertilizer applied). Each block included 2 rows and 8 columns (Fig. 1 and Fig. S1). Each treatment was replicated twice within each row. The intercropping treatments incorporated distinct seeding methods: alternate row and mixed row seeding. This study specifically concentrated on the alternate row seeding method. The crops were sown

on the 18th of October 2023 and harvested on the 28th of August 2024. For the cropping system factor, three treatment levels were evaluated, i.e. sole cropped winter wheat (*Triticum aestivum* L., variety Champion), sole cropped fava bean (*Vicia faba* L., variety Augusta), and their intercropping. The sowing ratios applied were 25 seeds m^{-2} for fava bean and 250 seeds m^{-2} for wheat. In the intercropping, the sowing ratio applied was half of the one used for each sole crop. The seeds were sown in rows with a width of 11 cm. Regarding the nitrogen (N) fertilization factor, two levels were evaluated: the standard N dose typically applied to wheat and the absence of fertilization. This corresponded to the application of 136 kg ha^{-1} of N for the sole wheat treatment, and to 68 kg ha^{-1} of N for the intercropping treatment. The AHL28 N fertilizer was applied as a solution which contained 28% N, of which 12% as carbamide N, 7.5% as nitrate N, and 7.5% as ammonium N. No N fertilization was applied to the sole-cropped fava bean. Half of the fertilizer dose was applied on the 18th of March and the second half on the 12th of April. All plots received the equivalent of 74.7 kg ha^{-1} of K, 18.09 kg ha^{-1} Mg, and 52.8 kg ha^{-1} of S.

The following abbreviations were employed in the crop performance plots: *INT-FB-SN* denotes intercropped fava bean – standard N; *INT-FB-ZN* denotes intercropped fava bean – zero N; *SC-FB-ZN* denotes sole-cropped fava bean – zero N; *INT-WW-SN* denotes intercropped winter wheat – standard N; *INT-WW-ZN* denotes intercropped winter wheat – zero N; *SC-WW-SN* denotes sole-cropped winter wheat – standard N; *SC-WW-ZN* denotes sole-cropped winter wheat – zero N. In the plots where the soil parameters are reported, the treatment names are as follows: *FB-WW-SN* for the intercropping – standard N; *FB-WW-ZN* for intercropping – zero N; *FB-ZN* for fava bean – zero N; *WW-SN* for sole wheat – standard N; *WW-ZN* for sole wheat – zero N.

Soil and plant sampling

Soil and plant samples were collected at seven distinct time points (Table 1). Bulk soil samples, from a depth of 0 – 10 cm, were obtained using a shovel, which was thoroughly cleaned after each collection. Rhizosphere soil was collected by detaching it manually from the roots after carefully extracting the plants and removing the excess soil that did not adhere. Bulk soil samples were collected at depths of 10 – 30 cm, 30 – 60 cm, and 60 – 90 cm using an auger. Aboveground plant biomass was sampled by cutting off the plants at the base. The plant biomass was then dried at room temperature until further processing, as described below. All the soil samples were immediately stored on ice, transported to the lab and an aliquot of each was frozen and kept at -20°C for the chemical analyses while another was used for the enzyme activities. Following the extraction of mineral nitrogen, as detailed below, the remaining portion of the sample was dried at 45°C for a minimum duration of 48 hours, until complete desiccation was achieved. For the measurement of the microbial biomass C and N, an aliquot of fresh soil was stored at 4°C. Soil samples were sieved to 2 mm in the field and all visible roots were removed. In instances where the high water content precluded the sieving, only the visible roots were excised. The rhizosphere soil samples from the intercrops were collected as a unique sample, as the roots of both species inhabit the same rhizosphere.

Sampling dates were grouped in three phases, i.e. the plant-establishment (sampling times 2 and 3), the active plant-growth (sampling times 4, 5 and 6) and the post-harvest phase (sampling time 7). The sampling time 1 was used as a reference of the basal soil conditions before the differences induced by the treatment were apparent. The details are reported in the “statistical analyses” section.

Table 1

Sampling dates. This table reports the dates on which the samplings were conducted, with the details on the phenological stages of the two crops. The type of samples that were collected is reported in the last column of the table.

Sampling time	Date	Wheat phenological stage	Fava bean phenological stage	Sample type
1	2024/02/26	Tillering	Leaf development	Bulk and subsoil
2	2024/03/21	Tillering	Side shoot formation	Bulk soil
3	2024/04/18	Early stem extension	Stem elongation	Bulk soil, plant biomass
4	2024/05/07	Stem extension	Flowering	Bulk and rhizosphere soil, plant biomass
5	2024/06/06	Grain filling	Fruit development	Bulk and rhizosphere soil, plant biomass
6	2024/07/17	Maturity	Maturity	Bulk and rhizosphere soil, plant biomass
7	2024/09/24	Post harvest	Post harvest	Bulk soil

Crop yield measurement

Crop grain yield was measured at the maturity stage, after harvesting the whole plots with a combine harvester. Residual crop moisture was measured gravimetrically, and the crop yields were standardized to 13% residual moisture. At the same time, the tiller density of the wheat and the shoot density of the fava bean were measured. The yields were calculated per hectare and per plant, that is, by normalizing the yields by the sowing density to account for different sowing ratios. The yield per hectare was employed to assess the overall land productivity and facilitate the comparison with the sole cropping systems, as it represents the most suitable metric for evaluating the intercropping efficiency (Willey 1979). Conversely, the yield per plant was analyzed to elucidate the effects of the interspecific interactions (Francis 1986). The Land Equivalent Ratio (LER) was calculated according to Wit and Bergh (1965) and Willey and Osiru (1972) as:

$$LER = \frac{Intercopped\ cereal\ yield\ \left(t\ ha^{-1}\right)}{Sole-cropped\ cereal\ yield\ \left(t\ ha^{-1}\right)} + \frac{Intercropped\ legume\ yield\ \left(t\ ha^{-1}\right)}{Sole-cropped\ legume\ yield\ \left(t\ ha^{-1}\right)}$$

Soil texture measurement

An aliquot of one sample from each plot collected at the sampling time 6 was used to determine the soil texture through particle-size distribution analysis by laser diffraction (Helos/KR, Sympatec GmbH, Clausthal-Zellerfeld, Germany). Particle-size classes < 2, 2–63, and 63–2000 µm were used to classify clay, silt, and sand, respectively (Jahn et al. 2006). Prior to the analysis, the soil was pretreated with 0.05 M sodium pyrophosphate ($Na_4P_2O_7{\cdot}10H_2O$) and subjected to fully automated sonication at 60 W (Quixel, Sympatec GmbH, Clausthal-Zellerfeld, Germany) to ensure a complete dispersion (Koza et al. 2021; Polakowski et al. 2023). Further details are provided in the supplementary materials in the Section 2 and the Fig. S3.

Soil nutrient extraction and quantification

Ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N) were extracted from fresh soil samples with a 12.5 mM solution of $CaCl_2$ (1:10 soil to solution ratio, w:v) and quantified spectrophotometrically using an autoanalyzer (SAN-plus, Skalar Analytical B.V., Breda, The Netherlands). The sum of NH_4^+-N and NO_3^--N represents the plant available mineral N fraction (N_{min}-N). Soil water content (WC) was determined gravimetrically after drying one aliquot of the sample at 105°C for 24 hours. Different plant-available macro- (phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca)) and micro-nutrients (zinc (Zn), copper (Cu), iron (Fe), and manganese (Mn)), and aluminum (Al) were quantified in this study by following the methodology described in Heuermann et al. (2022). Briefly, the elements were extracted with the Mehlich 3 solution from dry soil (1:10 soil:solution ratio, w:v) by shaking for 5 mins. Subsequently, the samples were centrifuged at 3000 × g for 10 mins. Before the measurement, the clear extract was diluted 1:10 with 8 ml of water and 1 ml of HNO_3 10%. Elements were measured with an ICP-OES (Varian 725-ES, Palo Alto, California). The sum of the concentrations of these elements (measured as cmol (cations) kg^{-1}) was used to calculate the effective cation exchange capacity (CEC_{eff}).

Soil and plant organic carbon and total nitrogen

Dry soil and plant samples were milled using a ball mill and completely dried at 45°C overnight. For organic C (OC) and total N (TN) analyses, between 50 to 60 mg of soil samples and 5 to 10 mg of plant samples were weighed into tin boxes. Organic C and TN concentrations and their stable isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) were measured using an Elementar Vario MICRO cube EA C/N analyzer (Elementar Analysen systeme GmbH, Hanau, Germany) coupled to an Elementar IsoPrime 100 IRMS (IsoPrime Ltd., Cheadle Hulme, UK). Isotope discrimination was calculated using the following equation (Farquhar and Richards 1984):

$$\Delta\,^{13}\mathrm{C} = \frac{(\delta\,^{13}C_s\ -\ \delta\,^{13}C_p)}{(1\ +\ \delta\,^{13}C_p)}$$

where C_s is the $\delta^{13}C$ signature of the atmosphere as the source and C_p is the signal of the plant. For the atmosphere, we apply − 8‰ as an average source signal (Seibt et al. 2008).

Nutrient and carbon stocks

Soil N_{min}-N stocks were measured for the whole profile at the beginning and at the end of the experiment. For this purpose, soil samples were collected up to a depth of 90 cm. The sampling depth was divided into four and three depths at the first and last sampling times, respectively. The soil nutrient and OC stocks were measured at all the sampling times in the topsoil. To measure soil bulk density (BD) gravimetrically, samples were collected using a 100-cm^3 stainless-steel cylinder. Afterward, the soil was dried at 105°C for 24 hours. OC and nutrient stock differences were calculated for the topsoil. The formula used was the following:

$$Stocks = Nutrient\ or\ OC\ concentration\ \times\ Bulk\ density\ \times\ Soil\ depth\ \times\ Conversion\ factor$$

Soil microbial biomass carbon and nitrogen

Soil microbial biomass C and N (MBC and MBN) were extracted with a modified version of the traditional chloroform fumigation and extraction method (Brookes et al. 1985), as described by Wachendorf and Joergensen (2011). Fresh soil samples were stored at 4°C and processed within less than 7 days after the collection. For each sample, two aliquots of 10 g of fresh soil were weighed into 50 ml glass tubes, one to be fumigated and one to be used as the non-fumigated reference. A pre-extraction of the excess N_{min}-N was performed with 0.5 M K_2SO_4 for 15 mins, followed by a centrifugation for 5 mins at 3500 g. The supernatant was then discarded. Afterward, the extraction of the unfumigated aliquots was done in the same way, without any incubation time, since it was demonstrated by Witt et al. (2000) that the extraction of C and N from the unfumigated samples does not change significantly between 0 and 48 hours of incubation at room temperature. Ethanol-free chloroform (300 µL) was directly added to fumigate the samples. They were then shaken and incubated at room temperature with the lids closed for 48 hours. Thereafter, chloroform was evacuated at atmospheric pressure by removing the caps and allowing it to evaporate for at least 1.5 h under a flow hood. The extraction of the fumigated samples was carried out as previously described for the other aliquots. An empty tube was used for the unfumigated blank, while an empty tube was used for the fumigated sample in which chloroform was added, incubated, and evacuated. Extracts were vacuum filtered using Whatman 42 filters. The total OC (TOC) and the TN content in the soil extracts were measured after the dilution with water (1:4) and the acidification with HCl (25 µL into 10 mL) using a Liqui TOC II elemental analyzer (Elementar Analysen systeme GmbH). The MBC and MBN values were calculated as the difference between the TOC and the TN in the fumigated and unfumigated samples. The recovery factors used for this modified version of the protocol were MBC = 50,497 + 2,3736*$TOC_{difference}$ and MBN = 24,405 + 1,877*$TN_{difference}$. The details of the calculation of the new conversion factors are reported in the supplementary materials (Section 3, Figs. S4 and S5). The stocks were calculated as described in the previous paragraph.

Soil enzyme activities and microbial carbon use efficiency

Soil enzyme activities were measured with the traditional colorimetric method that uses molecules, either linked to para-nitrophenol (pNP) or para-nitroaniline (pNA), which mimic the natural substrates of the enzymes. The enzyme activities quantified for this study were the β-glucosidase (BG), the acid and alkaline phosphatases (ACP and ALP), the N-acetyl-β-d-glucosaminidase (NAG), and the L-leucine aminopeptidase (LAP). The methods used for the β-glucosidase and the phosphatases were the ones described in Alef and Nannipieri (1995). The NAG activity was assessed according to the standard operative procedure described by the Soils Lab (University of Illinois Urbana-Champaign 2021a). The L-leucine aminopeptidase activity was quantified based on the standard operative procedure described by the Soils Lab (University of Illinois Urbana-Champaign 2021b). Slight modifications were made to these two protocols by using half the amount of soil (0.5 g instead of 1 g) and half the volume of all the reagents. The standards used were solutions of pNP and pNA incubated and terminated like samples and appropriately diluted after the incubation. Depending on the method, they were prepared either in the appropriate buffer solution, or in water. 200 µl of each standard and sample was pipetted in triplicate into Sarsted 96-well flat-bottom transparent microplates. The measurement was done in a SPECTROstar Nano microplate reader (BMG Labtech, Freiburg, Germany) by reading the absorbance at either 410 nm (for pNP-linked substrates) or 380 nm (for pNA-linked substrates). The linearity of the calibration curve was checked by considering a R^2 value > 0.99. The results were expressed as µg pNP or pNA g^{-1} dry soil h^{-1}.

The C:N microbial C Use Efficiency (CUE) was calculated according to the stoichiometric model developed by Sinsabaugh et al. (2016). Briefly, the Ecoenzymatic Activity C:N ratio ($EEA_{C:N}$) was calculated as follows:

$$EEA_{C:N} = \frac{BG}{NAG + LAP}$$

The substrate availability ($S_{C:N}$) was calculated as follows:

$$S_{C:N} = \frac{1/EEA_{C:N}}{MBC : MBN \ / \ Soil\ C : N}$$

Finally, the $CUE_{C:N}$ was calculated as follows:

$$CUE_{C:N} = 0.6 * \left(\frac{S_{C:N}}{S_{C:N} + 0.5} \right)$$

where 0.6 is the maximum possible CUE value, due to thermodynamic limitations, while 0.5 is the value of the half-saturation constant (K_x) of the $S_{C:N}$ scalar.

Statistical evaluation

All the statistical analyses were performed in R version 4.5.2 (R Core Team 2021). Before each of the statistical evaluations, the distribution of the variables was analyzed for the skewness and the appropriate transformations were performed. Soil heterogeneity across rows and columns at the beginning of the experiment was evaluated through linear mixed-effect models and plotted as heating plots. The results are described in the supplementary materials (Section 4, Fig. S6). Six variables were significantly different. Since biochemical proxies (the dependent variables) are particularly affected by the edaphic parameters such as the pH, OC or texture (the confounding variables), they were included as covariates in linear models. Identifying and adjusting for confounding variables is crucial to accurately determine the true nature of the relationship between variables under study and to draw valid conclusions from the statistical analyses. For the main dependent variables, i.e. the crop performance, plant elemental and isotopic composition, soil mineral N stocks, and biochemical variables, simple or mixed-effect linear models were used by implementing linear (*lm*) or mixed-effect model functions (*lmer*) (Bates et al. 2003), respectively. Specifically, in the case of the crop variables, simple models were used for each of the combinations of the cropping phase and the plant species without implementing any covariates. In the case of the mineral N stocks and the biochemical parameters, the seven sampling times were divided in the three phases described above. The sampling time 1 was used as an indicator of the soil basal conditions at the beginning of the experiment. The edaphic factors measured at time 1 were used as the covariates for the models in the other sampling times. They were chosen based on their relevance for the response variable of interest. The choice of using a simple or a mixed-effect model depended on the number of sampling times included in each phase. The treatment was here intended as the combination of the cropping system (sole or intercropping) and the fertilization level (standard or zero N level). The function *dredge* of the MuMIn package was used for choosing the best model (Bartoń 2010). The model residuals were inspected for the deviation from normality and homoskedasticity. The estimated marginal means and their 95% confidence intervals were calculated and plotted over the raw data (red dots and lines) through the *emmean* and the *cld* functions of the emmeans and lsmeans packages, respectively (Lenth and Piaskowski 2017). A type 2 ANOVA was used to assess the significance of the treatment followed by a pairwise comparison with Bonferroni's correction. If the treatment had a significant effect, and there were significant differences among the groups, the letters indicating the significance levels were reported in the plots.

Non-metric multidimensional scaling (NMDS) was used to evaluate the differences in the soil bio-chemical parameters among the treatments, and the physicochemical drivers of said differentiations. This was done after

transforming the biochemical and the physicochemical variables when they were affected by skewness with the Yeo-Johnson transformation through the bestNormalize package (Peterson 2017). Subsequently, the biochemical variables were Z-standardized. The NMDS coordinates were calculated through the *metaMDS* function of the vegan package (Oksanen et al. 2001) based on the Euclidean distance. The significance of the treatment effect was assessed with the adonis2 function (vegan package). Differences among the groups were evaluated with the pairwise.adonis function by making 999 permutations (https://raw.githubusercontent.com/pmartinezarbizu/pairwiseAdonis/master/pairwiseAdonis/R/pairwise.adonis.R). The groups were considered significantly different at adjusted p-value < 0.05. The 95% confidence intervals of the centroid were extracted and plotted. The correlation with the soil physicochemical properties was evaluated with the *envfit* function (vegan package) with 999 permutations. Significantly correlated parameters ($p \leq 0.05$) were plotted as gradients in the NMDS plots by implementing the *ordisurf* function (vegan package).

In the absence of the N treatment for the sole-cropped fava bean, a fully factorial analysis of the cropping system and N fertilization was not feasible. Instead, we conducted targeted comparisons of the cropping systems within each distinct N fertilization level (zero or standard N). The effects of the fertilization were evaluated exclusively within the sole wheat and intercropping systems. This methodological approach prevents confounded comparisons, such as comparing the zero-N sole wheat with the standard-N intercropping, thereby ensuring that the variations in the soil and the plant properties are interpreted within contextually relevant agronomic parameters.

The Spearman correlation was plotted between the grain yield and the top-soil nutrient stock changes between the sampling times 1 and 6, and between the soil biochemical and physicochemical properties. All the scripts and the original data are reported at the following link: 10.5281/zenodo.20035719

Results

Crop yields

The results show that both the cropping system and the N fertilization exerted a significant impact on the yield. The total crop yield per hectare was markedly higher in the sole wheat with the standard N and in both the intercropping treatments compared to the zero-N sole wheat (Fig. S7). The wheat yield per-plant reached its peak in the sole wheat with the standard N, whereas the intercropping with the zero N resulted in the lowest per-plant yield (Fig. S8a). In contrast, the performance of the fava bean was markedly improved through the intercropping (Fig. S8b). The highest per-plant yields were observed in the zero-N intercrop (22.33 ± 2.10 g plant^{-1}), more than doubling the yield of the sole crop (10.94 ± 1.48 g plant^{-1}). Furthermore, the intercropping led to a significant increase of the LER under the zero-N treatment (1.4 ± 0.15), but it was not significantly higher than 1 under the standard N (1.1 ± 0.15) (Fig. S9).

Crop elemental composition

The results indicated that both the N fertilization and the cropping systems exerted a significant influence on the $\Delta^{13}C$ in the wheat biomass across the various growth stages of the wheat (Fig. 2). Specifically, the fertilization led to a notable increase in the sole wheat $\Delta^{13}C$, rising from 21.00 ± 0.14 to 21.65 ± 0.14‰ during the early stem-extension stage, from 18.67 ± 0.28 to 20.11 ± 0.28‰ in the grain-filling stage, and from 18.96 ± 0.04e-02 to 19.83 ± 0.13‰ in the maturity stage (Fig. 2a, c, and d). Furthermore, the intercropping induced a significant increase in the wheat $\Delta^{13}C$, from 21.01 ± 0.14 to 21.75 ± 0.14‰ during the early stem-extension stage, and from 18.67 ± 0.28 to 21.21 ± 0.28‰ in the grain-filling stage under the zero N (Fig. 2a and c). Figure 2e shows that the wheat biomass

$\Delta^{13}C$ exhibited a significant positive correlation with the TN throughout the entire growing season. However, the fava bean biomass $\Delta^{13}C$ was not influenced by any treatment throughout the growth period, except during the flowering stage, where the biomass $\Delta^{13}C$ was significantly higher in the sole cropping compared to the intercropping under the zero N (Fig. S10b).

The fertilization significantly increased the TN content in the biomass of the sole-cropped wheat, from 1.89 ± 0.19% to 3.32 ± 0.19% during the early stem-extension stage, and from 1.12 ± 0.09% to 1.83 ± 0.09% during the stem-extension stage (Fig. S11a and b). A comparable effect was observed in the intercropping system, where the fertilization led to an increase from 2.07 ± 0.19% to 3.15 ± 0.19% in the early stem-extension stage (Fig. S11a). In the absence of the N fertilization, the intercropping stimulated the N accumulation in the wheat biomass, increasing from 0.72 ± 0.06% to 1.17 ± 0.06% during the grain-filling stage, and from 0.73 ± 0.06% to 1.16 ± 0.06% during the maturity stage (Fig. S11c and d). No significant changes in the TN content were observed in the fava bean across the different growing phases (Fig. S12).

Soil physicochemical properties

Mineral N stocks

The topsoil total N_{min}-N, NH_4^+-N, and NO_3^--N stocks were significantly influenced by the fertilization during the sampling times 2 and 3 (Fig. S13a, b, and c). Specifically, the N_{min}-N, NH_4^+-N, and NO_3^--N stocks were significantly higher under the fertilized sole wheat and the intercropping compared to the relative unfertilized treatments at the sampling 2. The trend was similar at time 3, but only for NO_3^--N, while the N_{min}-N and NH_4^+-N stocks only differed under the wheat between the two N fertilizer levels. Furthermore, at the sampling time 2, all three stocks were significantly higher under the fertilized sole wheat than the intercropping. In addition to this fertilizer-induced effect, at the post-harvest sampling time, the NO_3^--N stocks were significantly higher under the intercropping and the sole fava bean compared to the wheat treatment at the corresponding N fertilizer level.

Under the zero-N treatment, the N_{min}-N stocks and their net differences at the end of the cropping season were significantly higher in the sole-cropped fava bean and the intercropping systems compared to the sole wheat, with values of 95.87 ± 7.39 and 80.30 ± 10.46 vs 21.81 ± 10.46 kg ha^{-1}, and 72.38 ± 7.74 and 67.9 ± 10.94 vs 8.21 ± 10.94 kg ha^{-1}, respectively (Figs. 3a and S14a). A similar pattern was observed for the NO_3^--N stocks and their net differences, with values of 89.23 ± 6.67 and 73.07 ± 9.44 vs 18.43718 ± 9.44 kg ha^{-1}, and 70.36 ± 7.04 and 65.26 ± 9.95 vs 9.50 ± 9.95 kg /ha^{-1} 1, respectively (Figs. 3c and S14c). The NH_4^+-N stocks, however, were not significantly influenced by the treatment (Fig. 3b and Fig. S14b).

Soil water content

The results indicate that the effects of the treatment on the soil WC are the most evident during the active-growth phase, while they dissipate entirely following the harvest (Fig. 4). During the active plant growth, the fava bean exhibited the lowest WC among all the treatments in both the bulk soil and the rhizosphere (Fig. 4b and 4c). Conversely, the winter wheat under the zero N demonstrated significantly higher moisture retention during this period. These differences were temporary, as all the treatments converged to a uniform moisture level of approximately 21% during the post-harvest phase (Fig. 4d). Nevertheless, when assessing the entire cropping season, the results suggest that the winter wheat under the zero N consistently maintained higher soil moisture compared to the fava bean (Fig. 4e).

Soil pH

The soil pH was significantly influenced by the cropping system throughout the entire cropping season, except for the plant-establishment stage (Fig S15). Notably, the soil pH was considerably lower in the sole fava bean compared to the sole wheat under the zero N in the active-growth phase, in the post-harvest phase, and over the whole season (Fig. S15b, c d, and e). The alterations were consistent across the rhizosphere and the bulk soil compartments (Fig S15b and c).

Soil OC

The OC stocks remained stable across the various treatments, as no significant differences were observed due to either the cropping system or fertilization (Fig S16a). Furthermore, no differences in the OC concentration were observed in the rhizosphere soil in the active plant-growth phase (Fig. S16b).

Soil macro- and micronutrients

The N fertilization and the cropping system exerted a significant influence over the soil macro- and micronutrient stocks throughout the growing season (Table S1). The standard N application generally enhanced the stocks of Ca, Fe, Mn, and Zn in the sole-cropped wheat compared to the treatment without the N. This fertilization effect was consistent also under the intercropping system in the case of the Zn stocks. In contrast, the available K stocks were significantly higher under the zero than the standard N in the wheat systems. Regarding the cropping system, the sole wheat exhibited higher Ca stocks than the intercropping under the standard N, whereas the sole fava bean demonstrated higher stocks of Fe, Mn, and Zn under the zero N compared to the wheat.

In the rhizosphere soil, the changes in the nutrient concentrations were limited in scope. Specifically, the K concentration was significantly higher under the sole wheat than the fava bean, while the Mn concentration followed the opposite trend (Table S2). The intercropping only had an impact on the Fe availability by inducing its decrease compared to the sole wheat under the zero N dose.

The correlation between the plant yield and the soil nutrient stock changes

The yield of the sole wheat with the standard N fertilization exhibited a significant positive correlation with the available NH_4^+-N and K net stock changes (Fig. S17). In contrast, the wheat yield under the other treatments, whether in the sole or the intercropping, did not demonstrate any significant correlation with the soil nutrient stock changes. The sole fava bean yield was significantly positively correlated with the Al and Mn stock differences, while it was negatively correlated with the Zn stock changes and the mean water availability across the season. Furthermore, the fava bean yield under the intercropping with the zero N exhibited a significant negative correlation with the Fe and Mn net stock differences. The correlation analysis also revealed that none of the net differences in the available soil nutrients had a significant positive correlation with the LER (Fig. S18). The LER under the zero N treatment showed a significant negative correlation with the Fe and the Mn net stock changes, whereas the LER under the standard N treatment had a significant positive correlation with the mean water content across the season.

Soil biochemical properties

The LAP was significantly higher in the intercropping compared to the sole fava bean under the zero N, but only by assessing the entire cropping season (Fig. 5e). No significant changes were observed among any treatments during the independent growth phases (Fig. 5). Although the BG, the ALP and the CUE were significantly affected by the treatment, the differences were observed only among the treatments with both contrasting fertilization levels and cropping systems (Table S3). No significant changes were detected for the ACP and the NAG activity under any treatment during any growth period. Moreover, a similar trend was also observed for the MBC, MBN, both the stocks and the concentrations, and the MBC:MBN (Tables S1 and S3).

The NMDS analysis demonstrated that the treatment exerted a significant influence on the data ordination during both the plant-establishment and active plant-growth phases, in both the bulk and the rhizosphere soil (Fig. 6a, b, and c). However, no significant differences were detected in the pairwise comparisons during the plant-establishment phase and the active plant-growth phase within the rhizosphere soil (Fig. 6a and c). The observed differences among the groups during the active plant-growth phase in the bulk soil were attributed to the interaction between the cropping system and the fertilization (Fig. 6b).

Furthermore, during the plant-establishment phase, the primary factors influencing the differences among the treatments were the pH, WC, Mg, Ca, Fe, and K (Fig. S19a to f). In the active plant-growth phase within the rhizosphere soil, the main drivers of the differences among the treatment groups were the pH, WC, OC, Mg, and Ca (Fig. S20a to e). In the post-harvest phase, the key factors contributing to the differences among the groups were BD, the C:N, and CEC_{eff} (Fig. S21a to c).

Discussion

The crops perform equally or better in the intercropping compared to the sole cropping

The results of this study indicate that the wheat exhibited a comparable grain yield per plant, regardless of whether it was cultivated as a sole crop or in an intercropping system (Fig. S8a). In contrast, the fava bean showed a higher yield per plant in the intercropping system compared to the sole cropping (Fig. S8b). This suggests that the intercropping significantly increases the fava bean plant productivity, potentially by reducing the intraspecific competition or by promoting facilitative interactions with the wheat (Xiao et al. 2018; MacLaren et al. 2023). The enhanced plant performance can be attributed to the increased availability of the water and nutrients in the soil for the fava bean within the intercropping system (see below). Furthermore, the wheat yields remained notably consistent in both the zero and the standard N intercropping systems. This suggests that the biological N fixation by the fava bean adequately fulfilled the nutrient demands of the wheat, thereby eliminating the need for supplementary N to maintain the productivity (Zhang et al. 2024). Previous research indicates that the N fixed by the legumes which is then uptaken by the cereals can be substantial, from 3.9 to 35.3 kg N ha^{-1} over the whole season (Zhao et al. 2022) or 8.1% of the triticale biomass N (Hupe et al. 2021). This amount is largely dependent on the cereal and legume species combination, as well as their interaction with environmental variability (Zhao et al. 2022). The increase of the LER from the fertilized to the unfertilized treatments demonstrates that the complementarity effects between the wheat and the fava bean were significant in the intercropping system under the zero N fertilization (Ehrmann and Ritz 2013; Duchene et al. 2017). This may be attributed to the limited N availability in the soil for the sole wheat under the zero N conditions (Fig. S13), a limitation that was mitigated in the intercropping system due to the presence of the fava bean. Overall, our findings underscore that legume-cereal intercropping can

serve as an effective strategy to enhance the crop yield while minimizing the need for external N inputs, thereby supporting our first hypothesis.

The intercropped wheat plants increase their photosynthetic activity

The wheat demonstrated significant benefits from the intercropping, particularly in terms of the investment in photosynthetic activity and biomass TN accumulation (Figs. 2a and c, S11c and d), especially under the zero N application. This finding further supports our first hypothesis. The phenomenon may be attributed to the C3 photosynthetic pathway of the wheat. Specifically, the carbon isotopic ratio in C3 plants is primarily determined by the discrimination processes during the photosynthesis, which is influenced by the physiological status of the plant and its interaction with the environment (Peterson and Fry 1987; Brüggemann et al. 2011). The plants discriminate against the heavier ^{13}C isotope through two primary mechanisms: the diffusion of CO_2 via the stomata and the enzymatic carboxylation of CO_2 by the Rubisco. As proposed by Condon et al. (2002), isotope fractionation is influenced by the magnitude of the stomatal conductance and the ratio of the CO_2 concentration within the leaf intercellular air spaces (c_i) to the ambient CO_2 concentration in the atmosphere (c_a). The ci/ca ratio is elevated when the CO_2 supply surpasses the demand, which is the consequence of the plants maintaining the stomata open for relatively extended periods. This phenomenon underscores the intricate relationship between the C acquisition and the potential water loss at the stomatal level. Consequently, $\delta^{13}C$ values are frequently employed as indicators of intrinsic water use efficiency (Seibt et al. 2008). A high ci/ca ratio induces the plants to preferentially fix the lighter ^{12}C isotope, thereby discriminating against the heavier ^{13}C isotope, resulting in increased $\Delta^{13}C$ values. The stomatal conductance and the photosynthetic capacity are closely linked to the environmental conditions and the nutrient status of the plant.

In our study, we observed a significantly enhanced ^{13}C discrimination in the unfertilized treatments when the wheat plants were cultivated as intercrops compared to the sole crops (Fig. 2a and c). Moreover, the observed correlation between the $\Delta^{13}C$ and the biomass TN content indicated that the ^{13}C discrimination may be intricately linked to the N supply available to the wheat plants (Fig. 2e). Additionally, the soil water was abundantly accessible to the plants throughout the whole growing season (Figs. 4 and S2). The soil moisture levels consistently remained above 15%, significantly exceeding the soil permanent wilting point of approximately 10% of the dry weight at the given soil texture (Fig. 4). The increased ^{13}C discrimination observed in the intercropped wheat plants under the zero N conditions and sufficient water availability can likely be attributed to the enhanced stomatal conductance compared to the sole-cropped wheat under the same N conditions. This elevated CO_2 demand suggests heightened photosynthetic activity (Condon et al. 2002). Most likely, the intercropped wheat plants augment their CO_2 supply through an increased stomatal conductance to a degree that more than compensates for the drawdown from the enhanced carboxylation. Under the favorable conditions of higher N and adequate water availability, the intercropped wheat can sustain a higher stomatal conductance, thereby effectively utilizing the water to maximize the C gain. Although the intercropped fava bean may have experienced a temporary disadvantage in its interaction with the wheat (Fig. S10b), this effect was negligible over the whole season.

The increased accumulation of TN in the wheat biomass under the intercropping with the zero N application observed from the grain-filling stage onward (Fig. S11c and d) may have driven the enhanced photosynthetic activity. This phenomenon could be attributed to the critical role of N in the assembly of photosynthetic proteins, whose abundance is associated with the stomatal conductance (Condon et al. 2002; Seibt et al. 2008). The

increased photosynthetic activity in the wheat may be directed toward maximizing the seed production and enhancing their TN content, resulting in a greater number of seeds with a higher protein content. Additionally, the impact of the N fertilization under the intercropping conditions was not apparent in the later growth stages, suggesting that the soil N levels were likely sufficient to satisfy the plant requirements (Fig. S11c, and d). This might be attributed to the fact that the majority of the N release from the legume crops occurs near the maturity, which may account for the absence of the effects in the earlier stages (Pappa et al. 2011; Zhang et al. 2024). Most likely, this explains the observed increase of the protein content in the cereals intercropped with the legumes compared to those grown in the monoculture (Li et al. 2023). Comprehensively, the findings indicate that the cereals derive significant benefits from the intercropping, thereby corroborating our initial hypothesis (Fig. 7).

The fava bean induced a net N mineralization in the soil, independently from the cropping system employed

Our findings indicate that the fava bean stimulated a net NO_3^--N accumulation in the topsoil and in the whole profile at the end of the growing season, independently from whether it was grown in sole or in intercropping, and this was the driver of the N_{min}-N accumulation. Specifically, the values of the residual N_{min}-N in the soil profile from 0 to 90 cm under the fava bean treatment were nearly double those observed under the fertilized wheat (Fig. 3a). This elevated N_{min} stocks might be susceptible to leaching during the winter months and the establishment of a catch crop is highly recommended (Gentsch et al. 2022). The substantial increase in the N_{min}-N observed under the fava bean at the end of the growing season, both in the topsoil and the whole profile, can be attributed to two mechanisms: (1) the contribution of newly fixed N from the fava bean to the rhizosphere (Wichern et al. 2007; Qiao et al. 2024), and (2) the enhanced N mineralization from the soil organic matter (SOM). The intercropping resulted in a reduction of the residual N_{min}-N load in the whole profile by 16.3% without the fertilization and 20.3% with the fertilization in comparison to the fava bean monoculture. Nevertheless, the residual N_{min}-N following the intercropping remains sufficiently high (76.37–80.30 ± 10.46 kg ha^{-1}) to meet the nutrient requirements of a cover crop or a winter crop (Heuermann et al. 2022; Gentsch et al. 2022). Although the changes in the soil TN were not statistically significant (Table S1), the mineral N stocks constituted only a minor portion of the total pool, i.e., less than 2%. Furthermore, the comparable stocks observed between the two intercropping treatments imply that the N fertilization may have limited the legume N fixation capacity (Zhao et al. 2020). Collectively, these findings provide a clear confirmation of our second hypothesis, that the intercropping enhances the soil N availability, even surpassing that of the sole wheat cultivation under the standard N conditions (Fig. 7). Thus, adjusting the sowing ratios could serve as a strategy to align the residual N_{min}-N with the N demand of the subsequent crop, thereby mitigating N losses by leaching and denitrification in humid climates.

The soil pH and water shape the nutrient availability patterns

The utilization of the water and the nutrients in the soil differed between the sole and the intercrops. The wheat exhibited a lower depletion of the soil water by evapotranspiration compared to the fava bean (Fig. 4b and c). Consequently, the water availability under the intercropping was intermediate compared to the two sole cropping systems throughout the entire season. This suggests that the competition between the two crops for the water resources was unlikely; instead, the water was utilized complementarily during this non water-limited cropping season. The alterations in the soil pH induced by the different crops were most likely driven by the nitrification processes (Fig. S15b, c, d, and e) (Tong and Xu 2012). This inference is supported by the corresponding patterns observed in the NO_3^--N stocks and the pH levels.

Some distinct patterns of nutrient utilization have been identified. Under the sole wheat with the standard N, the oxidation of the NH_4^+-N contained in the fertilizer to NO_3^--N led to a consistent trend of a negative correlation between the soil pH and the grain yield, and a positive correlation between the yield and the NH_4^+-N net stock differences (Fig. S17). Consequently, the decreasing pH could have contributed to the increase of the K availability in soil, thus explaining the positive correlation between its net stock differences and the grain yield. Conversely, under the zero N conditions, an opposite trend was noted, with higher yields associated with a greater depletion of NH_4^+-N and K, and a trend of a positive correlation with the pH.

The impact of the intercropping on the stocks of the Fe, Mn, Al, and Zn appears to be associated with soil water utilization. Specifically, under the intercropping with the zero N dose, the net differences in the Fe and the Mn stocks were lower at higher fava bean yields (Fig. S17). In contrast, the WC throughout the growth period and the differences in the Zn stocks exhibited a positive correlation with the yield. Under the sole fava bean, contrasting trends were observed. This suggests that the increased water availability resulting from the intercropping reduced the oxygen availability in the soil, which facilitated the reduction and the utilization of the Fe and Mn (Khabaz-Saberi et al. 2006; Xue et al. 2016; Kronberg et al. 2024). Conversely, this may have enhanced the formation of insoluble Zn compounds (Scott Angle et al. 2003; George et al. 2012; Xue et al. 2016). The observed correlation trends between the soil resources and the LER were likely driven by the fava bean relation with soil resources, probably because of its higher contribution to the net yield increase compared to the relative sole cropping system under the ZN (Fig. S18).

The topsoil organic C stocks remained stable across the treatment groups, indicating that the treatment effect was not consistent enough to shape its accrual under the tested conditions (Scalise et al. 2015; Tang et al. 2016; Peng et al. 2024). These findings indicate that the intercropping had a stronger effect on the biogeochemical parameters such as the water, pH and nutrients rather than the C input to build up stable organic matter fractions (Fig. 7). Furthermore, they support our second hypothesis with limitation to the water and the nutrient utilization by the crops.

The changes in the soil biochemical functioning are limited to the N cycling

The hypothesis that the soil microbes may allocate more resources to the enzyme production and growth under the intercropping can only be accepted with limitations. Under the zero-N conditions, the elevated LAP activity observed in the intercropping as opposed to the sole-cropped fava bean (Fig. 5e) may suggest an increased rate of N mineralization from the organic matter (Baraniya et al. 2016). The mechanism underlying this shift in microbial metabolism may involve a functional selection process facilitated by the wheat, aimed at enhancing the availability of N from organic sources (Philippot et al. 2013; Zechmeister-Boltenstern et al. 2015). This is suggested by the observed positive correlation of the BG activity with the NO_3^--N availability (Fig. S22). Nevertheless, the soil enzymes should be considered as a pool of resources allocated by the microbial communities, rather than being directly linked with their nutrient limitations (Nannipieri et al. 2018; Mori 2024; Puissant 2025).

The heterogeneity in soil physicochemical properties throughout the experimental field obscured the potential impacts of the cropping system and the N fertilization (Figs. S19 to S21). Despite the inclusion of covariates in the statistical models to adjust for initial soil conditions, it was not possible to entirely eliminate confounding factors. The pH, WC, Mg, and Ca availability were identified as the strongest confounding variables influencing the ordination of the enzyme activities and the accrual of C and N in microbial biomass. Among these, the water and Ca

availability exhibited a significantly relevant gradient in the field at the onset of the experiment. The availability of the resources to the soil microbes, particularly the water, C, and nutrients, are the major determinants of the microbial resource allocation to enzyme production (Zechmeister-Boltenstern et al. 2015). The pronounced influence of the confounders may explain why the variations in the BG and the ALP activities, as well as the CUE, were observed exclusively between treatments that combined contrasting cropping systems and fertilization levels. Specifically, the variations in the BG activity and the CUE were detected between the sole wheat cultivation with the zero N and the intercropping with the standard N (Table S3). However, some clear trends were observed, indicating a higher BG activity and a lower CUE in systems with high compared to low N_{min}-N availability (Table S3). Additionally, the BG activity demonstrated a strong positive correlation with NO_3^--N concentration (Fig. S22). These patterns likely resulted from the alignment of the microbial demands for available C and N resulting from the increased N_{min}-N availability and the likely sufficient inputs of newly-fixed C. Such conditions may have enabled the microbes to satisfy their elemental stoichiometric requirements for degrading soil organic matter (Favaro et al. 2025). As a result, the microbial metabolism in the soil may have transitioned toward a rapid C mineralization from the organic matter when sufficient N_{min}-N is available, thereby likely generating a positive priming effect (Favaro et al. 2025). As a matter of fact, the decreasing CUE could indicate that the C availability for the microbes might be increasing, thus reducing the need to recycle it within the community (Hu et al. 2025; Kuzyakov et al. 2026). This conclusion is further supported by the strong correlation between the MBN and the NH_4^+-N availability (Fig. S22). Nevertheless, the lack of effects on the CUE, whether induced by the cropping system or the N fertilization aligns with the findings of a recent study (Schroeder et al. 2025). The authors found that different crop diversification practices did not have a significant effect on the microbial CUE across a pedoclimatic gradient. However, this is in contrast with the results reported by Domeignoz-Horta et al. (2024), who identified a significant positive correlation between microbial CUE and the crop species richness within a barley cropping system, ranging from an increase of 7.4% with one species to 25.2% with four species. Overall, the intercropping increased the potential for enzymatic N mineralization from organic sources (Fig. 7). This indicates a higher efficiency of microbial N cycling. The CUE and the BG activity in this experiment indicated that a high N availability might be triggering a positive priming effect.

Conclusions

Our study presents compelling evidence that, under zero N conditions, fava bean-winter wheat intercropping significantly increased the yield of the fava bean by reducing the intraspecies competition, while enhancing the wheat investment in photosynthesis. This effect may be attributed to the enhanced N nutrition of the intercropped wheat. The overall system performance, as measured by the LER, was significantly greater than one, indicating consistent facilitative effects under N-limited conditions. Additionally, the fava bean utilized the soil water resources more compared to the wheat, with the water availability tending to increase under the intercropping with the zero N. This likely contributed to a higher availability of Fe and Mn, and a lower availability of Zn for the crops throughout the plant development. Under the intercropping, the wheat may have influenced the soil microbial functionality, resulting in a significantly higher LAP activity compared to the fava bean. Consequently, this process might have contributed to the sustained N nutrition of the intercropped wheat under the zero N comparably to the standard N conditions. However, confounding variables arising from the natural field heterogeneity impeded the identification of other effects on the soil microbial biochemical functionality attributable to either one of the two tested factors. Only a trend suggesting a possible stimulation of the priming effect by increasing the mineral N availability was observed. Overall, this study suggests that intercropping fava bean with winter wheat is a viable cropping strategy to enhance the yield per hectare with a reduced reliance on external nutrient inputs. However, the effects on the microbial biochemical functionality were limited to the N cycling. Further research should investigate the

contributions of different soil and root microbial groups to the ecosystem service provision by this cropping system compared to the respective sole crops.

Declarations

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Competing interests

The authors declare that they do not have any competing interests.

Author contributions

Riccardo Picone: sample collection, laboratory analyses, statistical analyses, manuscript conceptualization, writing - original draft, writing - reviewing and editing, creation of images; **Giacomo Pietramellara**: funding acquisition, project administration, reviewing; **Georg Guggenberger**: funding acquisition, reviewing; **Andre Gohlke**: field trial management, crop yield assessment; **Moritz Koza**: soil texture analysis, reviewing; **Gemma Marchetti**: contribution to the laboratory analyses, reviewing; **Shamina Imran Pathan**: funding acquisition, project administration, experiment design, supervision, statistical analyses, manuscript conceptualization, reviewing, editing; **Norman Gentsch**: funding acquisition, experiment design, sample collection, supervision, statistical analyses, manuscript conceptualization, reviewing, editing.

Data availability

All the datasets and the related scripts for the statistical analyses of the data are available at the following link: 10.5281/zenodo.20035719

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Figures

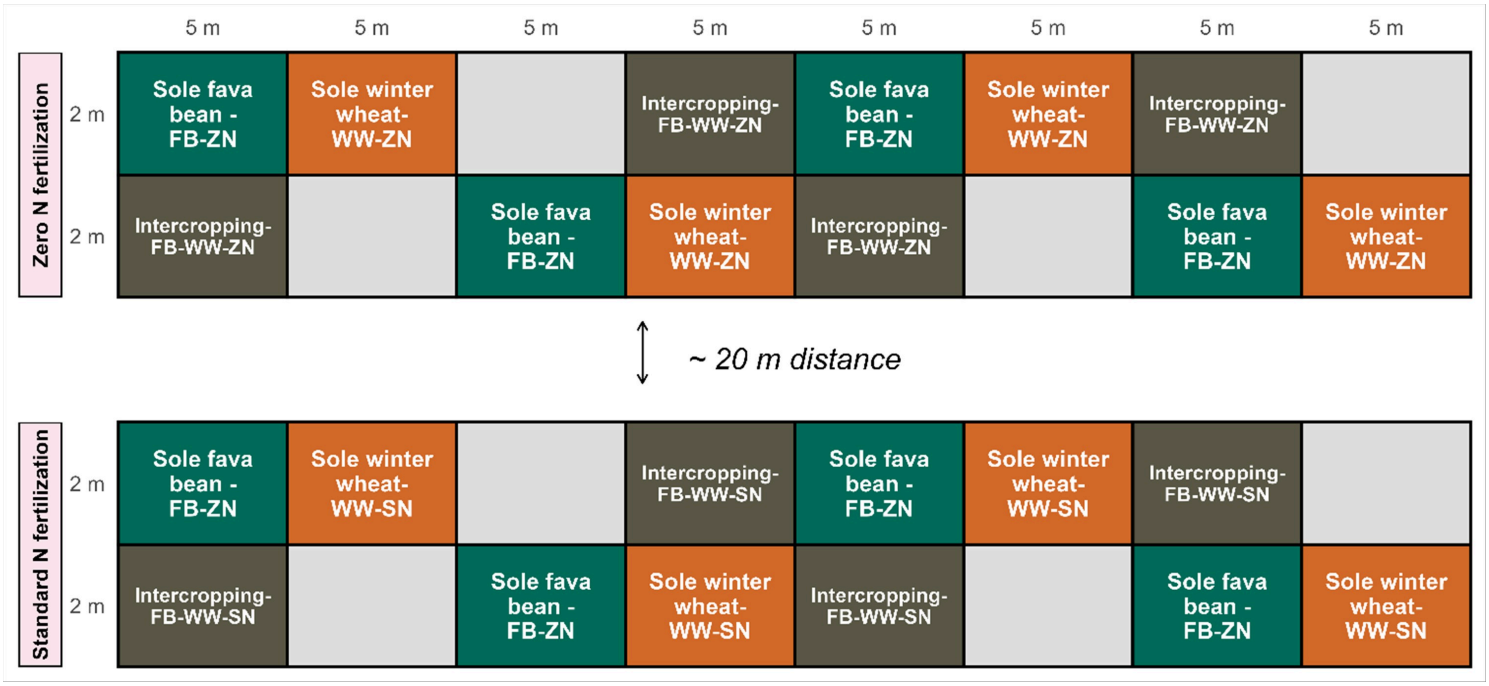


Figure 1

The experimental field trial. This figure reports a schematic visualization of the experimental field design. Plots were 2 x 5 m big. Between the fertilized and unfertilized treatments there were about 20 m of distance.

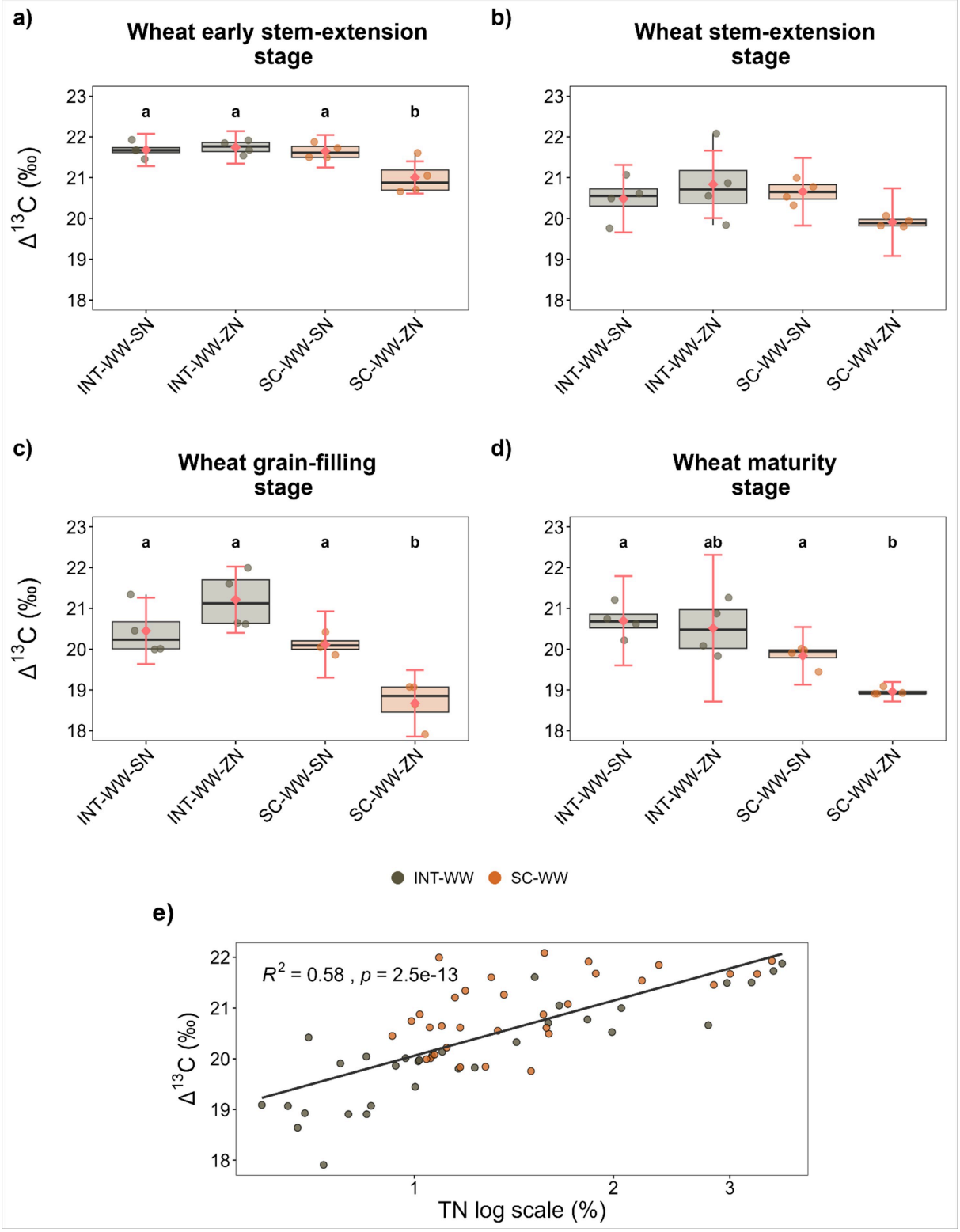


Figure 2

The wheat biomass $\Delta^{13}C$ throughout the four phenological stages and its correlation with the total N. The above plot reports the wheat biomass $\Delta^{13}C$ in the early stem-extension (a), the stem-extension (b), the grain-filling (c), and the maturity stages (d). The plot e reports the correlation of the wheat biomass $\Delta^{13}C$ with the total N (TN) across the whole cropping season. *INT-WW-SN* denotes intercropped winter wheat – standard N; *INT-WW-ZN* denotes intercropped winter wheat – zero N; *SC-WW-SN* denotes sole-cropped winter wheat – standard N; *SC-WW-ZN*

denotes sole-cropped winter wheat – zero N. In Fig. 2 a-d, the red dots represent the estimated marginal means, while the red lines represent their 95% confidence intervals. Different letters indicate significant differences among the groups (P < 0.05).

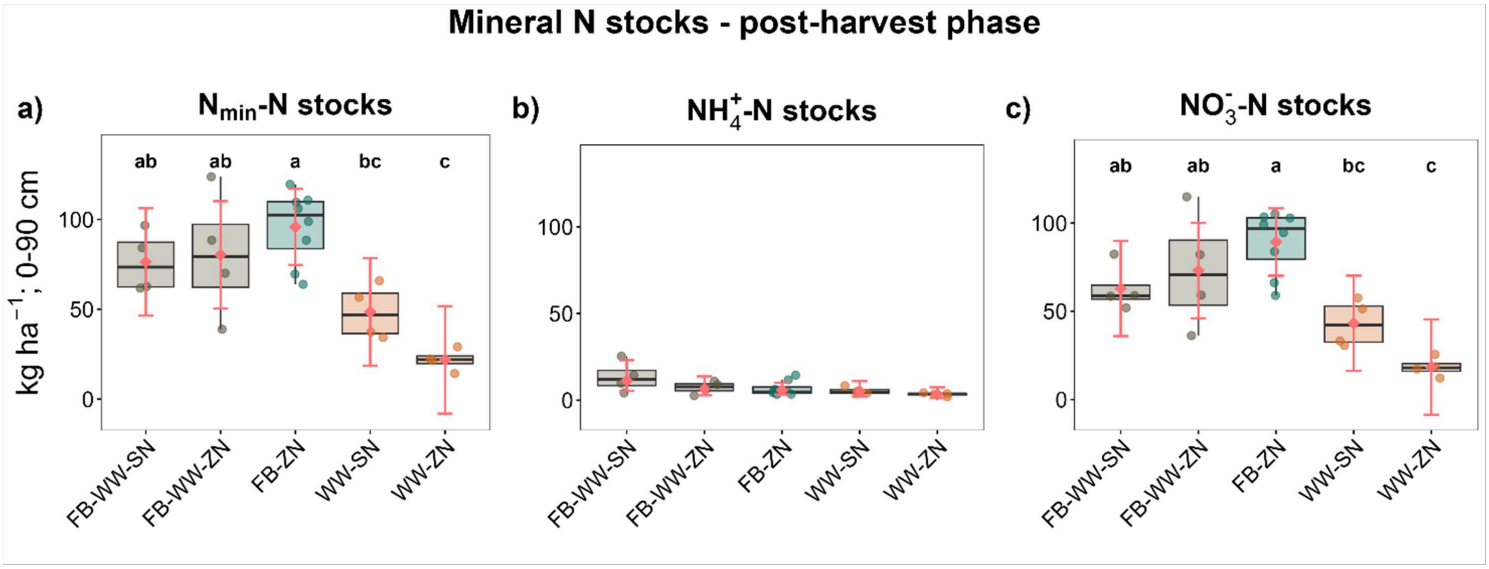


Figure 3

The mineral N, ammonium, and nitrate stocks in the post-harvest phase in the 0–90 cm soil profile. The plot reports the mineral N (N_{min}-N), the ammonium-N (NH_4^+-N), and nitrate-N (NO_3^--N) stocks in the first 90 cm of soil at the end of the season, i.e. after the harvest. The abbreviations of the treatments are as follows: *FB-WW-SN* denotes intercropping – standard N; *FB-WW-ZN* denotes intercropping – zero N; *FB-ZN* denotes sole-cropped fava bean – zero N; *WW-SN* denotes sole-cropped wheat – standard N; *WW-ZN* denotes sole-cropped wheat – zero N. The red dots represent the estimated marginal means, while the red lines represent their 95% confidence intervals. Different letters indicate significant differences among the groups (P < 0.05).

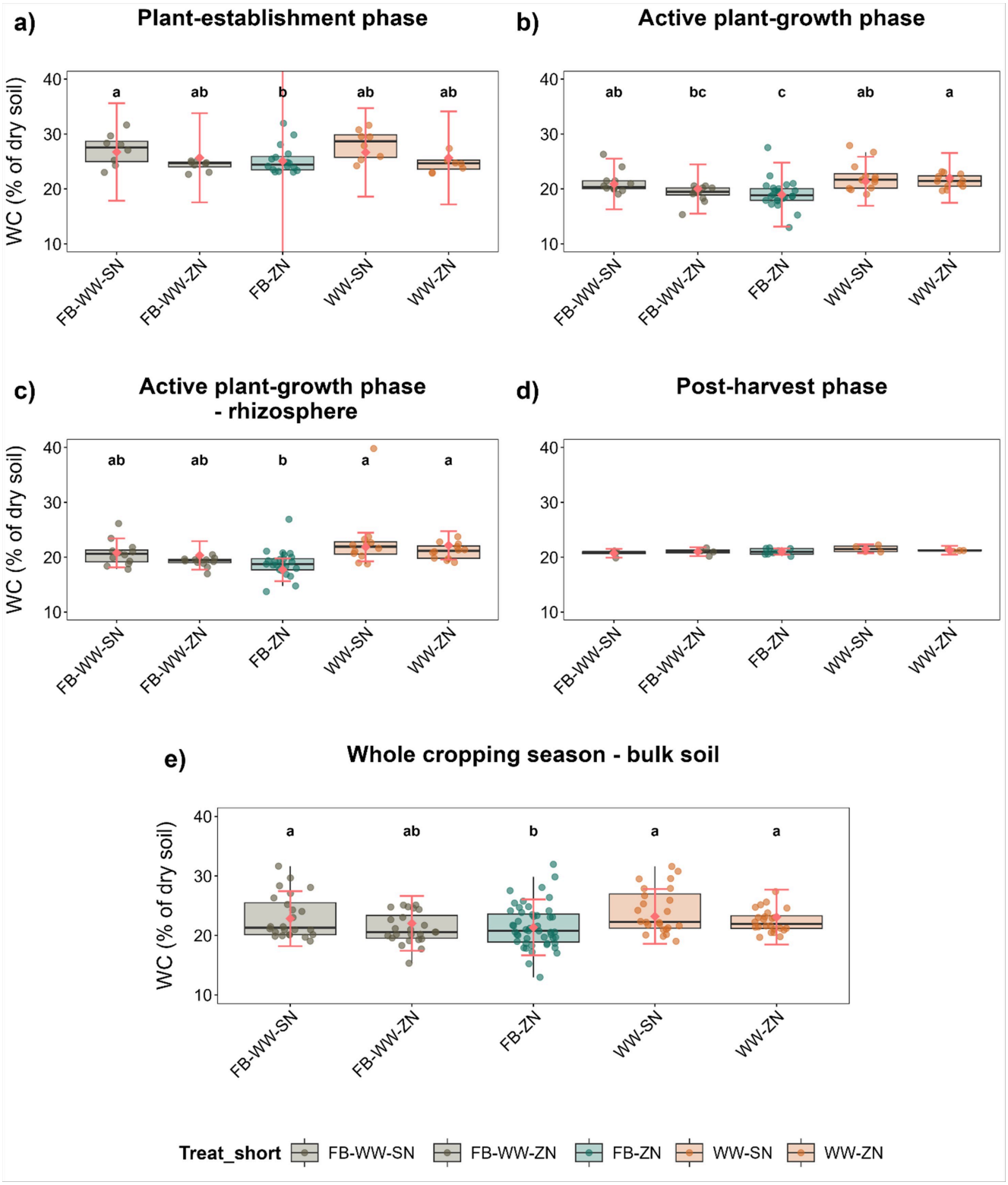


Figure 4

The topsoil water content in the bulk soil across the whole cropping season and in the rhizosphere in the active plant-growth period. The figure reports the topsoil water content (WC) in the plant-establishment phase (a), the active plant-growth phase in both the bulk (b) and the rhizosphere soil (c), the post-harvest phase (d), and over the whole cropping season in the bulk soil (e). The abbreviations of the treatments are as follows: *FB-WW-SN* denotes intercropping – standard N; *FB-WW-ZN*denotes intercropping – zero N; *FB-ZN* denotes sole-cropped fava bean – zero N; *WW-SN* denotes sole-cropped wheat – standard N; *WW-ZN* denotes sole-cropped wheat – zero N. The red

dots represent the estimated marginal means, while the red lines represent their 95% confidence intervals. Different letters indicate significant differences among the groups (P < 0.05).

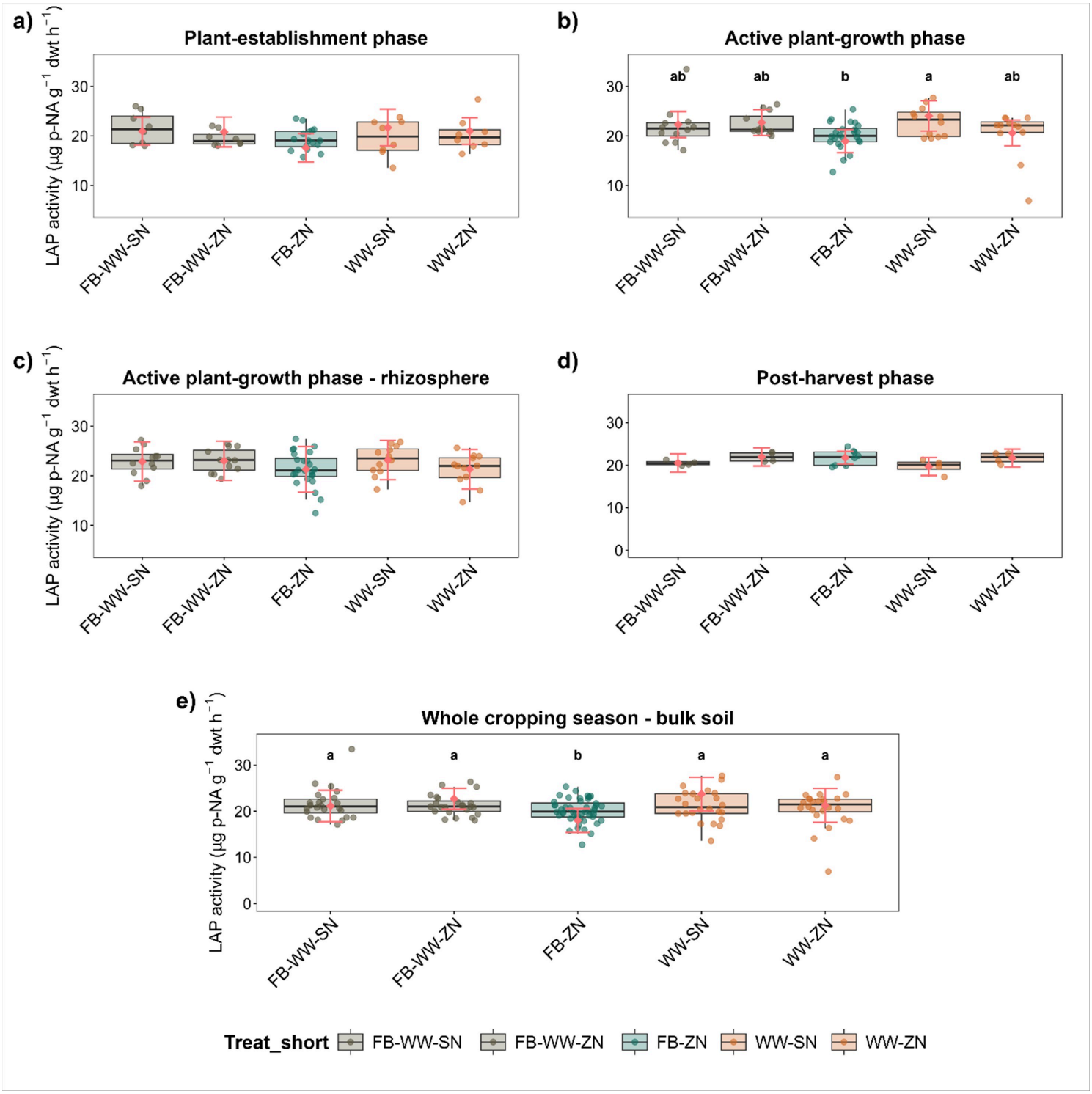


Figure 5

The L-leucine aminopeptidase activity in the bulk soil across the whole cropping season and in the rhizosphere in the active plant-growth period. The figure reports the L-leucine aminopeptidase activity (LAP) in the plant-establishment phase (a), the active plant-growth phase in both the bulk (b) and the rhizosphere soil (c), the post-harvest phase (d), and over the whole cropping season in the bulk soil (e). The abbreviations of the treatments are as follows: *FB-WW-SN* denotes intercropping – standard N; *FB-WW-ZN* denotes intercropping – zero N; *FB-ZN*

denotes sole-cropped fava bean – zero N; *WW-SN* denotes sole-cropped wheat – standard N; *WW-ZN* denotes sole-cropped wheat – zero N. The red dots represent the estimated marginal means, while the red lines represent their 95% confidence intervals. Different letters indicate significant differences among the groups (P < 0.05).

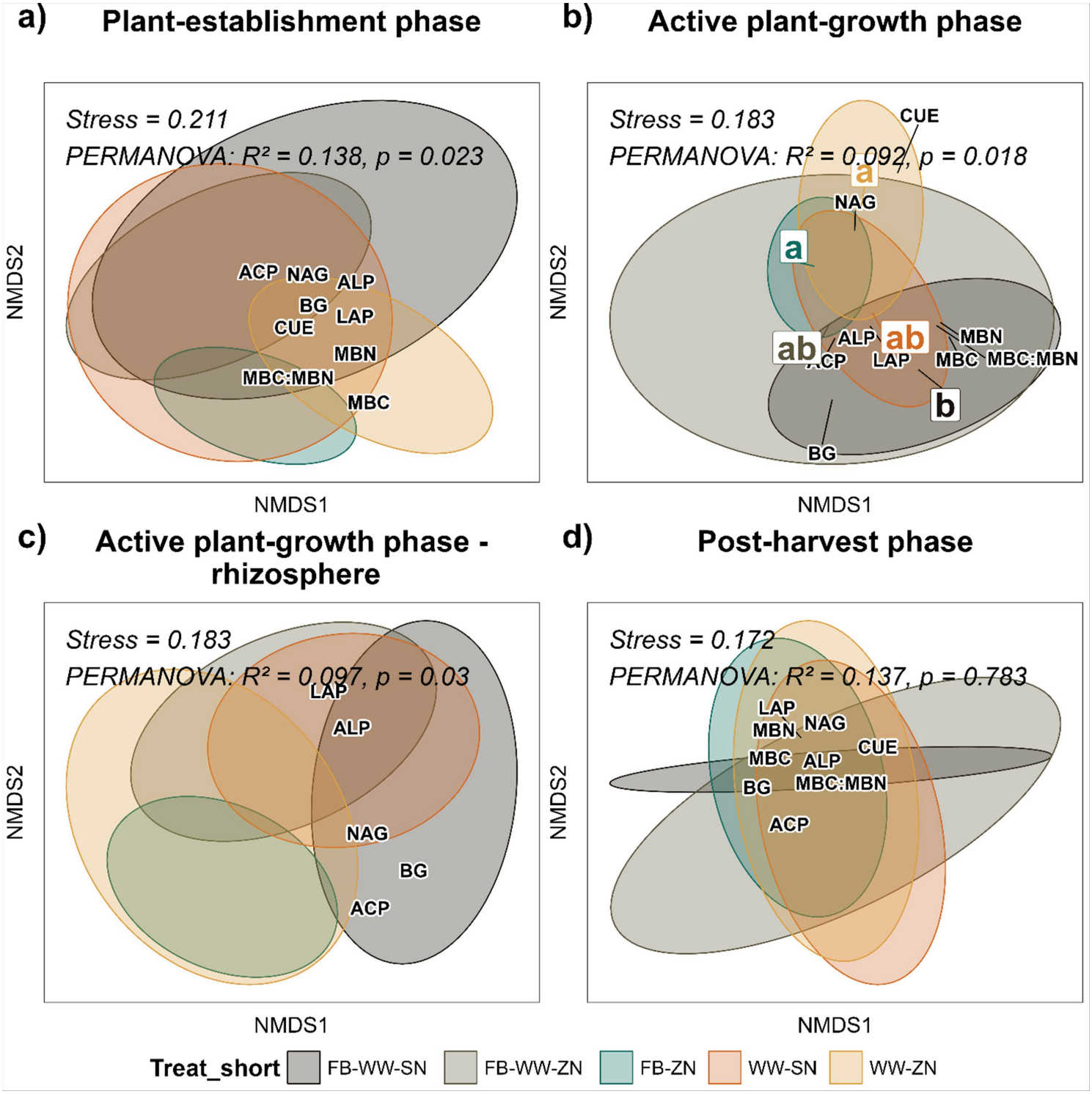


Figure 6

The NMDS ordination analyses on the biochemical properties in the bulk soil across the whole cropping season and in the rhizosphere in the active plant-growth phase. The above plot reports the NMDS ordination of the soil biochemical properties in the plant-establishment phase (a), the active plant-growth phase in both the bulk (b) and the rhizosphere soil (c), and in the post-harvest phase (d). The abbreviations of the treatments are as follows: *FB-*

WW-SN denotes intercropping – standard N; *FB-WW-ZN* denotes intercropping – zero N; *FB-ZN*denotes sole-cropped fava bean – zero N; *WW-SN* denotes sole-cropped wheat – standard N; *WW-ZN* denotes sole-cropped wheat – zero N. The ellipses represent the 95% confidence intervals of the centroid. Different letters indicate significant differences among the groups ($P < 0.05$).

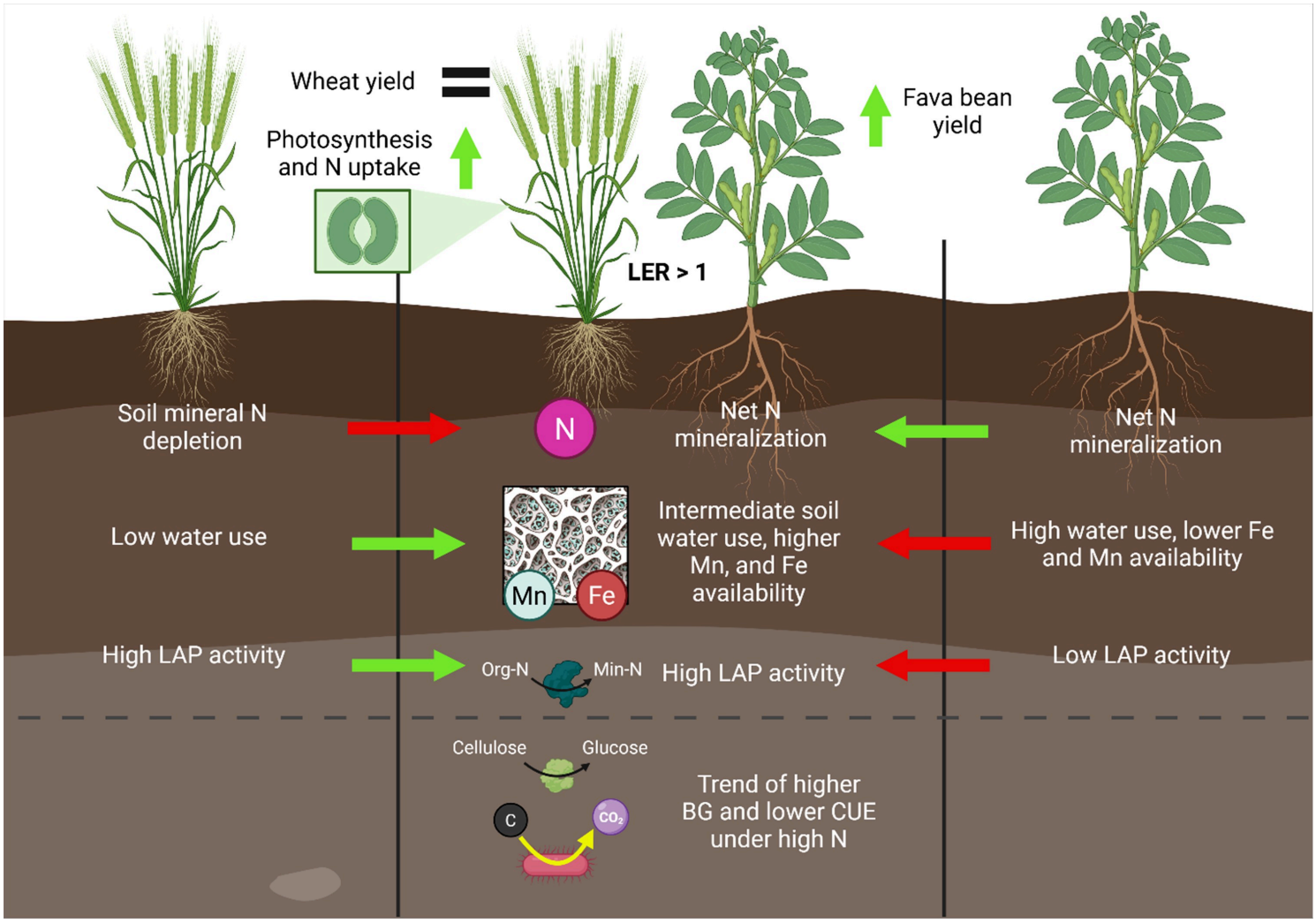


Figure 7

The effect of intercropping fava bean with winter wheat on the soil nutrient and C cycles. The figure above summarizes the findings of this study. The green lines represent an enhancement or a stimulatory effect over a process, while the red arrows represent a decrease or a suppressive effect. The bottom part of the figure separated by a dashed line reports some relevant trends revealed in this study. The abbreviations are as follows: LER indicates the Land Equivalent Ratio; org-N indicates organic N; min-N indicates mineral N; LAP indicates the L-leucine aminopeptidase activity; BG indicates the β-glucosidase activity; CUE indicates the microbial C use efficiency.

Supplementary Files

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