



Driving crop yield, soil organic C pools, and soil biodiversity with selected winter cover crops under no-till

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ABSTRACT

No-till (NT) and cover crops (CCs) have been repeatedly recommended for building-up resilience of agro-ecosystems, enhancing soil biodiversity, and steering efficient nutrients cycling and yield. Yet, the overall impact of CCs on soil properties and dynamics during transition may highly change depending on CC species and interactions with field condition.

In the present 3-yr field study, we (i) examined how selected CCs (i.e. rye [*Secale cereale* L.]; phacelia [*Phacelia tanacetifolia* Benth.] + white mustard [*Sinapis alba* L.]; Italian ryegrass [*Lolium multiflorum* Lam.] + crimson clover [*Trifolium incarnatum* L.] + Persian clover [*Trifolium resupinatum* L.]; hairy vetch [*Vicia villosa* Roth] + crimson clover) affect yield performance in a crop sequence of maize (*Zea mays* L.), soybean (*Glycine max* L. Merr.), and maize under NT, and (ii) assessed the effects of CCs on inputs to the soil (i.e. biomass, carbon [C], and nitrogen [N]), soil organic C (SOC) and pools, as well as microarthropods and earthworms abundance and diversity.

Grain yield during the initial 2-yr period was on average reduced with CCs by 1–23% in maize, and 1–33% in soybean. This effect was less evident with CC residues having low C:N ratio (< 20; i.e. hairy vetch + crimson clover) and erect habitus after termination (i.e. rye). Thereafter, CCs had no effect on maize yield the third year.

Soil organic C and pools indicated that (i) the effect of our CC treatments over a 3-yr application is limited to the topmost 5 cm of soil, and (ii) the biomass input with CC residue and its C:N ratio are crucial for boosting soil C cycling. This was also the case for earthworm-related indicators, while arthropods mainly responded to different CCs in terms of evenness. Yet, our results on soil fauna showed that different groups or species need different time for showing effects, thus suggesting that responses may be fully effective in a >3-yr term.

We concluded that CC mixtures that allow the best compromise between the high amount of residue and the low residue C:N ratio should be preferred for: (i) reducing possible detrimental effects on grain yield of maize and soybean, and (ii) enhancing soil C cycling and biodiversity. Therefore, selecting appropriate CC species in mixtures represents the main challenge at the field level for pursuing both objectives in the shortest timeframe. Within all options in summer crop sequences, here we reported that mixtures including leguminous cover crops might be primarily considered.

1. Introduction

Sustainable land use and management are essential in the delivery of agro-ecosystem services, including biodiversity conservation, landscape preservation, climate regulation, and food provision (FAO, 2019). Various strategies for improving soil quality and nutrient cycling at the

field level have been defined by the Environment Directorate General of European Commission (2016). Conservation agriculture practices were reported within these options as effective alternatives to conventional management approaches. Main reasons are positive contribution to (i) building-up resilience of farming systems, (ii) steering efficient nutrients cycling and yield, (iii) enhancing soil biology, and (iv) promoting

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climate change mitigation and adaptation (Lal, 2015). However, Pitelkrow et al. (2015) in a global meta-analysis documented a yield reduction for a number of field crops during the transition from conventional tillage (i.e. moldboard plowing plus rotary harrowing) to no-till (NT). These authors showed also that introducing cover crops (CCs) within NT systems should be recommended to limit unfavorable effects during such a transition.

Cover crops indeed increase the rate of biomass input to the soil, thus promoting soil organic matter accumulation (Blanco-Canqui and Ruis, 2020). In addition, CC roots act as “bio-drillers” improving soil structure (Fiorini et al., 2018), and indirectly provide pabulum for the entire biotic community in soil (Menta et al., 2020). Yet, the overall impact of CCs on soil properties and dynamics may highly change depending on CC species within each agroecosystem. Gramineous CCs have the highest potential of biomass production, thus targeting nutrient re-cycling and soil organic matter accumulation (Adetunji et al., 2020; Duval et al., 2016). For instance, the concomitant adoption of NT and rye (*Secale cereale* L.) as CC was shown to sustain yield performance of main crops (Boselli et al., 2020), while enhancing soil quality parameters, and keeping nitrous oxide emissions under control (Fiorini et al., 2020a). On the other hand, brassicaceous CCs are widely recognized as highly-effective catch crops and often indicated as the best choice to remediate soil compaction (Blanco-Canqui and Ruis, 2020). Last, leguminous CCs are recognized as the most effective whether maximizing nitrogen (N) input become the priority (Gabriel and Quemada, 2011).

At the field level, CCs are usually cultivated in mixture to pursue more than one agro-ecological function, complementing and synergizing the effects. For instance, leguminous grown together with graminaceous generally promote facilitation effects by transferring biologically fixed N, thus increasing biomass production (Rasmussen et al., 2013). Diverging functional plant traits (complementarity) indeed increase niche differentiation (Hooper, 1998) to produce a more complete use of resources (e.g. soil N) (Fridley, 2001). It was previously reported that growing together Italian ryegrass (*Lolium multiflorum* Lam.) and clovers maximizes such a resource use complementarity (Ryan-Salter and Black, 2012). However, while their use as forage crops has been widely studied, there is a lack of knowledge concerning the responses of soil quality parameters and crop yield of the following main crop to Italian ryegrass and clovers cultivation in mixture as CC.

Positive effects due to complementarity of plant traits or facilitation were reported to be boosted also by mixing species belonging to the same botanical family (Elsalahy et al., 2019). This is especially the case when these species have contrasting above- and below-ground growing traits. For instance, the cultivation in mixture of hairy vetch (*Vicia villosa* Roth) and clovers may potentially follow this statement and enhance the functional differentiation of above- and below-ground community traits, over space and time. Yet, these hypotheses still need to be corroborated with a multi-year experimental approach.

Other combinations at the field level could be selected with mixtures targeted to different agro-ecological functions, as the case of phacelia (*Phacelia tanacetifolia* Benth.) and white mustard (*Sinapis alba* L.). While earlier studies reported the positive effects of phacelia and white mustard cultivated as CC monocultures, their combined effects on soil quality and crop yield of main crops is still missing. The common trait of these two species in temperate climates is a very fast growth before winter (Brust et al., 2014). In addition, they both are excellent N and P scavengers and their residues have relatively low percentage of lignin and C:N ratio (Justes et al., 2009; Liu et al., 2013; Stivers-Young, 1998), which is pivotal for fast and efficient organic matter humification.

Indeed, differences of biomass inputs (in terms of amount and physico-chemical properties, e.g. C:N ratio) to the soil – as derived by different CC species or mixtures – highly affect the degradation of biomass itself and the inclusion of the deriving fresh organic carbon (C) into soil organic carbon (SOC) through humification (Nicolardot et al., 2001). Concentrations and proportions of SOC fractions between available and recalcitrant C pools are useful indicators of decomposition

kinetics and humification degree (Vieira Guimarães et al., 2013). The same difference in biomass inputs may have an impact also on soil fauna through modifications in their microhabitat and food resources (Bardgett and Cook, 1998; Menta et al., 2020). Beyond its important role in maintaining soil quality and providing ecosystem services, soil fauna has also been included in soil monitoring programs as bio-indicator (Menta and Remelli, 2020).

Diversity, often using synthetic indices approach (such as Simpson, Pielou and Shannon), and abundance, are the most used parameters applied to soil fauna. Unfortunately, the use of these biological parameters alone can be inadequate to explain soil health and quality exhaustively, since they do not take into account neither the ecological role of each taxon nor alteration in community structure. It is known that some soil fauna groups are particularly sensitive to changes in soil management and may ultimately be informative of soil quality variations (Parisi et al., 2005). However, to select a battery of indicators relevant for specific purposes (such as soil quality assessment), the comparison of different biological descriptors is recommended (Pérez et al., 2011). Some studies have suggested that earthworms can be useful in soil quality assessment in different land uses, due to their key functional role in soil ecosystems and their sensitivity to changes in soil properties and plant cover. According to this feature, earthworm biodiversity, abundance and biomass are also considered useful indicators of soil biological activity and quality (van Ekeren et al., 2009; Kanianska et al., 2016).

The objectives of this study were: (i) to examine how different widespread CCs (i.e. rye [*Secale cereale* L.]; phacelia [*Phacelia tanacetifolia* Benth.] + white mustard [*Sinapis alba* L.]; Italian ryegrass [*Lolium multiflorum* Lam.] + crimson clover [*Trifolium incarnatum* L.] + Persian clover [*Trifolium resupinatum* L.]; hairy vetch [*Vicia villosa* Roth] + crimson clover) affect yield performance of main crops under NT as compared with no-CCs, during a 3-yr crop sequence (i.e. maize (*Zea mays* L.), soybean (*Glycine max* L. Merr.), and maize); (ii) to assess the 3-yr effects of CCs treatments on inputs (i.e. biomass, C, and N) to the soil, SOC and C pools concentrations, as well as soil fauna (i.e. microarthropods and earthworms) abundance and diversity. The following hypotheses were tested: (i) crop yield is increased by CCs, especially yield of maize by leguminous-based CCs; (ii) CCs treatments with the highest biomass production and lowest biomass C:N ratio are the most effective for enhancing soil quality.

2. Materials and methods

2.1. Field site and treatments

A three-year field study was conducted between September 2016 and October 2019, at the commercial “Ciato farm”, located in Panocchia (44°40'20.3"N 10°18'04.5"E; 174 m asl), near Parma, Po Valley, Northern Italy. The soil had a clay loam texture (sand 339, silt 368, and clay 293 g kg⁻¹) in the upper layer (0–30 cm), and was classified as a loamy, mixed, mesic Fluventic Ustochrepts, according to the Soil Taxonomy (NRCS Soil Survey Staff, 2014). Initial soil physical and chemical properties in the 0–30 cm soil layer were: pH 6.5, SOC 10.9 g kg⁻¹, total N 1.1 g kg⁻¹, available P 34 mg kg⁻¹, exchangeable K 131 mg kg⁻¹, and cation exchange capacity 21 cmol⁺ kg⁻¹. The climate is temperate (Cfa as Köppen classification), mean annual temperature is 13.1 °C and annual precipitation is 830 mm.

The field experiment was conducted on a three-year summer-crop sequence with maize (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.), and maize again. Experimental treatments were established in September 2016. Conversion to NT occurred with the experiment starting, since the entire field was previously managed with conventional tillage practices (i.e. moldboard plowing plus rotary harrowing, without cover crops). Cover crops were cultivated from September to middle March in the 2016–2017 winter season, from September to end of March in the 2017–2018 winter season, while from October to middle

March in the 2018–2019 winter season. In detail, treatments were: (1) Control, a no-CC treatment as a control; (2) R, a rye cover crop; (3) PM, a two-species mixture composed by phacelia (62%) and white mustard (38%); (4) RCC, a three-species mixture composed by Italian ryegrass (48%), crimson clover (38%), and Persian clover (14%); (5) VC, a two-species mixture composed by hairy vetch (35%) and crimson clover (65%). As a result, the experiment design was a randomized complete block (RCB) with three blocks and five treatments corresponding to the five winter cover crops. Plot size was 2600 m² (20 m width and 130 m length). Sowing of CCs took place with a sod-seeder each year, two weeks after having harvested the previous main crop. Seeding rates of CCs were: 100 kg ha⁻¹ for R, 25 kg ha⁻¹ for PM, 65 kg ha⁻¹ for RCC, and 50 kg ha⁻¹ for VC. Cover crop termination took place each year right before planting the main crop by spraying 3 L ha⁻¹ of Roundup Platinum (Glyphosate 79.5%) in all CC treatments, and in Control treatment to suppress spontaneous weeds. Main crops (i.e. maize and soybean) were planted at a 70 cm row distance. Maize was planted at the beginning of April (in both years); soybean at the beginning of May. Number of plants per square meter was 7.5 and 7.7 for maize (in 2017 and 2019, respectively), and 38.5 for soybean. Both maize and soybean were irrigated by traveling sprinkler. Fertilizations occurred for maize (220 kg N ha⁻¹ as urea), with two applications (100 kg N ha⁻¹ at V2-3 and 120 kg N ha⁻¹ at V6-7), at the same rate for all treatments. Harvest took place at the beginning of September for maize (in both years), and at the end of September for soybean.

2.2. Plant biomass sampling and analyses

Total aboveground biomass of the main crops and CCs was measured every year right before harvest and termination, respectively. Biomass samples were collected from three random areas of 6 m² within each plot for main crops, and of 4 m² for CCs. In the case of main crops, grain was manually separated from the crop residue. Right after, the main crop was harvested by combine and all the grain from each plot was weighted and sampled separately.

Once in the lab, grain samples were dried at 105 °C for 24 h and weighted to determine crop yield. Residue samples (of the main crops and CCs) were dried at 65 °C until constant weight and then ground at 1 mm size. Then C and N concentrations were determined for all residue samples by the Dumas combustion method with an elemental analyzer (Vario Max CNS, Elementar, Germany). Residue-derived C and N inputs to the soil for each crop (main crop and cover crop) was calculated by multiplying the weight of biomass by their C and N concentrations. 3-yr cumulative biomass, C, and N, input with main crop and cover crop residue separately were calculated. 3-yr average C:N residue of both main crop and cover crops was also computed.

2.3. Soil sampling and analyses

Soil sampling took place at the end of the experiment (October 2019), immediately after harvesting maize. Within each plot, six soil sub-samples at 0–30 cm depth were collected using a coring device with a 15-mm diameter auger. After extraction, each soil core was divided into three portions according to the three different soil layers: 0–5 cm, 5–15 cm, and 15–30 cm. The six sub-samples of each layer for each plot were pooled together and mixed. As a result, the total number of soil samples was 45. Samples were then air dried, passed at 2-mm sieve and analyzed. Soil organic carbon (SOC) concentration was determined as Walkley & Black method (Nelson and Sommers, 1996). Total extractable carbon (TEC), and humic and fulvic acid carbon (HA + FA) were determined according to Nelson and Sommers (1996) with the dichromate oxidation method. Not humified and more labile C fraction (NHC) was calculated as follows:

$$\text{NHC} = \text{TEC} - (\text{HA} + \text{FA})$$

Not extractable organic carbon (NEC), conventionally defined as humin (a pool of organic carbon recalcitrant to microbial degradation), was calculated as the difference between SOC and TEC (2):

$$\text{NEC} = (\text{SOC} - \text{TEC})$$

Humification rate (HR) was determined according to Francaviglia et al. (2017) as follows:

$$\text{HR} = (\text{HA} + \text{FA} \times 100) / \text{SOC}$$

2.4. Microarthropod-based soil quality evaluation

For soil arthropod extraction, within each plot, three soil cubes of 10 × 10 × 10 cm were collected using a spade after removing the superficial litter. Also in this case, soil sampling took place at the end of the experiment (October 2019), immediately after maize harvesting. The soil samples were carried to the lab within 24 h. Arthropods were extracted using the Berlese-Tullgren funnel (2 mm mesh size, extraction time 10 days) and preserved in a 70% ethanol and 30% glycerol solution. The extracted specimens were identified at class level for Myriapoda and order level for Crustacea, Hexapoda and Arachnida using a stereomicroscope (20–40 ×). All the specimens belonging to each taxon were counted to obtain abundance data (expressed in individuals m⁻²). For each plot, Simpson Index of diversity (1-D), Shannon Diversity Index (H) and Pielou's evenness (J) were applied to arthropod data.

To evaluate the microarthropod-based soil quality, QBS-ar index was applied (Parisi et al., 2005). This index is based on the positive relation between the number of arthropod groups adapted to soil and the soil biological quality. Indeed, soil arthropods show morphological characters revealing their adaptation to soil habitat. Higher morphological adaptation to soil indicates higher sensitivity to chemical and physical variation, and, consequently, to soil degradation. Therefore, a higher soil quality would be related to a higher number of well adapted microarthropod groups. QBS-ar index is based on the morphological characters mentioned above, assigning at each taxon, an Eco-Morphological index (EMI), ranging between 1 and 20, in relation to the adaptation level to soil (1 = no adaptation; 20 = best adaptation). QBS-ar results from the sum of each maximum EMI score assigned at each taxon identified in the soil sample. For more details, see Menta et al. (2018).

2.5. Earthworm sampling and counting

Three undisturbed soil cubes of 8000 cm³ (20 × 20 × 20 cm) were collected from each plot by a spade and brought to the lab within 24 h. Thereafter, earthworms were manually separated from the soil and counted to determine the number of individuals (Shepherd et al., 2008). Before being weighted, earthworm intestines were voided according to (Dalby et al., 1996). Thus, the earthworm density (number of earthworms per square meter) and the earthworm biomass (g of earthworms as dry biomass per square meter) were calculated by multiplying the number and the dry biomass of earthworms extracted from each undisturbed soil cubes by 25.

2.6. Statistical analyses

Data on (i) grain yield of main crops (i.e. maize 2017, soybean 2018, and maize 2019), (ii) 3-yr inputs (i.e. biomass, C, and N) to the soil due to main crop and CC residues, (iii) SOC and C pools concentrations (i.e. TEC, HA+FA, NHC, and NEC), as well as (iv) humification index (i.e. HR), were statistically analyzed with linear analyses of variance (ANOVA) by using the “agricolae” package of RStudio 3.3.3 (R Core Team, 2020). Similarly, ANOVA was applied to test for differences between treatments on both microarthropod and earthworm data. The

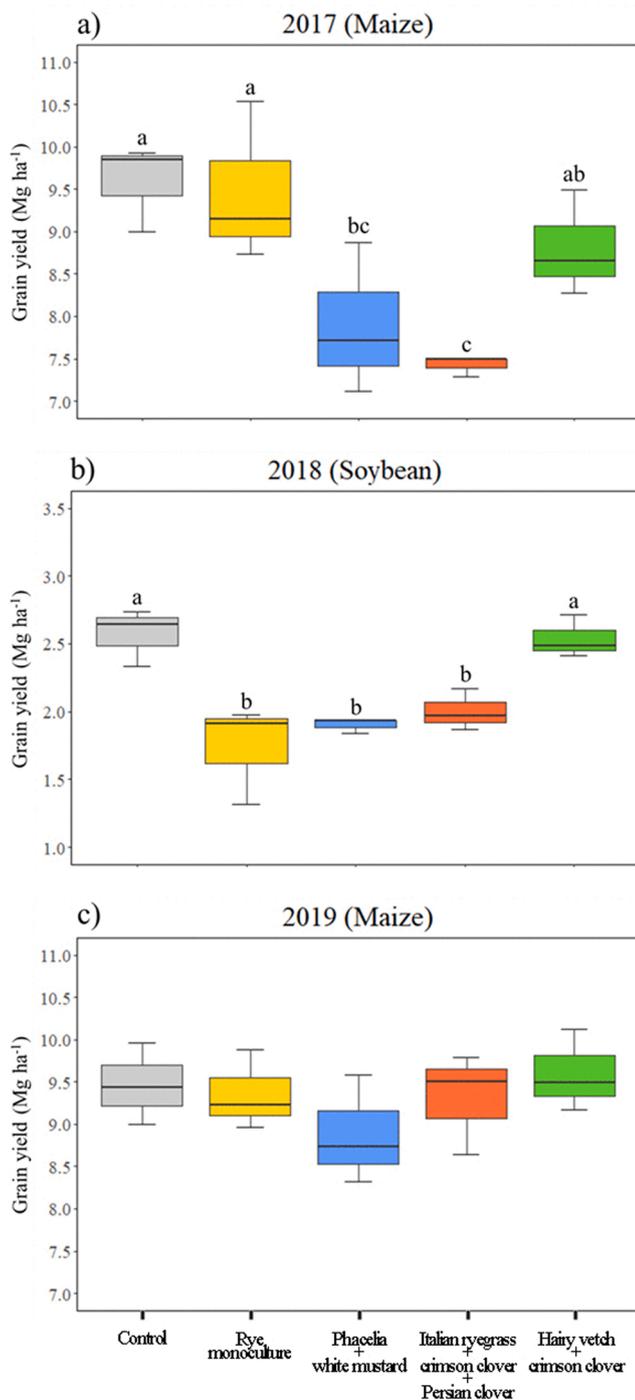


Fig. 1. Grain yield (Mg ha^{-1}) of maize (a), soybean (b), and maize (a) during the 3-yr field study as affected by treatment: control (Control); rye (R); phacelia + white mustard (PM); Italian ryegrass + crimson clover + Persian clover (RCC); hairy vetch + crimson clover (VC). Different letters above bars mean significant differences between treatments.

variables considered were arthropod total abundance, abundance of arthropods showing EMI 20, number of eco-morphological groups, and number of eco-morphological groups with EMI 20, the indexes (i.e. Simpson, Shannon, Pieolu's evenness and QBS-ar), earthworm's density and weight. Tukey test was performed as *post-hoc* in within treatments comparisons; while Dunnett test, using R package "DescTools" (Signorell et al., 2020), was used to compare treatments with Control. Models for multiple linear regression were carried out with microarthropod and earthworm variables and biomass input and C:N ratio (of main crop +

Table 1

3-yr cumulative biomass, C (Mg ha^{-1}), and N input (kg ha^{-1}), as well as average C:N ratio, as affected by treatments, in main crop (upper part) and cover crop (lower part) residue. Control; R: Rye; PM: Phacelia and white Mustard; RCC: Italian Ryegrass, crimson Clover, and Persian Clover; VC: hairy Vetch and crimson Clover. Lowercase letters indicate differences among treatments within the same type of residue. P-values by ANOVA are also reported.

| Residue | Treatment | 3-yr cumulative biomass input (Mg ha^{-1}) | 3-yr cumulative C input (Mg ha^{-1}) | 3-yr cumulative N input (kg ha^{-1}) | 3-yr average C:N ratio |
|--------------------|-----------------------|---|---|---|------------------------|
| Main crops residue | Control | 35.60 | 16.84 | 259.61a | 62 |
| | R | 33.77 | 16.29 | 218.28b | 75 |
| | PM | 29.51 | 14.67 | 220.36b | 67 |
| | RCC | 27.42 | 13.46 | 212.63b | 64 |
| | VC | 33.29 | 16.45 | 255.00a | 65 |
| | <i>p</i> (<i>F</i>) | 0.0675 | 0.0740 | 0.0288 | 0.1235 |
| CCs residue | R | 7.42a | 3.68 | 157.59b | 24c |
| | PM | 6.61b | 2.90 | 150.16b | 19b |
| | RCC | 7.65a | 3.62 | 157.59b | 24c |
| | VC | 4.18c | 2.00 | 193.92a | 10a |
| | <i>p</i> (<i>F</i>) | 0.0125 | 0.0522 | 0.0426 | 0.0034 |

CC residues) as terms.

All variables were examined for normality with Shapiro-Wilk test and for homogeneity of variances with Levene's test prior to perform the analyses. Mean values were separated with Tukey's test ($\alpha = 0.05$) by using the "multcomp" package. Only TotEMG data, which did not show a normal distribution, were square root transformed.

Arthropod community matrix was square root transformed to minimize the influence of the most abundant groups and Bray-Curtis dissimilarity index was calculated. Then, permutational multivariate analysis of variance (PERMANOVA) was conducted on the dissimilarity matrix, considering treatments as independent variables, and using the R package "RVAideMemoire" for pairwise comparisons. Data were visualized with non-metric multidimensional scaling (NMDS) and hierarchical clustering. An analysis of similarity percentages (SIMPER) was then performed to test which arthropod groups were driving the differences in assemblages. Ordination, PERMANOVA and SIMPER were all performed using the R package "vegan" (Oksanen et al., 2008).

3. Results

3.1. Grain yield

Grain yield was significantly affected by CC treatment in maize 2017 (Fig. 1a) and in soybean 2018 (Fig. 1b), while not in maize 2019 (Fig. 1c). In detail, maize grain yield in 2017 was the highest under Control and R, and progressively decreased in the order $\text{VC} \geq \text{PM} \geq \text{RCC}$.

Control had the highest grain yield also with soybean in 2018 (together with VC in this case), followed by RCC, PM, and R (Fig. 1).

3.2. Residue-derived biomass, C, and N inputs

The 3-yr cumulative biomass and C inputs to the soil due to main crops residue were not affected by CC treatment, although values tended (*p* between 0.06 and 0.08) to be higher under Control, R, and VC, and lower under RCC and PM (Table 1). The 3-yr cumulative N input due to main crop residue was significantly higher under Control and VC than under RCC, R, and PM. No difference was found in 3-yr average C:N ratio.

The cumulative 3-yr biomass input to the soil due to CC residue (which excluded Control by definition) was significantly lower under VC than under the other CC treatments (Table 1). The cumulative 3-yr C input was not statistically affected. Nevertheless, VC tended ($p = 0.0522$) to have the lowest 3-yr cumulative values also in this case.

Table 2

Concentration (g kg^{-1} soil) of soil organic carbon (SOC), total extractable carbon (TEC), humic and fulvic acid carbon (HA+FA), not humified carbon (NHC), and not extractable organic carbon (NEC), as well as humification rate (HR), in different soil layers (0–5 cm; 5–15 cm; 15–30 cm) as affected by 3-yr cover crop treatment. Control; R: Rye; PM: Phacelia and white Mustard; RCC: italian Ryegrass, crimson Clover, and persian Clover; VC: hairy Vetch and crimson Clover. Lowercase letters indicate differences among treatments within the same type of residue. P-values by ANOVA are also reported.

| Soil depth | Treatment | SOC | TEC | HA+FA | NHC | NEC | HR |
|------------|-----------------------|--|--------|--------|--------|---------|--------|
| | | Pool amount (g kg^{-1} soil) | | | | | |
| 0–5 cm | Control | 12.19c | 1.99b | 1.11b | 0.89 | 10.50b | 8.88b |
| | R | 13.17b | 2.32a | 1.32a | 1.00 | 10.85ab | 10.04a |
| | PM | 13.70a | 2.28a | 1.28a | 1.02 | 11.22a | 9.31ab |
| | RCC | 13.15b | 2.00b | 1.10b | 0.91 | 11.17a | 8.34b |
| | VC | 12.99b | 2.21ab | 1.19ab | 1.02 | 10.54b | 9.17ab |
| | <i>p</i> (<i>F</i>) | 0.0337 | 0.0427 | 0.0458 | 0.5887 | 0.0403 | 0.0478 |
| 5–15 cm | Control | 11.06 | 1.73 | 1.03 | 0.71 | 9.33 | 9.29 |
| | R | 12.11 | 1.82 | 1.12 | 0.71 | 10.28 | 9.23 |
| | PM | 11.68 | 1.86 | 1.18 | 0.68 | 9.82 | 10.09 |
| | RCC | 11.16 | 1.87 | 1.12 | 0.74 | 9.29 | 10.04 |
| | VC | 11.76 | 1.80 | 1.10 | 0.71 | 9.95 | 9.33 |
| | <i>p</i> (<i>F</i>) | 0.0875 | 0.4063 | 0.3073 | 0.9812 | 0.0799 | 0.2696 |
| 15–30 cm | Control | 10.59 | 1.62 | 0.92 | 0.70 | 8.96 | 8.74 |
| | R | 11.32 | 1.82 | 1.08 | 0.74 | 9.50 | 9.53 |
| | PM | 10.77 | 1.76 | 1.04 | 0.72 | 9.01 | 9.68 |
| | RCC | 10.40 | 1.71 | 0.98 | 0.73 | 8.69 | 9.41 |
| | VC | 11.43 | 1.82 | 1.02 | 0.80 | 9.61 | 8.91 |
| | <i>p</i> (<i>F</i>) | 0.1282 | 0.1080 | 0.5171 | 0.7828 | 0.2017 | 0.7521 |

Table 3

Abundance of eco-morphological groups (ind. m^{-2}), total arthropods abundance (ind. m^{-2}), abundance of arthropods with EMI 20 (ind. m^{-2}), total number of eco-morphological groups and number of eco-morphological groups with EMI 20, as affected by 3-yr cover crop treatment. Control; R: Rye; PM: Phacelia and white Mustard; RCC: italian Ryegrass, crimson Clover, and persian Clover; VC: hairy Vetch and crimson Clover. Mean values \pm Standard Error. Different superscript letters in the variables used for statistical analysis mean significant differences between treatments.

| | Control | R | PM | RCC | VC |
|---------------------------------------|------------------------------|-------------------------------|-------------------------------|------------------------------|-------------------------------|
| Acari | 155.69 \pm 37.45 | 162.76 \pm 69.70 | 169.84 \pm 0.00 | 134.46 \pm 7.08 | 127.38 \pm 32.43 |
| Araneidae | - | 7.08 \pm 7.08 | 7.08 | - | - |
| Isopoda | - | 14.15 \pm 7.08 | - | - | - |
| Chilopoda | - | - | 7.08 \pm 7.08 | 7.08 \pm 7.08 | 7.08 \pm 7.08 |
| Symphyla | - | 7.08 \pm 7.08 | 7.08 \pm 7.08 | - | - |
| Coleoptera | 14.15 \pm 14.15 | 14.15 \pm 14.15 | - | - | 35.38 \pm 7.08 |
| Collembola | 99.07 \pm 35.38 | 56.61 \pm 7.08 | 70.77 \pm 7.08 | 134.46 \pm 35.38 | 70.77 \pm 14.15 |
| with EMI 20 | 7.08 \pm 7.08 | 7.08 \pm 7.08 | - | 21.23 \pm 0.00 | 35.38 \pm 18.72 |
| Diplura | 7.08 \pm 7.08 | 7.08 \pm 7.08 | 7.08 \pm 7.08 | 21.23 \pm 0.00 | 7.08 \pm 7.08 |
| Hemiptera | - | - | - | - | 7.08 \pm 7.08 |
| Hymenoptera | 21.23 \pm 0.00 | 14.15 \pm 14.15 | 7.08 \pm 7.08 | 14.15 \pm 14.15 | 42.46 \pm 0.00 |
| Psocoptera | 21.23 \pm 0.00 | 63.69 \pm 32.43 | - | 14.15 \pm 7.08 | 28.31 \pm 18.72 |
| Others | - | 7.08 \pm 7.08 | - | 7.08 \pm 7.08 | - |
| Holometabola | - | 7.08 | - | 7.08 | - |
| Coleoptera (larvae) | - | 21.23 \pm 12.26 | - | 42.46 \pm 42.46 | - |
| Total abundance | 325.53 \pm 67.51 | 382.14 \pm 139.21 | 275.99 \pm 42.46 | 396.29 \pm 78.80 | 360.91 \pm 63.69 |
| of which with EMI 20 | 14.15 \pm 7.08 | 21.23 \pm 12.26 | 21.23 \pm 21.23 | 49.54 \pm 7.08 | 49.54 \pm 18.72 |
| n° of eco-morphological groups | 4.67 \pm 0.67 | 6.33 \pm 1.33 | 6.00 \pm 0.58 | 5.33 \pm 0.88 | 5.67 \pm 0.66 |
| of which with EMI 20 | 1.33 \pm 0.33 ^b | 2.00 \pm 0.58 ^{ab} | 3.00 \pm 0.58 ^{ab} | 3.33 \pm 0.33 ^a | 2.33 \pm 0.33 ^{ab} |

Conversely, the cumulative 3-yr N input due to CC residue was significantly higher under VC than under RCC, R, and PM. This turned into the lowest C:N ratio for VC residues.

3.3. Soil organic C and pools

3-year CC treatments significantly affected SOC concentration in the 0–5 cm soil layer (Table 2): PM had the highest SOC concentration, Control was the lowest, while all the others CC treatments were not different from both the former and the latter. Such a significant effect of CC treatment in SOC concentration was not recorded in the 5–15 cm and in the 15–30 cm soil layer (Table 2). Nevertheless, differences among treatments were close to be significant (at least in the 5–15 cm, with a *p*-value of 0.0875), but the treatments hierarchy did not follow the same pattern as in the 0–5 cm soil layer. In detail, R and VC treatments tended to increase SOC concentration in the 5–15 cm and 15–30 cm soil layer, respectively; RCC and Control had always the lowest SOC concentration values; PM led to an intermediate value of SOC concentration in both soil layers.

Total extractable carbon (TEC) and HA+FA concentrations in the 0–5 cm soil layer were significantly affected by CC treatment (Table 2). Both R and PM had the highest concentrations in this case, then RCC and Control had the lowest TEC and HA+FA, while VC showed intermediate values. As regard the 5–15 cm and the 15–30 cm soil layers, TEC and HA+FA concentrations were not statistically affected by treatment (Table 2). Nevertheless, concentration values were always the lowest for both the two fractions and two soil layers under C.

Similarly to SOC concentration, NEC was found to have the highest concentration under PM (and also under RCC in this case) in the 0–5 cm soil layer, while under R (although without statistical significance; *p*-value 0.0799) in the 5–15 cm soil layer (Table 2).

The HR showed significant differences among CC treatments in the 0–5 cm soil layer, while not in the deeper ones. In detail, R had the highest HR, Control and RCC the lowest, while PM and VC showed intermediate values (Table 2).

3.4. Soil arthropods

Our results on the abundance of eco-morphological groups showed that a minimum of 4 and a maximum of 9 groups were identified, for a total number of arthropods ranging between 191 ind. m^{-2} and 552 ind. m^{-2} (Table 3). Among all arthropods extracted, 43% were Acari, 29%

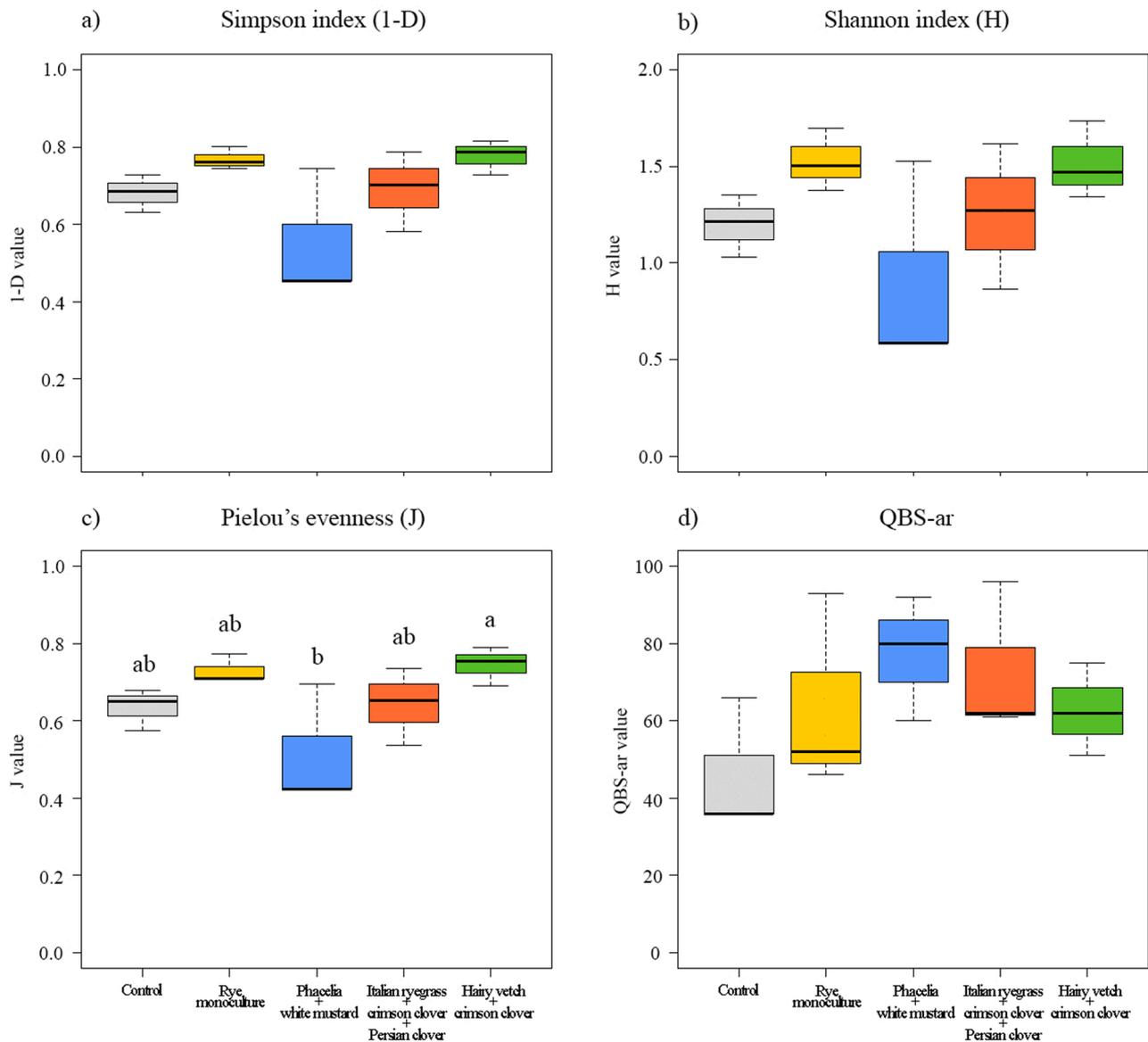


Fig. 2. Box-plots of (a) Simpson index, (b) Shannon index, (c) Pielou's evenness, and (d) QBS-ar index for each treatment. The bottom and top of each box represent the lower and upper quartiles respectively, the line inside each box shows the median and whiskers indicate minimal and maximum observations. Different letters above bars mean significant differences between treatments: control (Control); rye (R); phacelia + white mustard (PM); Italian ryegrass + crimson clover + Persian clover (RCC); hairy vetch + crimson clover (VC).

Collembola, 7% Coleoptera (adults:larvae in a ratio of 1:1), 7% Psocoptera, 6% Hymenoptera, 3% Diplura and 1% Chilopoda. Araneidae, Isopoda, Symphyla, Hemiptera and others Holometabola account each one for less than 1%. No significant difference was found in total abundance and in abundance of microarthropods with EMI 20, and neither in the total number of eco-morphological groups while the number of groups having EMI 20 showed a significant increase under RCC compared to the Control ($p < 0.05$) (Table 3). Such a difference was mainly due to the presence of Collembola with EMI 20 and Chilopoda.

In the present study, neither Simpson index (1-D) nor Shannon index (H) differed significantly within treatments and between them and the Control, while Pielou's evenness (J) differed only within treatments ($p < 0.05$; Fig. 2a, b, and c, respectively). Nevertheless, PM always showed the lowest value, while R and VC constantly highlighted the highest ones (although significantly only with Pielou's). Last but not least, the QBS-ar index was also not significantly affected by treatment (Fig. 2d) in our experiment, although Control tended to have the lowest value.

No arthropod-based variable resulted explained by multiple linear regression models with C:N ratio and biomass input terms (data not shown).

PERMANOVA analysis showed that arthropod assemblages differed between treatments ($p < 0.01$; Fig. 3a); however, no pairwise comparison resulted significant. SIMPER analysis showed that treatment communities differed one to each other for less than 50%, with major dissimilarities in R vs PM and R vs RCC. Those differences were driven in both cases by Psocoptera and Coleoptera larvae, with the addition of Acari in the first contrast and Collembola in the second one (Table A1). Overall, Coleoptera was one of the most important taxa for discriminating between treatments. From the community structure analysis, three clusters emerged: R on one side, PM and RCC on the other, and VC and Control in the middle, thus supporting the NMDS representation (Fig. 3b). In this background, Hymenoptera, despite their lower abundance, was the group that more often influence treatments community dissimilarities.

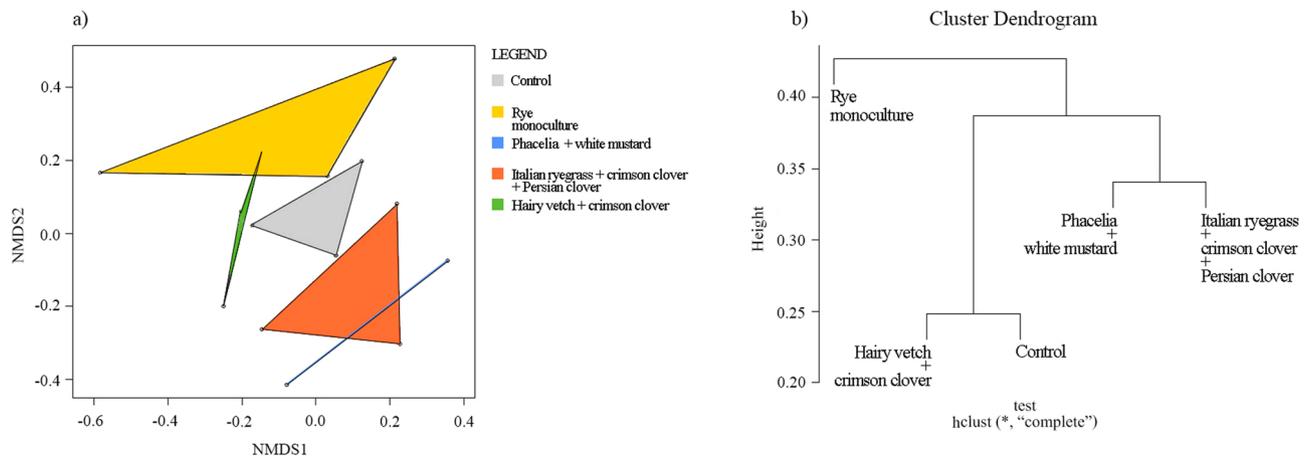


Fig. 3. (a) NMDS ordination plot (stress: 0.1636321), and (b) hierarchical clustering on Bray-Curtis dissimilarities in arthropods communities according to treatments: control (Control); rye (R); phacelia + white mustard (PM); Italian ryegrass + crimson clover + Persian clover (RCC); hairy vetch + crimson clover (VC). Different letters above bars mean significant differences between treatments.

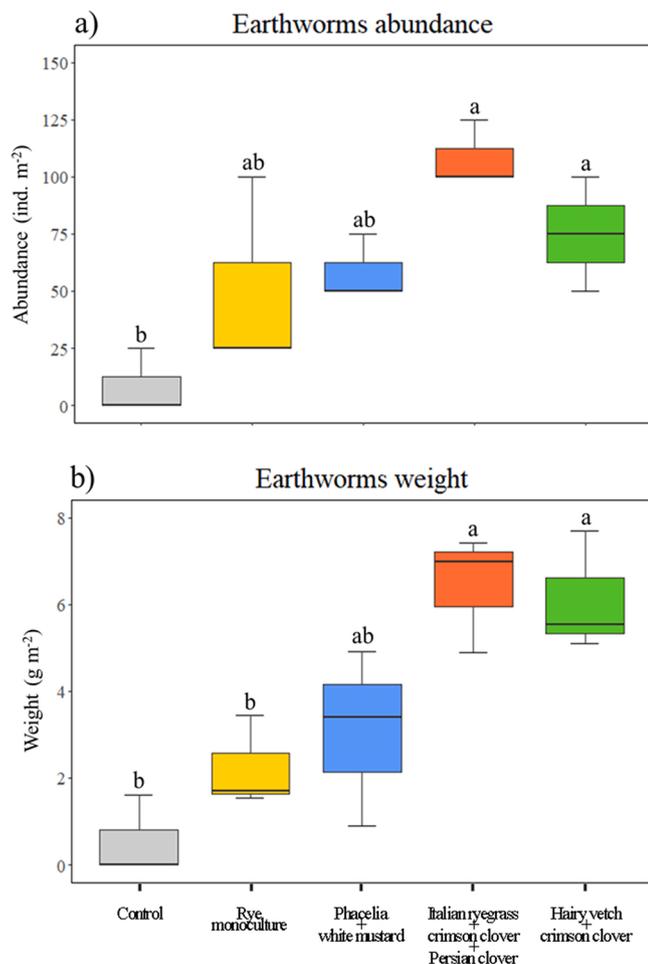


Fig. 4. Box-plots of (a) earthworm abundance and (b) earthworm weight for each treatment. The bottom and top of each box represent the lower and upper quartiles respectively, the line inside each box shows the median and whiskers indicate minimal and maximum observations. Different letters above bars mean significant differences between treatments: control (Control); rye (R); phacelia + white mustard (PM); Italian ryegrass + crimson clover + Persian clover (RCC); hairy vetch + crimson clover (VC).

3.5. Earthworms

The treatment RCC had the highest value of earthworm abundance, followed by VC, while Control was the lowest. Both RCC and VC abundance significantly differed from the Control (Fig. 4a). Earthworm weight showed a similar pattern, with the highest value in RCC and VC and the lowest in Control. (Fig. 4b). Differences were observed between treatments ($p \leq 0.01$), with RCC and VC significantly higher when compared to R, while no differences were observed when compared with PM. Conversely, both earthworm abundance and weight were explained (for at least 50% of their variance and with a $p < 0.01$) by a multiple regression model using C:N ratio and biomass input terms, in the present study. Nevertheless, the C:N ratio was the only one affecting (negatively) the dependent variables, both for abundance and weight model ($p < 0.01$ and $p < 0.001$, respectively).

4. Discussion

4.1. Effect of cover crops on grain yield of maize and soybean

The highest maize yield under Control in the present study is in apparent contradiction with previous outcomes reporting that negative effects of NT on crop yield in the initial years might disappear in the case of concomitant inclusion of CCs (Boselli et al., 2020; Pittelkow et al., 2015). Main reasons reported for such a positive effect of CCs were: the increased soil organic matter and nutrient cycling due to extra-inputs of biomass (Blanco-Canqui et al., 2011), as well as the “bio-drilling” function of CC roots improving soil structure (Fiorini et al., 2018). However, other authors (Calonego and Rosolem, 2010) showed that soil compaction during transition to NT remains a main issue in the very initial years in spite of the concomitant inclusion of CCs, since their actions are gradual, being fully effective in a 3- to 4-year term. Our results are in agreement with this second statement and showed no positive yield effects of CCs in the very short term (2017 and 2018).

In addition, the highest grain yield under Control also with soybean in 2018 (together with VC in this case) suggests that yield responses to CC treatment in our experiment were associated to other than factors related to differential soil compaction and/or root development. A possible explanation is that CC residue may have had negative effects on the initial phenological phases of main crops. It is well known indeed that NT per se reduces soil temperature and delays emergence and initial rooting of crops planted in early spring under temperate climate (Wang et al., 2012). Then, combining certain (i.e. PM and RCC on maize in 2017, R, PM, and RCC on soybean in 2018) CCs and NT may have further boosted this effect, thus leading to a reduced yield under certain CC

treatments compared with under Control in our study. Similar results were previously reported by [Salmerón et al. \(2011\)](#) under similar soil-climate conditions.

Yet, certain other CCs (i.e. VC and R on maize in 2017, VC on soybean in 2018) had no effect on crop yield in our experiment. This was probably because of novel aspects not considered before: on one side, beyond the highest related N input, VC residue – with a low C:N ratio – underwent to a fast decomposition in both years, thus limiting the effect of reducing soil temperature; on the other side, R residue might have behaved as VC residue with respect to soil temperature, but mainly because of its erect habitus also after termination, and only in the case of early termination timing (middle of March as in 2017) with a relatively low residue amount.

4.2. Responses of residue-derived biomass, C, and N input

Overall, Control in our study increased (or tended to increase) biomass, C, and N input due to main crops residue compared with CC treatments probably because of a differential growth performance as a consequence of presence/absence of CC residues (as discussed above). Previous findings indeed suggested that a delayed plant growth during the initial stages often results into a reduced plant height, which negatively affect the amount of biomass, C, and N input to the soil as crop residue ([Dam et al., 2005](#)).

On the other hand, our results on CC residue showed that the gramineous-based CCs had the highest values of cumulative 3-yr biomass and C input, while VC had the lowest ones. Conversely, VC the highest cumulative 3-yr N input, which turned into the lowest C:N ratio for VC residues. Taking into account climate variability, these results confirmed that gramineous-based CCs are generally those with the highest productivity potential (both in term of biomass and C) under NT ([Duval et al., 2016](#)). Yet, whether maximizing N input become the priority legumes will be more effective ([Gabriel and Quemada, 2011](#)).

4.3. Impact of cover crop on soil organic C and pools

Our results showed that SOC concentration in the 0–5 cm soil layer was increased by all the tested 3-year cover crops, being PM the CC treatment leading to the highest SOC increase. Conversely, no significant effect was recorded in the 5–15 cm and in the 15–30 cm soil layer, although R and VC tended to have the highest SOC concentration in the 5–15 cm and 15–30 cm soil layer, respectively. RCC and Control had always the lowest SOC concentration values; PM led to an intermediate value of SOC concentration in both soil layers. These results highlight that the tested CCs have the potential to boost SOC accumulation, even though not at the same extent. Additional biomass (and C) input due to CCs cultivation may indeed increase SOC concentration due to the extra-amount of crop residues ([Duval et al., 2016](#)). However, such an effect is often limited to the topmost centimeters of soil if NT is adopted and direct inputs to the deeper soil by plowing are suspended ([Boselli et al., 2020](#)).

A 3-year period of PM cultivation as winter CC was the best option to increase SOC concentration in the 0–5 cm soil layer in the tested soil-climate condition. PM was better than gramineous-based CCs (i.e. R and RCC), although R and RCC had higher 3-yr cumulative biomass input (and tended to have also higher 3-yr cumulative C input) to the soil than PM. This suggests that other than biomass and C inputs were the main drivers regulating SOC concentration, at least in the short term prospective. In particular, the C:N ratio of PM residues was lower than that of R and RCC, which may have promoted the degradation of residues and the inclusion of the deriving C into the SOC through humification ([Nicolardot et al., 2001](#)). Higher humification coefficients with lower C:N residue are widely recognized ([Hättenschwiler and Gasser, 2005](#); [Nicolardot et al., 2001](#)). However, this was not the case of VC treatment although the lowest C:N ratio of residues and the highest humification rate, because of the much lower biomass input (between –

37% to – 65%) than all the other CC treatments.

As regards soil C pools, TEC and HA+FA concentrations in the 0–5 cm soil layer were observed to be increased by R in the present study. This was probably due to (i) the relatively high rhizodeposition reported for rye in earlier studies ([Austin et al., 2017](#)), and (ii) the increased 3-yr biomass inputs to the soil with main crops and CC residue under R (41.19 Mg ha⁻¹), which together stimulated TEC and HA+FA accumulation processes ([Francaviglia et al., 2017](#)). While for PM, the low C:N ratio of CC residue may explain TEC and HA+FA concentrations since it is indicative of a fast decomposition rate and a high humification degree ([Guimarães et al., 2013](#)). Then, no difference was recorded in the 5–15 cm and 15–30 cm soil layers. Nevertheless, we found that Control had the lowest values for both TEC and HA+FA concentrations in both layers. This could be ascribed to the lower abundance and activity of soil fauna (i.e. earthworms) under Control (as reported above), which may have reduced the incorporation of organic matter down to deeper soil layers ([Pulleman et al., 2005](#)).

As for SOC concentration, also NEC was found to have the highest values under PM in the 0–5 cm soil layer, while under R in the 5–15 cm soil layer. According to [Camilli et al. \(2016\)](#), higher concentration of NEC indicates the presence of a C pool less sensitive to mineralization and stabilized in chemically or physically protected stable forms. Results presented here corroborates this previous finding and a very close relationships between NEC and SOC concentration.

Last, also HR showed significant differences only in the 0–5 cm soil layer and the ranking was $R \geq PM = VC \geq Control = RCC$. Since HR refers to the humified C fraction compared to SOC, a high HR ratio is generally indicative of a low degree of humification ([McCallister and Chien, 2000](#)). The high HR observed under R is assumed to be related to the greater content of non-humic substances and non-decomposed material, thus corroborating the slow decomposition rate of the residues left onto the soil surface under this CC treatment.

4.4. Effects on soil fauna

The most popular parameters observed to characterize soil invertebrate communities are diversity and abundance ([Menta and Remelli, 2020](#)). In this study, no evidences of cover crop impact on those parameters were highlighted, a result that is in apparent agreement with findings of [Menta et al. \(2020\)](#), who reported that those variables are often affected more by main crop type or sequence, rather than by residue management or cover cropping. Our results also suggest that leguminous-based CCs (as RCC and VC in our study) may have some positive effects in a longer period of time, at least on the abundance of arthropods which are more adapted to soil (EMI 20), and consequently more sensitive to soil conditions. Highly-adapted Collembola and Chilopoda may have taken advantage especially from RCC conditions, in accordance with [Salamon et al. \(2004\)](#), who found that the identity of plant species in a mixture is an important determinant for springtails, especially if legumes are involved. Indeed, they argued that legumes increase Collembola diversity through increasing microbial (particularly fungal) biomass in the rhizosphere. Furthermore, the N-rich litter of legumes forms an attractive food resource for both Collembola and Chilopoda ([Menta and Remelli, 2020](#)). Previous results by [Fernández et al. \(2008\)](#), who studied the contribution of CCs in the development of sustainable agriculture scenarios, found that legumes constantly hosted the highest arthropods biodiversity. In this study, biodiversity indexes results suggested that PM gave the worst effects on soil fauna, however from QBS-ar emerged that this cover crop hosted arthropods better adapted to soil; only legumes maintain overall high values for all the parameters considered. Nevertheless, QBS-ar results substantially agrees with previous studies ([Sapkota et al., 2012](#); [Fiorini et al., 2020b](#)) suggesting that (i) biomass vs no biomass input could be considered as a main driver of QBS-ar pattern, and (ii) results can be significant only in the long term.

Analyzing arthropods community structure emerged that some

Table A1

Results of SIMPER analysis. Most influential arthropod groups are shown, accounting for a cumulative dissimilarity between distances from the seepages of 70%. Overall (%): average contrast dissimilarity; Ratio: average contribution to overall dissimilarity to sd ratio; Cum. (%): ordered cumulative contribution of each arthropod group.

| Contrasts between treatments | Overall % | Most influential groups | Ratio | Cum. % | |
|------------------------------|-----------|-------------------------|---|------------------------------|-------------------------------------|
| C | -R | 34.39 | AcariHymenopteraColeoptera larvaeIsopodaColeopteraPsocoptera | 1.792.481.311.250.831.10 | 14.0327.8040.7051.4961.2770.37 |
| | -PM | 31.20 | PsocopteraHymenopteraCollembolaAcariColeoptera | 4.691.300.750.950.66 | 23.9741.7852.2062.1672.06 |
| | -RCC | 30.42 | HymenopteraCollembolaDipluraColeoptera larvaeAcari | 2.121.211.280.662.60 | 17.4132.5347.0260.5670.64 |
| | -VC | 24.81 | ColeopteraAcariCollembolaPsocopteraDiplura | 1.511.231.611.240.84 | 22.5737.2151.3564.6074.73 |
| R | -PM | 42.71 | PsocopteraAcariColeoptera larvaeIsopodaHymenopteraOthers Holometabola | 3.941.911.281.220.820.65 | 23.6035.9147.3356.8566.1472.25 |
| | -RCC | 40.01 | Coleoptera larvaeCollembolaPsocopteraDipluraAcariHymenopteralsopoda | 1.461.691.211.291.430.801.24 | 14.1827.2340.0450.6759.6968.5677.33 |
| | -VC | 39.80 | ColeopteraHymenopteraPsocopteraAcariColeoptera larvaeIsopodaCollembola | 1.471.241.421.461.321.261.44 | 13.4725.8837.8348.2158.4466.9873.61 |
| | PM | 34.07 | CollembolaDipluraColeoptera larvaePsocopteraHymenopteraChilopoda | 1.451.280.661.250.910.84 | 17.3232.4545.5358.0868.0375.76 |
| RCC | VC | 38.74 | ColeopteraHymenopteraPsocopteraAcariChilopoda | 5.701.801.260.780.82 | 21.6341.4556.7064.8472.03 |
| | -VC | 36.65 | ColeopteraHymenopteraDipluraPsocopteraColeoptera larvaeCollembola | 5.981.311.291.100.661.31 | 19.6035.6046.5657.0767.4876.61 |

groups are worthy of attention in studying the effect of CCs on soil fauna. Indeed, leguminous-based cover crops were those with a community structure more similar to Control, while other CCs, such as R, induced changes in arthropods composition. Overall, Coleoptera was one of the most important taxa for discriminating between treatments, thus corroborating results by [Vasconcellos et al. \(2013\)](#) and [Martins et al. \(2018\)](#), which indicated that this order could be an efficient bio-indicator of soil quality. On the other hand, Hymenoptera, despite their lower abundance, was the group that more often influence treatments community dissimilarities, moreover their major abundance in VC, corresponding with the higher Pielou's evenness value, confirmed its role as indicator of other arthropod taxa changes ([Menta and Remelli, 2020](#)).

Nevertheless, C:N ratio and biomass input terms failed in building predictive models for arthropod-based variables. The reason could lie in the chosen variables on which we worked, since C:N ratio and biomass could affect arthropods depending on the trophic level to which they belong. An explanation that is grounded in [Ebeling et al. \(2014\)](#), where the abundance of decomposers was positively associated with increased plant biomass, whereas herbivore abundance increased with increasing C:N ratio. By altering parameters like root biomass and soil structure, CCs could affect soil biota food webs; for example [van Eekeren et al. \(2009\)](#) observed that with clover the availability of easily decomposable material in the rhizosphere and litter quality aspects, such as plant defensive compounds, may reduce bacterial and fungal biomass and the proportion of herbivorous nematodes, as well as increase the proportion of bacterivorous nematodes. Since those parameters directly affect some of the arthropod food habits, multiple mechanisms may combine to drive abundance and diversity patterns in mesofauna dynamics, suggesting that the introduction of grasses in CCs mixtures could be beneficial for promoting arthropods biodiversity.

CCs impact on soil fauna was more evident on earthworms, probably because organic materials are the main limiting factor for earthworm communities in cultivated sites ([Pèrès et al., 2011](#)). Earthworms higher abundance and biomass in RCC and VC further highlight the role of introducing leguminous species in a mixture for enhancing soil biology, as previously suggested by [van Eekeren et al. \(2009\)](#). For instance, these authors found that the introduction of clover in a grass sward often results into increased density and biomass of earthworm population and ascribed the reasons in the increased amount of above-ground dry matter production as residues. Moreover, as supported by the results obtained in this study, [van Eekeren et al. \(2009\)](#) also noted that the earthworm biomass had a negative relationship with the C:N ratio of residues, suggesting that the quality of residues – rather than the quantity – plays a key role in driving earthworm abundance. Finally, our

results are also consistent with [Shipitalo et al. \(1988\)](#), who reported large weight gains in earthworms on diets of legumes, which had the lowest C:N ratio. So that, those results highlight the importance, especially in short-term studies, of integrate traditional biodiversity indexes with QBS-ar and community composition analysis, as well as different bioindicators, in order to have a broader view of the impact of agricultural management in soil dynamics.

5. Conclusions

Our 3-yr field study examined the effects of selected winter cover crops under NT on grain yield of maize and soybean, cumulative biomass, C, and N input to the soil, as well as soil C pools and biodiversity in a clay loam soil of a temperate region devoted to intensive crop production.

We observed that introducing cover crops for damping negative effects of transition from conventional tillage to no-till on crop yield and biomass is not always effective. Cover crop residues may indeed affect negatively plant biomass and grain yield in maize and soybean. Such an unfavorable effect could be avoided with cover crop residues with fast decomposition (as in our mixture hairy vetch plus crimson clover, which also allows to maximize N input) and erect habitus after termination (as in our rye cover crop, in the case of early termination date).

In addition, we found that CCs need to be also targeted at producing residues with low C:N ratio (as that of our mixture phacelia plus white mustard), rather than only high rate of residues (i.e. high biomass and C input), in order to promote soil C cycling by enhancing total soil organic C and pools. For this reason, mixtures of selected cover crops species that allow the best compromise between a reasonable amount of residue and low residue C:N ratio should be preferred. Yet, any change in soil C concentration and distribution seems to be limited the topmost 5 cm of soil.

Last, the inclusion of extra-biomass amount into the soil with leguminous-based cover crops may also positively affect soil biodiversity. Therefore, our results suggested that properties of leguminous biomass could be considered efficient drivers to define the complexity of arthropod and earthworm communities. Nevertheless, most robust trend can be highlighted applying long-term studies.

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Author contributions

A.F., P.M., and V.T. conceived the ideas and designed the methodology; A.F., S.R., R.B., and F.A. collected the data; A.F. and S.R. analysed the data; A.F., S.R., M.T., C.M., and V.T. led the writing. All authors contributed critically to the drafts and gave final approval for publication.

Declaration of Competing Interest

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

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Appendix

See [Table A1](#) in appendix section.

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