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REGULAR ARTICLE

Nutrient stoichiometry of a plant-microbe-soil system in response to cover crop species and soil type

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Abstract
Aims The theory of ecological stoichiometry mostly builds on studies of natural terrestrial ecosystems, whereas only limited stoichiometry information is available in response to agronomic practices.
Methods We designed a greenhouse experiment in order to disentangle the specific role of cover crop identity and soil characteristic in affecting nutrient stoichiometry of a plant-microbe-soil system.
Results Nutrient ratios of cover crop biomass were species-specific and the growth rate explained, for most species considered, the stoichiometric differences in response to soil type. In contrast, the nutrient stoichiometry of soil microbes was more homeostatic and did not respond to either cover crop identity or soil type. Compared to bare soil, the presence of cover crop enhanced microbial phosphorus immobilization in the clay-rich soil, whereas it promoted microbial carbon biomass and microbial nitrogen immobilization in the sandy-rich soil. A greater microbial cumulative respiration in clay soils, where a higher microbial biomass C at the beginning of the incubation was observed, suggested a major role of soil type, compared to cover crop identity, in affecting microbial metabolism.
Conclusions By understanding the stoichiometric constraints in the plant-microbe-soil system, our findings can help to implement agro-ecological practices by selecting appropriate cover crop species in relation to soil type in order, for example, to avoid nutrient limitation due to microbial nutrient immobilization.

Keywords Agricultural soil · Ecological stoichiometry · Soil texture · Metabolic quotient · Cumulative soil respiration · Homeostasis

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Introduction

Cover crops, also known as green manure crops, represent an important management component of conservation agriculture in association with crop rotation and no-tillage (Hobbs et al. 2008). Cover crops are typically used between two cash crops (i.e. main crops) as single species or a mixture of species in order to provide various ecological and agronomical benefits. Indeed, cover crops can, for example, protect the soil from erosion, reduce water and nutrient losses, improve soil organic matter content, promote soil biological activity, stabilize cash crop yields, and control weeds (e.g. Abdalla et al. 2019; Büchi et al. 2020; Finney et al. 2017; Martínez-García et al. 2018; McDaniel et al. 2014; Smith et al. 2008; Vukicevich et al. 2016; Wittwer et al. 2017). However, to optimize the benefits provided

by cover crops, management practices must be adapted to local soil and climatic conditions (Abdalla et al. 2019; Romdhane et al. 2019).

Different studies have shown that cover crops can increase soil microbial biomass and modify the structure of microbial communities (Buyer et al. 2010; Finney et al. 2017; Hontoria et al. 2019; Martínez-García et al. 2018; Schmidt et al. 2019). Accordingly, the selection of certain cover crop species can provide a tool for steering soil microbial composition in order to assure an adequate nutrient availability for subsequent cash crops (Bender et al. 2016; Mariotte et al. 2018; Verzeaux et al. 2017; Vukicevich et al. 2016). Such a management strategy must rely on an accurate knowledge of plant-microbe-soil interactions, in particular considering that microbial metabolism can be affected by root exudates of cover crops, nutrient demands of cover crops, soil type and local climatic conditions (Bell et al. 2014; Carrillo et al. 2017; Kim et al. 2020; Mukumbareza et al. 2016; Rosenzweig et al. 2017;). To this aim, the application of ecological stoichiometry concepts to the plant-microbe-soil system can provide helpful insights on how soil type, in combination with cover crop diversity, can affect not only the nutrient balance but also the functions of soil microbes and, ultimately, nutrient cycling in agroecosystems (Bertrand et al. 2019). This is particularly important if we want to optimize microbial and plant performances not only to secure crop yields, but also to improve soil carbon (C) sequestration and, more broadly, to properly adopt ecologically sustainable agricultural practices (Kallenbach et al. 2019; Ptacnik et al. 2005).

The theory of ecological stoichiometry focuses on the balance of elements (i.e. the nutrient ratio) from individual-scale to ecosystem-scale in relation to available resources. The founding biogeochemical principles of ecological stoichiometry assume that organisms have consistent nutrient ratios, in particular C to nitrogen (N) to phosphorus (P), and that the abundance of nutrients in a system is regulated by the interactions between organisms and their environment (Elser et al. 1996; Sterner and Elser 2002). Stoichiometric homeostasis is defined as the degree to which the organism, or system, can maintain a relatively stable chemical composition (i.e. nutrient ratios) in response to variations in the composition and availability of external resources (Spohn 2016). For terrestrial ecosystems, it has been shown that, at global scale, autotrophic organisms (plants) are generally rather plastic (i.e. they have a greater stoichiometric flexibility) in terms of nutrient ratios compared to heterotrophs (e.g., soil organisms) that generally show more constrained nutrient ratios, i.e. they tend to be more homeostatic (Cleveland and Liptzin 2007). Ecological stoichiometry represents a helpful conceptual framework to understand the cycling of C, N and P nutrients at different spatial scales (Buchkowski et al. 2019; Sterner and Elser 2002).

Currently most of ecological stoichiometry studies focus on aquatic ecosystems or natural terrestrial ecosystems with few studies relating ecological stoichiometry of crops, soil, and microbes to agricultural practices (Bertrand et al. 2019). To fill this knowledge gap, we designed a greenhouse experiment to understand the interactions between soil type (a clay versus a sandy soil), cover crop identity (four different species widely used in conservation agriculture) and soil microbes to answer the following questions: 1) How do plant and microbial stoichiometry vary in relation to soil type? 2) To what extent does the identity of cover crops affect soil microbial stoichiometry? 3) How do plant species and soil type interact in affecting microbial metabolism?

Materials and methods

Experimental design

A greenhouse pot experiment was carried out at Agroscope-Changins (Nyon, Switzerland) during the period June–July 2018. A clay-rich and a sandy-rich soil were used that were, respectively, characterized by pH (in H_2O) 7.8 and 5.8, sand content 281 and 519 (g kg^{-1}), clay content 291 and 62 (g kg^{-1}), organic carbon (C_{org}) 19 and 16 (g kg^{-1}), total nitrogen (N_{tot}) 2.2 and 1.5 (g kg^{-1}), available P (Olsen-P) 29 and 50 (mg kg^{-1}), cation exchange capacity 68 and 143 meq kg^{-1} (Table S1). Sandy soil was collected at the Federal Agricultural Research Station (Agroscope) of Cadenazzo from a grassland strip at the border of a former trial with chestnut trees (*Castanea sativa*). The clay soil was collected at the Federal Agricultural Research Station (Agroscope) of Changins from a grassland field that was nor fertilized neither harvested during the three years preceding our experiment. Four species of cover crops with contrasting growth strategies were selected, namely *Avena strigosa* (oat), *Lupinus albus* (lupine), *Pisum sativum* (pea), and *Brassica juncea* (mustard). Oat has the highest root length and root area

among the studied species (Wendling et al. 2016). Lupine and pea are leguminous species capable to fix atmospheric nitrogen. In addition, lupine is a non-mycorrhizal species forming root clusters (i.e. proteoid roots), whereas pea is a mycorrhizal species with a higher specific root length than lupine (Erman et al. 2009; Nuruzzaman et al. 2005; Wamberg et al. 2003). Mustard is a non-mycorrhizal species with a pivotal root system (Tester et al. 1987) and with a recognized anti-nematodes role (Dutta et al. 2019). The cover crops were sown in monospecific pots (diameter 27 cm, height 24.3 cm) so to obtain a final density per pot of 5 plants for lupine, 20 plants for oat, 25 plants for mustard, and 7 plants for pea. Plant density in pots followed field density recommendations (Wendling et al. 2016; Wendling et al. 2017). The plants were manually watered to keep a constant soil moisture content (i.e. 70% - 80% of the field capacity). Pots were relocated every three weeks to avoid potential bias related to greenhouse heterogeneity. To ensure optimal photosynthetic conditions for plant growth, daily temperature was maintained between 18 °C and 25 °C. The natural daylight was provided with high-pressure sodium lamps (400 W m^{-2}) from 6 am to 8 pm when light intensity dropped below 250 W m^{-2}. In total 32 pots were here considered resulting from a combination of four cover crop species, two soil types, and four replicates. In addition, three replicates of bare soil were incubated in the same greenhouse conditions for each soil type.

Productivity and nutrient concentration in aboveground plant biomass

In each pot, the aboveground biomass of cover crop was harvested after 8 weeks, i.e. during the flowering period. In order to measure the oven-dried aboveground productivity, a subsample (20 g) of fresh biomass was dried at 55 °C for 72 h to estimate the water content. The oven-dried subsample was then ground using a Retsch rotor mill. Total C concentration in plant biomass was measured by calcination (480 °C), whereas total N concentration was measured by combustion using the Dumas method (Masson and Bussiere 2010). Total P concentration in plant biomass was determined by ICP-AES (Varian Vista RL Simultaneous or Varian 725ES Simultaneous) after incineration (480 °C for 5 h) and mineralization in hydrofluoric acid (Masson and Bussiere 2010). All nutrient concentrations were then converted to oven-dried mass at 105 °C.

Soil sampling and analysis

At the end of the cultivation period, along the entire depth of each pot, four soil cores (2.5 cm diameter) were sampled, then sieved (2 mm mesh size) and thoroughly mixed to obtain a composite soil sample. During sieving, plant roots were carefully removed. About 100 g of fresh soil were immediately stored in a cold chamber (4 °C for two weeks) for subsequent microbial biomass analysis whereas the remaining amount was air-dried before storing for chemical analyses. Organic carbon (C_{org}) was determined based on sulfochromic oxidation (NF ISO 14235). Total soil nitrogen (N_{tot}) was analyzed by dry combustion using an elemental analyzer (Thermo, flash 2000, USA) (NF ISO 13878). Total soil phosphorus (P_{tot}) was measured according to the molybdate colorimetric method following an extraction using 0.25 g of soil in 5 ml of hydrofluoric acid (40%) and 1.5 ml of $HClO_4$ (65%) (NFX 31–147). The available phosphorus (Olsen-P) was estimated after sodium bicarbonate (0.5 M Na-HCO_3) extraction and filtration (Whatman, Grade 42, 45 μm size) according to the protocol of Murphy and Riley (1962) (NF ISO 11263). All nutrient concentrations were referred to oven-dry soil weight (105 °C for 24 h).

Microbial biomass nutrients and microbial metabolism

Soil microbial C (C_{mic}), N (N_{mic}) and P (P_{mic}) were estimated using the chloroform fumigation extraction (Vance et al. 1987). Total C and N of fumigated and non-fumigated samples were analyzed using a TOC/TN auto analyzer (Shimadzu analyzer TOC-V CPH + NM-1) after 0.5 M K_2SO_4 extraction (ratio 1:10). Phosphorus was measured by a colorimetric method using a sulfomolybdic reagent (Olsen et al. 1954) after extraction (1:20) with 0.5 M $NaHCO_3$ (pH 8.5). Final values of C_{mic}, N_{mic} and P_{mic} were calculated according to the coefficient factors k_C, k_N and k_P of, respectively, 0.45, 0.54 and 0.40 (Brookes et al. 1985; Jenkinson et al. 2004). Cumulative respiration in sieved soil sample was used as a proxy of microbial metabolism and was measured after two days of incubation. Briefly, about 5 g of fresh soil used for determination of microbial biomass nutrients were put into a smoked glass bottle (121 ml), kept closed with a septum and pre-incubated in the dark at 25 °C for 1.5 days, so to avoid including the pulse respiration (the Birch effect associated to soil manipulation) during the incubation measurement. At

the start of the incubation, bottles were flushed with CO_2-free air and left in incubation for two days. At the end of the incubation, CO_2 concentration was measured by injecting a gas sample into an infrared gas analyzer (EGM-4, PP Systems, Amesbury, Massachusetts) using an airtight syringe so that cumulative soil respiration was calculated by considering the enclosure volume, air pressure, temperature and oven-dried soil weight (105 °C for 24 h). The microbial metabolic quotient was calculated as ratio between the cumulative respiration and the correspondent microbial biomass carbon at the beginning of the incubation (Anderson and Domsch 1993).

Statistical analyses

Principal component analysis (PCA) was used to characterize the entire dataset of pots (treatments) based on a set of biological and chemical parameters that were measured at the end of the experiment, i.e. aboveground cover crop productivity; total C_{so}, N_{so}, and P_{so} in bulk soil (so); total C_{pl}, N_{pl}, and P_{pl} in cover crop biomass (pl); total C_{mic}, N_{mic}, and P_{mic} in soil microbial biomass (mic). Pearson's correlation and univariate regression techniques were also applied. Data were tested for normality and homoscedasticity. One-way ANOVA with LSD-Fisher post-hoc comparisons was used to assess stoichiometric differences in relation to soil types and cover crop treatments. In the case that normality or homoscedasticity conditions were not met, then the permutational ANOVA was performed using R 4.0.2 software with the function pairwisePermutationTest in the *rcompanion* library. The relative response of microbial biomass stoichiometry in presence of different cover crop species (treatment) in clay and sandy soil was calculated as the log-transformed ratio in presence of cover crop and correspondent ratio in bare soil. Stoichiometric homeostasis (H) of soil microbes was calculated following Persson et al. (2010) as $H = \log_{10}(x)/((\log_{10}(y)-\log_{10}(c))$ where x is the bulk soil nutrient stoichiometry, y is the correspondent nutrient stoichiometry of microbial biomass and c is a constant (Sterner and Elser 2002). Therefore, 1/H represents the slope of the regression between $\log_{10}(x)$ and $\log(y)$ and can have values between zero and one. Strictly homeostatic organisms have an H of infinity (i.e. $\log_{10}(y) = \log_{10}(c)$), whereas non-homestatic organisms have $H = 1$ (i.e. $\log_{10}(y) = \log_{10}(x) + \log_{10}(c)$). If the regression is not significant ($p > 0.1$), 1/H is set to zero (see Makino et al. 2003) and the organisms are considered "strictly homeostatic". The relationship between cumulative respiration and soil variables was tested for the entire dataset, including soil type and cover crop species that, consequently, did not meet the condition of independence. Therefore, the effect of soil type and species identity was initially tested. In case of statistical significance, soil type and/or species effect were modeled simultaneously together with soil bio-geochemical variables to quantify the portion of the variance explained by selected soil variables and also explained (i.e. controlled) by soil type or species effect. These last effects were modeled through the helmert contrast to code for soil type and/or species effects as explanatory variables (Legendre and Legendre 2012). Then, variance partitioning and significance were computed using the functions rda (vegan package). Statistical analyses were performed using R 4.0.2 software (R Development Core Team 2018) and Statistica for Windows.

Results

Based on the selected variables, the PCA ordination showed a clear separation of experimental pots in relation to soil type along the first axis and in relation to cover crop identity along the second axis (Fig. 1). Along the first ordination axis, sandy soil pots were associated with higher total soil P concentration (P_{so}), whereas clay soils were characterized by higher soil total N (N_{so}) and organic C (C_{so}) concentration as well as by higher microbial biomass nutrients (Fig. 1, Table S2 & S3). Along the second ordination axis, pots hosting leguminous species, i.e. *P. sativum* and *L. albus*, had negative scores for both soil types in association with higher plant biomass N and C concentration (Table S4). Pots hosting *A. strigosa* and *B. juncea* showed positive scores along the second axis for both soil types in association with generally higher P concentration in plant biomass (Fig. 1). In general, crop species had a c. 50% higher productivity in sandy soil compared to clay soil, and leguminous species had a higher productivity (c. 40%) compared to the other two cover crop species in both soil types (Table S4).

In clay soil there was a positive correlation between plant C and N concentration (Pearson's $r = 0.78$, $p < 0.001$, $n = 16$), but none between plant C and P, or between plant N and P concentration ($p > 0.45$). In sandy soil there was a positive correlation between plant C and N concentration (Pearson's $r = 0.85$, $p < 0.001$, $n = 16$), but a negative correlation between plant C and P concentration and between plant N and P concentration (Pearson's $r < -0.63$, $p < 0.001$, $n = 16$). In the case of soil microbial biomass, there was a positive correlation between microbial C and N concentration in both clay and sandy soil (Pearson's $r > 0.65$, $p < 0.01$, $n = 16$), but no correlation was found between microbial C and P or between microbial N and P concentration ($p > 0.15$).

Plant C:N, C:P and N:P (mass-based) ratios were between 3 and 7 fold higher than the corresponding ratios of microbial biomass and the coefficients of variation were much broader for plants than microbes, with the exception of C:P ratio in sandy soil (Fig. 2). In the case of aboveground plant biomass in clay soil, C:N and N:P ratios were, respectively, lower and higher in leguminous species compared to the other two cover crop species, with C:P ratio significantly higher for *L. albus* (Table 1). In sandy soil (Fig. 2), leguminous species had lower C:N ratios, as well as higher C:P and N:P ratios compared to *A. strigosa* and *B. juncea* (Table 1). A comparison of nutrient ratios for the same plant species between the two soil types showed higher C:P ratio for *B. juncea* in clay soil, lower C:P and N:P ratio but higher C:N ratio for *A. strigosa* in sandy soils, lower C:P for *P. sativum* in clay soil, and higher C:P and N:P ratio for *L. albus* in clay soil (Table 1).

Regarding the microbial biomass, we did not observe any cover crop effect (i.e. treatment effect) on microbial biomass C:P and N:P ratio for both soil types, whereas the microbial C:N ratio had the lowest value in presence of *B. juncea* in clay soil and in presence of *P. sativum* in sandy soil (Table 2). By comparing the same plant species treatment between the two soil types, we found that nutrient ratios of microbial biomass did not differ in response to *B. juncea*, *A. strigosa*, and *L. albus,* whereas with *P. sativum* the microbial biomass C:N ratio was higher in clay soil (Table 2).

Along the entire gradient of soil types and cover crop identity ($n = 32$), the mean relative percentage difference of C:N ratio between microbial biomass and bulk soil was −34% (range from −58% to −6%), whereas for plant biomass and bulk soil it was 114% (range from −28% to 316%), overall indicating a lower C:N ratio in microbial biomass, but a higher C:N ratio in plant biomass compared to bulk soil (Fig. S1). In the case of C:P ratio, the mean relative percentage differences between microbial biomass and bulk soil and between plant biomass and bulk soil were, respectively, −4.5% (range from −55% to 69%) and 604% (range from 182 to 1313%), overall indicating a slightly lower C:P ratio in microbial biomass, but a higher C:P ratio in plant biomass compared to bulk soil (Fig. S1). Finally, for the N:P ratio the mean relative percentage differences between microbial biomass and bulk soil and between plant biomass and bulk soil were, respectively, 65% (range from −45% to 366%) and 389% (range from 5% to 1299%), indicating that for microbes and plants the N:P ratio was overall higher than the N:P ratio of bulk soil (Fig. S1).

The regressions ($n = 32$) of log-transformed C:N, C:P and N:P ratios of microbial biomass in relation to the correspondent (log-transformed) bulk soil ratios resulted in no-significant regressions (Fig. S2). More specifically, the 1/H was −0.19 for C:N ratio ($p = 0.34$), 0.14 for C:P ratio ($p = 0.19$) and 0.01 for N:P ratio ($p = 0.93$), suggesting a certain degree of homeostasis for microbes in response to changes of soil stoichiometry.

The effect of cover crop presence on soil microbial stoichiometry was calculated as relative response of microbial nutrient ratio with versus without cover crop (Fig. 3). The relative response of microbial C:N ratio was overall positive in both soil types for all the cover crop species, although the microbial C:N ratio in presence of cover crop did not differ significantly from the correspondent ratio of bare clay and sandy soil (t-Student > −1.7, $p > 0.15$, *d.f.* = 5) (Table 2). In clay soil (Fig. 3a), the relative response of microbial C:P and N:P ratio was negative for all cover crop treatments due to lower, although not significant (t-Student >0.69, $p > 0.16$, *d.f.* = 5), C:P and N:P ratio in presence of cover crop compared to correspondent ratios in bare clay soil (Table 2). In sandy soil (Fig. 3b), the relative response of C:P and N:P ratio was positive for all cover crop treatments due to higher ratios in presence of cover crops compared to the correspondent ratios in bare sandy soil (Table 2). Based on one-way ANOVA, the relative response of nutrient ratio in clay soil differed

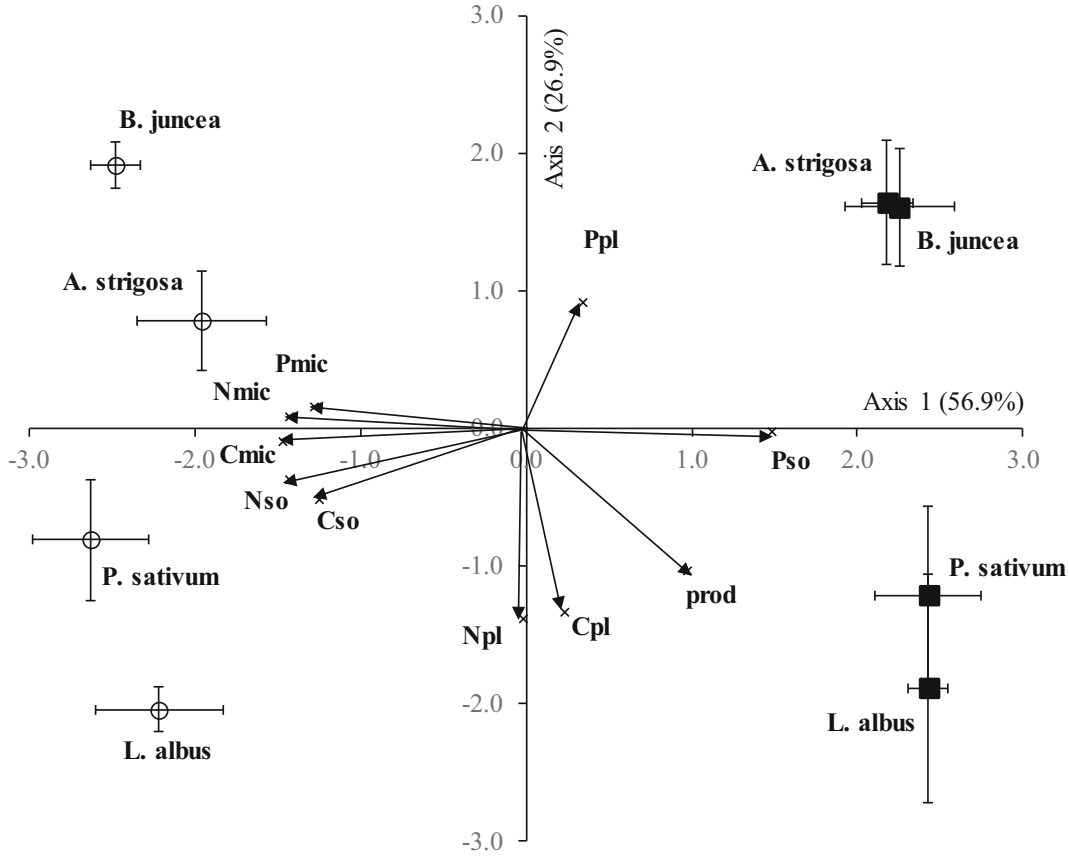

Fig. 1 Centroid ordination (PCA) of cover crop treatment in relation to aboveground plant productivity (prod) as well as in relation to total carbon (C), nitrogen (N) and phosphorus (P) concentrations in bulk soil (so), plant biomass (pl) and microbial biomass (mic). Open circles refer to clay soil pots, whereas full squares refer to sandy soil pots. For each treatment pot, the mean value of the ordination scores of corresponding four replicates are reported (mean centroid ± SD). The percentage of variance explained by each axis is reported between parentheses

significantly among cover crop species only for the microbial C:P ratio in the *L. albus* treatment (species effect $F = 4.1$, $p = 0.033$, *d.f.* = 3), whereas in sandy soil a species effect was detected for the relative response of C:P and N:P ratio in the *A. strigosa* treatment (species effect $F = 3.7$, $p = 0.041$ and $F = 4.4$, $p = 0.037$, respectively) (Fig. 3). A comparison of the relative response of nutrient ratio between soil type for the same cover crop species showed a significant soil effect for C:P and N:P ratio ($p < 0.05$), whereas the relative response of C:N ratio did not differ between soil type ($p > 0.05$), with the only exception of *B. juncea* treatment (t-Student = −4.8, $p = 0.003$, $n = 4$) (Fig. 3).

Mean cumulative soil respiration after two days of laboratory incubation was significantly higher in clay soil (39.9 ± 5.3 μgC-CO_2 g_{soil}^{-1}) than in sandy (12.9 ± 1.4) soil (t-Student = 10.2, $p = 0.02$, $n = 4$) (Fig. 4). The soil parameters primarily explaining the respiration differences between soil types were the microbial biomass elements, in particular the microbial biomass C (Table S5). Accordingly, greater cumulative respiration was measured in clay soils that were characterized by higher microbial biomass C at the beginning of the incubation (Fig. 4, Table S3). Within each soil type, there was no effect of cover crop identity on cumulative respiration (Table S5). The average metabolic quotient (qCO_2), i.e. the cumulative soil respiration per unit of microbial biomass C, was significantly lower (t-Student = 4.0, $p = 0.026$, $n = 4$) in clay soil (19.5 ± 3.3 μgC-CO_2 mgC_{mic}^{-1} d^{-1}) than sandy soil (30.4 ± 3.6 μgC-CO_2 mgC_{mic}^{-1} d^{-1}). In clay soil the plant species effect on metabolic quotient was detected only for *L. albus* ($F = 3.6$, $p = 0.047$, *d.f.* = 3), whereas no plant species effect was detected in sandy soil ($F = 0.59$, $p =$

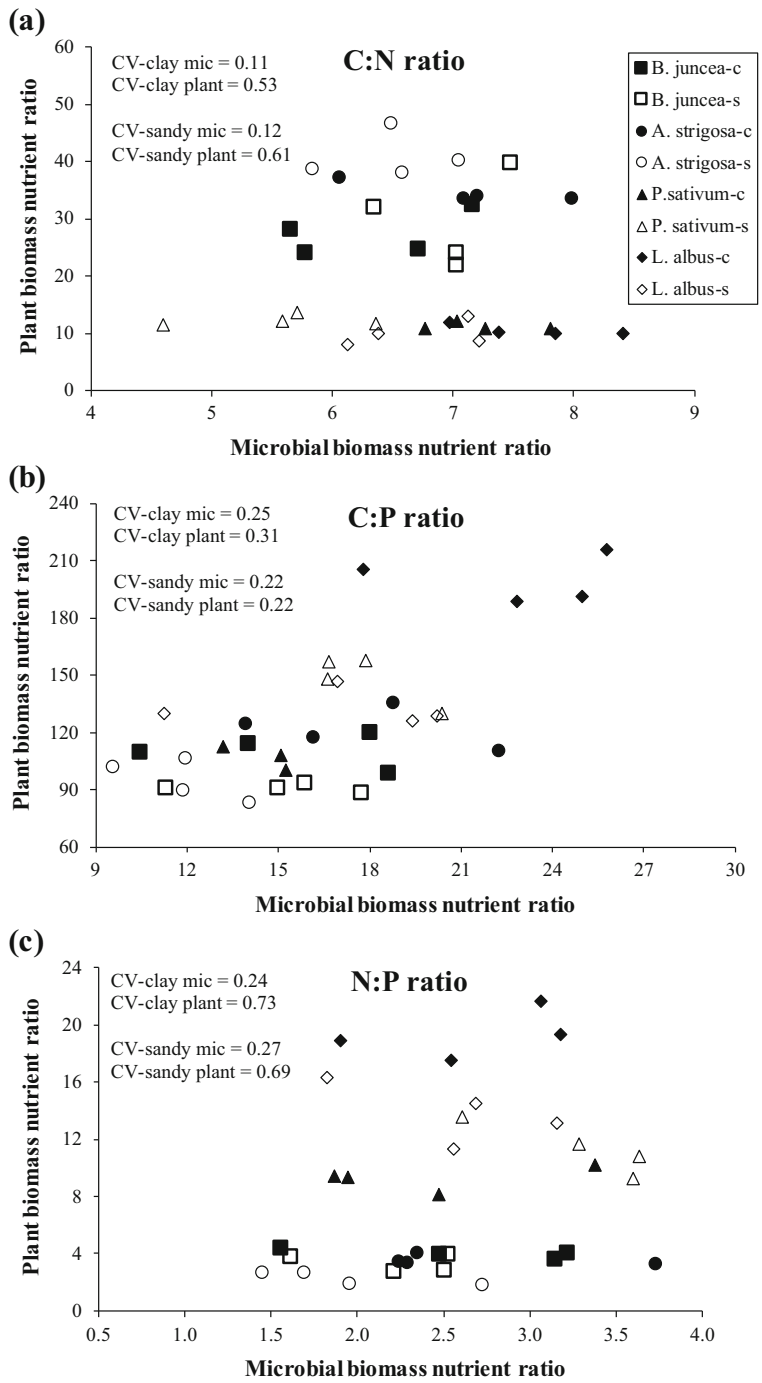

Fig. 2 Relationship between microbial nutrient ratio and corresponding plant nutrient ratio for each pot in clay (c) and sandy soil (s) (total number of pots per soil type = 16). CV is the coefficient of variation of nutrient ratio for microbial (mic) biomass and cover crop (plant) biomass

0.63, *d.f.* = 3). By merging the data from both soil types and all four cover crop treatments, the combined metabolic quotient was positively correlated with the soil C:N ratio (Pearson's $r = +0.89$, $p = 0.003$, $n = 8$) and negatively correlated with the soil C:P ratio (Pearson's $r = -0.83$, $p = 0.011$, $n = 8$).

Discussion

A better understanding of the interactions within the plant-microbe-soil system, in particular in relation to cover crop identity and soil type, is an essential component of agro-ecological practices. Our greenhouse

Table 1 Mean value (± SD, $n = 4$) of nutrient ratios in aboveground plant biomass for the different cover crop species (= plant treatment) in sandy and clay soil at the end of the experimental growth. Significant differences between plant treatments within the same soil type are indicated by different lower-case superscripts, whereas significant differences for the same cover crop between the two soil types are indicated by different upper-case superscripts (one-way ANOVA with Fisher LSD post-hoc comparisons or permutational ANOVA, $p < 0.05$)

	Plant C:N ratio				Plant C:P ratio				Plant N:P ratio			
	B. juncea	*A. strigosa*	*P. sativum*	*L. albus*	*B. juncea*	*A. strigosa*	*P. sativum*	*L. albus*	*B. juncea*	*A. strigosa*	*P. sativum*	*L. albus*
Clay soil	$27.6^{b,A}$ (3.9)	$34.6^{a,A}$ (1.8)	$11.1^{c,A}$ (0.6)	$10.5^{c,A}$ (0.9)	$111.4^{bc,A}$ (9.0)	$122.3^{b,A}$ (10.7)	$107.4^{c,B}$ (6.2)	$200.7^{a,A}$ (12.6)	$4.1^{c,A}$ (0.3)	$3.5^{c,A}$ (0.4)	$9.2^{b,A}$ (0.8)	$19.3^{a,A}$ (1.7)
Sandy soil	$29.6^{a,A.}$ (8.2)	$41.06^{a,B}$ (5.0)	$12.2^{b,A}$ (1.0)	$9.9^{b,A}$ (2.2)	$91.7^{c,B}$ (2.5)	$95.8^{c,B}$ (10.6)	$148.3^{a,A}$ (12.9)	$133.1^{b,B}$ (9.2)	$3.4^{c,A}$ (0.6)	$2.2^{c,B}$ (0.5)	$11.3^{b,A}$ (1.8)	$13.8^{a,B}$ (2.1)

experiment explored issues of ecological stoichiometry in an agricultural system by asking what was: i) the pattern of variation of nutrient ratios in plants and microbes in response to soil type, ii) the effect of cover crop identity on microbial nutrient stoichiometry, and iii) the combined effect of cover crop species and soil type on microbial activity. We examined two soil types with contrasting texture and four cover crop species.

General pattern of plant and microbial stoichiometry

Regarding the first question about plant and microbial stoichiometry, we observed that, within the same soil type, C:N and N:P ratio of plant biomass differed in different cover crops. The lower C:N and the higher N:P ratio of *P. sativum* and *L. albus* (Table 1) can be explained by their ability to symbiotically fix nitrogen (Adams et al. 2016; Novotny et al. 2007; Wolf et al. 2017). The lower biomass C:N ratio in both soil types for *B. juncea* compared to *A. strigosa* (Table 1) is in line with the recognized capacity of brassicas to efficiently take up soil inorganic N (Dean and Weil 2009; Kristensen and Thorup-Kristensen 2004; Vos and Van Der Putten 2004). The differences in C:P ratio between cover crop species within the same soil type (Table 1) can be explained by the dilution effect of plant productivity on P concentration (Luo et al. 2017; Medeiros et al. 1994; Yadav et al. 2019). This conclusion is supported by the positive correlation between plant productivity and C:P ratio in both clay (Pearson's $r = 0.63$, $p < 0.01$, $n = 16$) and sandy (Pearson's $r = 0.88$, $p < 0.001$, $n = 16$) soil.

After upscaling, the aboveground biomass productivity as observed in our pot experiment (i.e. 4.3–8.1 t ha^{-1} for clay soil and 6.2–11.9 t ha^{-1} for sandy soil) was within the same order of magnitude of open field conditions (Cherr et al. 2006). Lower values of biomass C:P and N:P ratios for *B. juncea*, *A. strigosa* and *L. albus* in sandy soil (Table 1) are in line with the growth rate hypothesis (Ågren 2008; Sterner and Elser 2002), stating that fast-growing organisms have a greater requirement for P for cell growth and metabolism (Rivas-Ubach et al. 2012). Indeed, we observed that all cover crop species had higher productivity in sandy soil compared to clay soil (Fig. 1, Table S4). The higher plant productivity in sandy soil can be primarily explained by the more favorable physical conditions. This hypothesis is supported by the C_{org} to clay content ratio (Table S1), an index of soil structure quality (Johannes et al. 2017), that had a value of c. 1:5 for the sandy soil, i.e. above the threshold for optimal structural quality (c. 1:8), compared to the clay soil (ratio c. 1:17), i.e. below the threshold indicative of a poor structural state (i.e. 1:13). The one exception to the growth rate hypothesis was represented by the stoichiometry of *P. sativum* that, in sandy soil, was characterized by higher biomass C:P ratio and, although not significant, higher biomass N:P ratio notwithstanding a higher productivity (Table 1, Table S4). A possible explanation is that P stoichiometry of *P. sativum* was more affected by the dilution effect associated to a higher productivity (Cadot et al. 2018). This is in line with the higher P concentration of *P. sativum* in clay soil where, notwithstanding a lower P availability (P_{min}) (Table S1), the biomass productivity was significantly lower (Table S4).

Table 2 Mean value (± SD, $n = 4$) of microbial nutrient ratios for the different cover crop species (= plant treatment) in the two soil types at the end of the experimental growth. As reference, the mean (± SD) value of microbial nutrient ratio in bare soil ($n = 3$) at the end of the experiment are also reported. Significant differences between plant treatments within the same soil type are indicated by different lower-case superscripts, whereas significant differences for the same cover crop between soil types are indicated by different upper-case superscripts (one-way ANOVA with Fisher LSD post-hoc comparison or permutational ANOVA, $p < 0.05$)

	Microbial C:N ratio					Microbial C:P ratio					Microbial N:P ratio				
	B. juncea	*A. strigosa*	*P. sativum*	*L. albus*	*Bare soil*	*B. juncea*	*A. strigosa*	*P. sativum*	*L. albus*	*Bare soil*	*B. juncea*	*A. strigosa*	*P. sativum*	*L. albus*	*Bare soil*
Clay soil	$6.3^{a,A}$ (0.7)	$6.8^{ab,A}$ (0.8)	$7.2^{ab,A}$ (0.4)	$7.7^{b,A}$ (0.6)	6.9 (0.9)	$16.3^{a,A}$ (3.7)	$17.8^{a,A}$ (2.2)	$19.1^{a,A}$ (4.1)	$20.7^{a,A}$ (3.6)	24.0 (7.3)	$2.4^{a,A}$ (0.7)	$2.6^{a,A}$ (0.2)	$2.7^{a,A}$ (0.6)	$2.9^{a,A}$ (0.3)	3.6 (1.4)
Sandy soil	$6.8^{a,A}$ (0.5)	$6.1^{ab,A}$ (0.5)	$5.9^{b,B}$ (0.7)	$6.7^{ab,A}$ (0.5)	5.6 (1.2)	$15.1^{a,A}$ (2.7)	$13.9^{a,A}$ (1.9)	$17.9^{a,A}$ (1.8)	$16.9^{a,A}$ (4.1)	7.5 (0.5)	$2.2^{a,A}$ (0.4)	$2.3^{a,A}$ (0.6)	$3.3^{a,A}$ (0.5)	$2.2^{a,A}$ (0.6)	1.5 (0.3)

In contrast to the nutrient stoichiometry of the plants, microbial biomass stoichiometry did not respond to either cover crop species or soil type (Table 2). This result, in combination with the smaller coefficient of variation of nutrient ratios (Fig. 2), indicates that soil microbes have a more constrained C, N, and P stoichiometry compared to plants (Cleveland and Liptzin 2007; Xu et al. 2015; Xue et al. 2019). This conclusion if further confirmed by the calculated H index of microbial biomass stoichiometry (Fig. S2) indicating a greater degree of homeostasis of microbes for C:N, C:P and N:P ratios (Cleveland and Liptzin 2007; Hartmann and Richardson 2013; Makino et al. 2003), at least along our relatively short gradient of soil nutrient concentration. It is worthy, in future studies, to understand to what extent soil microbial homeostasis can be explained by the physiological adjustment of microbes (Ballantyne et al. 2008) or by a process of species replacement (Danger et al. 2008). It is also interesting to note that microbes have lower C:N and C:P ratios than does bulk soil, in contrast to plants that have higher ratios than does the soil (Fig. S1) (Zechmeister-Boltenstern et al. 2015).

Effect of cover crop presence on microbial stoichiometry

Regarding the second question about the effect of cover crop presence on microbial stoichiometry, we found microbial stoichiometry to be more responsive to soil type than cover crop identity. In clay soil, the negative relative response of microbial C:P and N:P ratio is indicative of enhanced microbial P immobilization in presence of cover crop (Jin et al. 2014); however, this trend was not observed in sandy soil (Fig. 3). We hypothesize that in the clay soil, characterized by relatively lower P availability (Table S1), the presence of cover crops facilitated absorption of P by microbes through i) the direct effect of root exudates and exogenous phosphatases on soil P mobilization (Hallama et al. 2019; Hinsinger 2001), and ii) the indirect use of roots exudates by microbes as C source for producing P-solubilizing compounds (Patel et al. 2008; Spohn et al. 2013). It is interesting to note that the presence of *L. albus*, although promoting P immobilization in microbial biomass, appeared to reduce the facilitation effect compared to the other cover crop species (Fig. 3). This result may be related to the presence

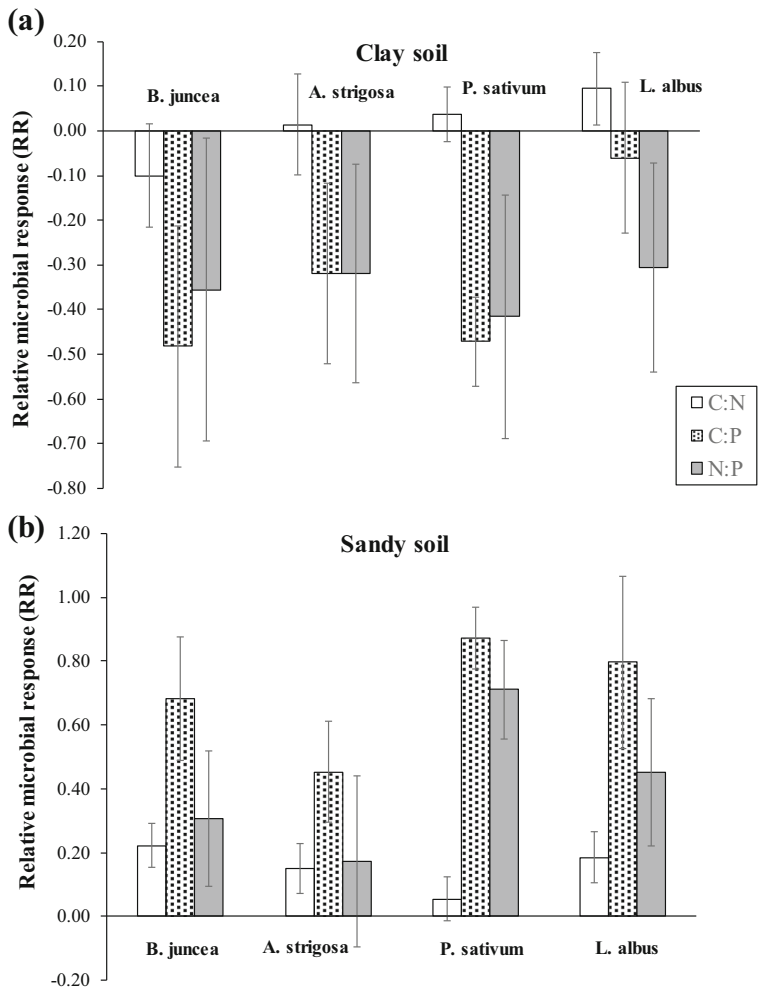

Fig. 3 Relative response of microbial biomass C:N, C:P and N:P ratio in presence of different cover crop species (plant treatment) in clay (**a**) and sandy (**b**) soil. Each value is the mean (± SD, $n = 4$) of the log-transformed ratio between microbial nutrient ratio in presence of cover crop and correspondent microbial nutrient ratio in bare soil

of cluster roots that increased the competitive ability of *L. albus* in recruiting soil microbes for P acquisition (Lambers et al. 2013; Schneider et al. 2019). In contrast, in sandy soil, where P is not limiting, but the amount of organic matter is (i.e. C_{org} to clay content ratio < 1:13), the presence of cover crop had no effect on microbial P content compared to bare soil, but promoted the immobilization of C and N in microbial biomass, ultimately resulting in a positive relative microbial response of C:P and N:P ratio (Fig. 3, Table 2). This result can be explained by the role of plants in providing organic matter and energy to microbes through root exudates, root turnover and plant litter that can, ultimately, sustain a greater microbial biomass (Rees et al. 2005; Vezzani et al. 2018). We are aware that extraction coefficients for estimation of P microbial biomass by chloroform fumigation should be specific to soil type due to the different sorption capacity of soils (Morel et al. 1996). However, even if microbial biomass P may be underestimated in our clay soil due to greater adsorption of released phosphate, the observed microbial immobilization of P would be further greater by applying a clay-soil specific coefficient, ultimately supporting our observed trends.

The presence of cover crop slightly increased the microbial C:N ratio in both clay and sandy soil in comparison to corresponding bare soil (Fig. 3, Table 2), with no differences associated with cover crop identity. Such a result supports a strict association between C and N acquisition by microbes, also supported by the positive correlation observed between C and N in microbial biomass, suggesting a strong homeostatic relationship between microbial C and N (Cleveland and

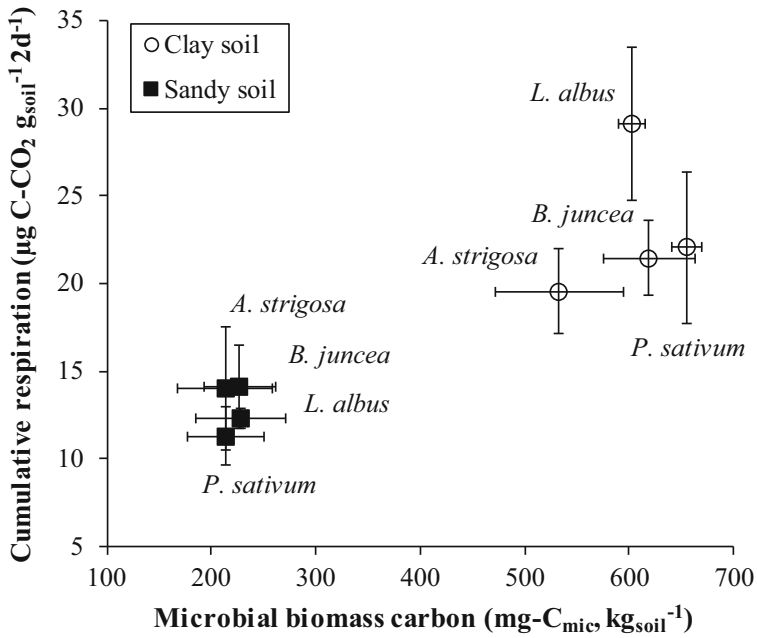

Fig. 4 Mean (± SD) cumulative respiration from clay and sandy soils (n = 4) after three days of incubation in relation to mean (± SD, n = 4) microbial biomass carbon at the beginning of soil incubation. Cover crop species (= plant treatment) were removed before incubation. Full squares refer to sandy soil, whereas open circles to clay soils

Liptzin 2007; Hartmann and Richardson 2013; Mooshammer et al. 2014).

Soil microbial respiration

Regarding the third question about microbial metabolism, the cumulative soil respiration was greater in clay soil in association with a higher microbial biomass C. We did not observe any effect of cover crop on cumulative respiration, despite literature evidence that plant identity and productivity affect microbial diversity and metabolism (Finney et al. 2017; Gao et al. 2017; Steinauer et al. 2016; Zhang et al. 2017). Our data suggest that soil type, i.e. abiotic factors, plays a major role in controlling microbial metabolism (Chodak and Niklińska 2010; Nunan et al. 2017). We hypothesize that the fine texture of clay soil, compared to the coarser texture of sandy soil, provides a physical environment more suitable for microbes (De Vries et al. 2012; Cuadros 2017; Hassink et al. 1993; Vinhal-Freitas et al. 2017), ultimately resulting in greater microbial biomass C and enhanced metabolism (Hartmann and Richardson 2013).

Even if our protocol for measuring the metabolic quotient was limited to only two days of incubation, we were able to observe significant differences in response to soil type. Indeed, the lower value of metabolic quotient in clay soil could be indicative of a more efficient use of nutrient resources by soil microbes (Sinsabaugh et al. 2017; Xu et al. 2017). This result implies that, in clay soil, the microbial communities have a different taxonomic composition compared to sandy soil in response to, for example, differences in soil texture (Sessitsch et al. 2001), bulk soil stoichiometry (Sinsabaugh et al. 2013; Spohn 2015), and soil pH (Wardle and Ghani 1995), ultimately selecting for microbes with different metabolic strategies (Fierer et al. 2007; Malik et al. 2020).

Conclusions

We found that nutrient stoichiometry of aboveground biomass of cover crops is species specific and the growth rate hypothesis can explain stoichiometric differences in response to soil type for most of the cover crop species here studied. Unlike for plants, nutrient stoichiometry of soil microbial biomass was not influenced by cover crop identity or soil type, and showed a higher degree of homeostasis compared to cover crops. The effect of cover crop presence on microbial stoichiometry was dependent on soil type: in clay soil the presence of cover crops promoted microbial P immobilization, whereas in sandy soil cover crops enhanced microbial C and N acquisition. For both soil types, the identity of cover crops did not affect the relative response of microbial stoichiometry. Finally, we observed

that cumulative soil respiration, here used as a proxy for microbial metabolism, differed in relation to soil physical properties (greater in clay than sandy soil) and to the amount of microbial biomass carbon. Our findings, although limited to a greenhouse experiment, can help to implement agro-ecological practices, for example by selecting the appropriate cover crop species in relation to soil type in order to avoid nutrient limitation due to microbial nutrient immobilization.

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Author contributions Luca Bragazza: conceptualization, formal analysis, writing – original draft. Mario Fontana: methodology, investigation, writing – review and editing. Thomas Guillaume: writing – review and editing. Kate M. Scow: writing – review and editing. Sokrat Sinaj: methodology, writing – review and editing.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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