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Long-term C and N sequestration under no-till is governed by biomass production of cover crops rather than differences in grass vs. legume biomass quality

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ABSTRACT

Agricultural activities through conventional and intensive practices contribute to climate change by increasing emission of reactive carbon (C) and nitrogen (N) from soils to the atmosphere. Minimum soil disturbance, appropriate residue management and cover cropping as conservation practices are perceived as key strategies to limit GHGs emissions by increasing soil C and N sequestration as soil organic matter (SOM), thus promoting soil aggregation and enhancing soil fertility. Yet, the actual contribution of conservation practices to C and N sequestration, as well as mechanisms behind chemical and biochemical stabilization of SOM in the long-term are still controversial. In the present 9-year field study on a wheat-maize-soybean rotation we investigated the effect of no-till (NT) coupled with grass vs. legume cover crop (i.e., rye [*Secale cereale* L., NT-R] or hairy vetch [*Vicia villosa* Roth, NT-V]) on main crop yields, C and N input by cover and main crops, soil aggregation and C and N sequestration rates, in comparison with conventional tillage (CT). We hypothesized that NT-R may lead to higher biomass input, C sequestration and comparable yield to CT, while NT-V may increase N input, N sequestration and lead to comparable yield to CT. We found that yield of winter wheat, maize, and soybean were never reduced under both NT treatments, neither during the transition phase, nor afterwards. Rye and hairy vetch provided the same amount of biomass and C input, although vetch doubled N input compared with rye. Moreover NT-V increased cumulative biomass and C input from main crop residues compared with NT-R. Both NT-R and NT-V promoted C (+0.4 Mg ha^{-1} y^{-1} and +0.6 Mg ha^{-1} y^{-1}, respectively) and N (+88 kg ha^{-1} y^{-1} and +145 kg ha^{-1} y^{-1}, respectively) soil sequestration, mainly due to the increase of macroaggregate-associated C and N, thus corroborating a major role of NT for macroaggregates formation and SOM stabilization within macroaggregates. Since no difference was found between cover crops in terms of biomass input, and C and N sequestration potential, we concluded that cover crop biomass production (rather than biomass quality) and retention onto the soil as residue were the main drivers of soil C and N sequestration. Therefore, both rye and hairy vetch may be combined with NT and promise significant potential as effective C farming practices.

1. Introduction

Growing population, land degradation and climate change are significant threats to food security and sustainable human development in the near future (Godfray et al., 2010; McNutt, 2013; Hossain et al., 2020). Agricultural activities are among the main causes of climate change by contributing to 23% of the total anthropogenic greenhouse gas (GHG) emission (Shukla et al., 2019). Nevertheless, increasing carbon (C) sequestration into agricultural soils have been identified as a significant tool to meet the ambitious goals of EU Green Deal for keeping under control the unfavourable effect of a changing climate (Dynarski et al., 2020). Therefore, the so-called Carbon Farming should lead to a net CO_2 sequestration into the soil, while preserving soil health, playing a major role in the adaptation of agroecosystems to climate change, sustaining food availability and lowering fertilizer demand (Oliver and Gregory, 2015) to meet goals of the Farm-to-fork strategy by EU (European Commission, 2020) and the Sustainable Development Goals by FAO (Sachs, 2012) at the same time.

Soil organic carbon (SOC) and total nitrogen (STN) contents are often used as indicators to monitor soil health or quality (Cardoso et al., 2013; Singh, 2018). Indeed, SOC regulates chemical, physical and biological processes in soil by affecting nutrient availability, water holding

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capacity, aggregation turnover and stability, and microbial activity (Herrick and Wander, 2018). Nitrogen (N) is instead the most important macronutrient for plant growth and metabolism (Mengel and Kirkby, 2001). Since the development of the Haber-Bosch process, N fertilizers are extensively used to address N deficiency in crops, leading to significant N losses especially when N inputs exceed plant needs or soil system capacity (Gruber and Galloway, 2008). Therefore, adopting innovative farming practices with the potential to concomitantly sequester C and N into the soil (as SOC and STN) is an effective way to increase soil fertility and lower the dependency of farmers on chemical fertilizers (Lal, 2004; Hansen et al., 2017).

Regular return of fresh organic matter (from crop residues and/or manure) to soil has been indicated as a measure to enhance SOC and STN content, particularly when applied together with conservation tillage or no-till (NT), which minimize SOC and STN losses (Lal, 2015; Fiorini et al., 2020a). The additional step is combining NT and cover crops (CCs) to further increase SOC and STN accumulation and conservation (Kong et al., 2005; Ogle et al., 2012; Boselli et al., 2020). Nevertheless, different CCs may play different agro-ecological functions. Gramineous CCs are particularly recommended to enhance soil organic matter accumulation and C sequestration because of producing more biomass residue during and after termination for decomposers (Adetunji et al., 2020), whereas legumes CCs maximize N input because of biological N-fixation, thus offering the opportunity to increase STN and reduce dependence on chemical N-fertilizers (Fiorini et al., 2022).

No-till and CCs together may be helpful also for reducing aggregate turnover, thus increasing the residence time of C and N into the soil (Six et al., 2002). It is well known that aggregates provide physical protection as well as chemical and biochemical stabilization to soil organic matter (SOM) by binding organic compounds to soil minerals and creating barrier between microorganisms and their substrate (Six et al., 2000a, 2002). Tillage promotes physical disturbance to soil, thus increasing soil aggregates turnover and, as a consequence, C and N mineralization (Perego et al., 2019). Yet, the real benefit of no-till for C sequestration, and therefore for climate change mitigation and soil fertility restoration, has been recently questioned (Powlson et al., 2014; Du et al., 2017). The main concern is that the increase of SOC stock in the most superficial layer is counteracted by the decrease of it in the deeper layers. In addition, the authors pointed out that the increase of C sequestration under NT may be short-term. To further complicate matters, contrasting information is available on selecting correct species of cover crops for SOC and STN sequestration: in fact, Poeplau and Don (2015) found that both legume and non-legume CCs have similar sequestration potential, whereas other studies suggest that legume CCs (Jian et al., 2020) or grasses CCs (Fageria et al., 2007) may sequester more C and N.

The main objective of the present study was to determine the effect of NT, combined with two different cover crops (rye [*Secale cereale* L.] and hairy vetch [*Vicia villosa* Roth]), on (i) crop yield during time; (ii) biomass, C and N input to the soil; (iii) C and N stabilization in soil aggregates along different soil layers; and (iv) C and N sequestration potential and efficiency, as compared with conventional tillage (CT) without CCs. We hypothesized that introducing conservation farming practices may enhance soil aggregation, thus providing stabilization of C and N. In particular, NT + rye (NT-R) may provide higher biomass input and therefore higher accumulation of SOC, while NT + vetch (NT-V) may increase STN and N stabilization into soil aggregates. Furthermore, we formulated the hypothesis that the effect on aggregation level and nutrient stabilization of NT is particularly pronounced in the topmost soil layer. Based on results by Boselli et al. (2020), an additional hypothesis was that no-till + CCs maintains main crop yield levels comparable to those of conventional tillage without CCs in the long-term (after a 5-yr transition period).

2. Materials and methods

2.1. Site description

We set up a nine-year field experiment at the CERZOO research farm in Piacenza (45°00′18.0′N, 9°42′12.7′E; 68 m above sea level), Po Valley, Northern Italy. This site is located on a flat landscape. The soil is a fine, mixed, mesic *Udertic Haplustalfs* (Soil Survey Staff, 2014), with a silty clay texture. The initial physico-chemical properties of soil in the top 0–30 cm layer were: organic matter 23 g kg^{-1}; pH in H_2O 6.8; bulk density 1.30 g cm^{-3}; sand 122 g kg^{-1}; silt 461 g kg^{-1}; clay 417 g kg^{-1}; STN (Kjeldahl) 1.2 g kg^{-1}; available P (Olsen) 32 mg kg^{-1}; exchangeable K (NH_4^+ Ac) 294 mg kg^{-1}; and cation exchange capacity 30 cmol$^+$ kg^{-1}. Meteorological data during the experiment were collected from an automatic station placed in the field. The site is characterized by a temperate climate (Cfa as Köppen classification), with an average annual temperature of 13.2 °C and annual rainfall of 839 mm, based on a 30-year average.

2.2. Experimental design

The experiment was established in autumn 2011 as a randomized complete block (RCB) with four replicates (blocks) and three treatments: (i) conventional tillage (CT; which included moldboard ploughing to 30-cm depth with crop residue incorporation and two rotary harrowing to 15–20 cm depth for seedbed preparation); (ii) no-till (NT; consisting of direct sowing on untilled soil with crop residue retained on the soil surface) plus rye (*Secale cereale* L.) as cover crop (NT-R); (iii) NT plus hairy vetch (*Vicia villosa* Roth) as cover crop (NT-V). CCs were sowed with a row width of 17.5 cm. Each plot was 22 m wide and 65 m long (1430 m^2). The seeding rate of CCs was 110 kg ha^{-1} for rye and 80 kg ha^{-1} for hairy vetch. Termination of CCs was conducted by spraying Glyphosate [N-(phosphonomethyl) glycine] at the rate of 3 L ha^{-1} about 14 days before seeding the following main crop (maize or soybean). During the nine-year trial, three courses have been iterated of the following rotation: winter wheat (*Triticum aestivum* L. subsp. *aestivum*), maize (*Zea mays* L.) and soybean (*Glycine max* [L.] Merr.). All the main crops were direct sowed on untilled soil in NT treatments, while conventionally planted in CT. Full details about soil-crop management are reported in Boselli et al. (2020).

2.3. Yield and biomass measurements

Aboveground biomass of CCs was assessed every year by manually harvesting three random areas of 3 m^2 in each plot right before termination. Yield components of main crops (total aboveground biomass, straw/stover and grain) were annually determined by randomly selecting and harvesting three areas of 6 m^2 in each plot. Thereafter, grain and biomass were manually separated, and dry matter yields were obtained by oven-drying sub-samples at 65 °C until constant weight. C and N concentrations were measured for all yield components by the Dumas combustion method with an elemental analyser (Vario Max CNS, Elementar, Germany).

Annual residue-derived C and N inputs to the soil (for both main and cover crops) were calculated by multiplying residue dry matter by C and N concentration. For 2013–2014, data on CCs are not available due to a severe slug attack during plant emergence, causing the failure of the cover crops. We calculated 9-yr cumulative biomass, C and N residue-derived input, and residue C:N ratio of main and cover crops as the sum of annual data. Average annual main crop yield was calculated, separately for the three crops (wheat, maize, and soybean), as the arithmetic mean of annual crop yield. In addition, we calculated average annual residue-derived C and N input from both main crops and CCs as the arithmetic mean of annual C and N input.

2.4. Soil sampling, analyses and calculations

Three soil samples were collected randomly from each plot at 30-cm depth in October 2020 after harvesting maize (9 years after no-till adoption) using a tube sampler with a diameter of 15 cm. Each sample was divided into three layers: 0–5, 5–15, and 15–30 cm. The three samples of each depth section for each plot were combined and mixed together. As a result, four composite samples of each depth were collected for each treatment. Then, soil samples were air-dried and sieved at 8-mm diameter. Moreover, sub-samples were sieved at 2-mm diameter to determine SOC and N content using Dumas combustion method. Soil carbonates correction was not performed due to the absence of carbonates in the soil.

Soil aggregate size distribution analysis was performed on 8-mm diameter samples, according to Elliott (1986) and to Six et al. (1998). In detail, 80 g of soil was submerged into deionized water for 5 min and wet sieved. Then, three sieves of 2000 μm, 250 μm, and 53 μm mesh were used to divide the four aggregate fractions: large macroaggregates (LM; >2000 μm), small macroaggregates (sM; 250–2000 μm), microaggregates (m; 53–250 μm) and silt-and-clay fraction (s + c; <53 μm). Each fraction was isolated by manually moving the sieve up and down 50 times during each phase (2 min). After each phase, soil aggregates remaining on the top of the sieve were transferred onto an aluminum pan, oven dried at 105 °C and weighed. Water and soil passing through the sieve were poured onto the smaller mesh sieve, thus starting the next phase.

We used the physical fractionation method developed by Six et al. (2000b) to isolate fractions within macroaggregates, namely coarse particulate organic matter (cPOM; >250 μm), microaggregates within macroaggregates (mM; 53–250 μm) and silt and clay (s + cM; <53 μm). Specifically, a composed subsample of large macroaggregates and small macroaggregates, in the same proportions obtained after wet sieving, was immersed in deionized water on top of a 250 μm sieve and sieved with 50 stainless steel beads (4 mm diameter) for 2 min. Once the macroaggregates had been cracked, organic fraction remaining on the top of the 250 μm was isolated and quantified as cPOM. Microaggregates and other released material passing through 250 μm ended up on 53 μm sieve and were sieved as in the wet sieving method. Soil aggregates retained by the sieve were isolated as mM, while those passed through the sieve as s + cM.

Correction for sand content was performed for all non-silt and clay fractions according to Elliott et al. (1991). C and N concentration of aggregate fractions was determined by using combustion method previously described.

The mean weight diameter (MWD) was calculated according to van Bavel (1950) as follows:

$$MWD = \sum_{i=1}^{n} x_i w_i \tag{1}$$

where x_i is the mean diameter of each aggregate-size fraction separated by sieving, and w_i is the proportion of each sand-free aggregate-size fraction out of the entire sample weight.

Sampling for soil bulk density (BD) determination was performed along with soil sampling for aggregate stability assessment. BD was then calculated by dividing the dry weight of each soil layer (0–5; 5–15 cm; and 15–30 cm) by its volume.

Three undisturbed soil cubes of 8000 cm^3 (20 × 20 × 20 cm) were collected from each plot using a spade on 6 October 2020 (at the end of the experiment) at 3 pm and immediately brought to the lab to assess earthworms abundance into the soil. The air temperature was around 18 °C during the sampling activity and the cumulative rainfall amount during the previous 30 days was 64 mm Earthworms were manually separated from the soil and counted (Shepherd et al., 2008). Thereafter, earthworms were weighted and oven-dried to determine dry biomass after voiding earthworm intestines according to Dalby et al. (1996). Then, earthworm density (number of earthworms per square meter) was calculated by multiplying the number of individuals and the dry biomass by 25.

2.5. Assessment of C and N sequestration rate in the soil

Soil organic C (SOC) and total N (STN) stocks (Mg ha^{-1} and kg ha^{-1}, respectively) were calculated for each soil layer by multiplying SOC or STN concentration, BD, and the depth of each soil layer. Then, SOC and STN stocks of the 0–30 cm soil layer were assessed as the weighted means of SOC and STN stocks of each soil layer. C and N sequestration (Mg ha^{-1} and kg ha^{-1}, respectively) in the soil was calculated as the difference between final (October 2020) and initial (October 2011) SOC and STN stocks of 0–30 cm soil layer. Average annual C and N sequestration rate (Mg ha^{-1} y^{-1} and kg ha^{-1} y^{-1}, respectively) was then calculated by dividing C and N sequestration by the duration of the experiment (9-yr). In addition, we calculated annual C sequestration efficiency (SE) as the ratio of average annual C sequestration and average annual C input. Average annual C input was considered as equivalent to the residue-derived C input from main crops and CCs.

2.6. Statistical analyses

We conducted a repeated measures analysis of variance (ANOVA) on main crop grain yield with soil management/CCs type, year, and block as fixed factors and replicate as random effect. Grain yield data were standardized using *Z-score* to perform repeated measures ANOVA because of crop diversity throughout the experiment. *Z-scores* were calculated as follows:

$$z = \frac{x - \mu}{\sigma} \tag{2}$$

where x is observed data point, μ is average grain yield for each year and σ is standard deviation. The same approach was used by Boselli et al. (2020) to manage crop diversity.

A linear model was applied to study the effect of treatments on: (i) crop, CCs, and total biomass return (for each year and final); (ii) C and N input to the soil from both main crops and CCs (for each year and final); (iii) soil aggregate fractions, within-macroaggregates fractions and MWD; (iv) aggregate-associated C and N; and (v) C and N sequestration rates and C sequestration efficiency. Shapiro-Wilk and Levene's tests were performed to check normality and homogeneity of variances of measured variables. When the ANOVA assumptions were violated, data were log-transformed prior to analysis and back-transformed after the post-hoc test. Tukey's honestly significant difference (HSD) was used as *post-hoc* to test significant differences among treatments with a *p*-value of 0.05 as threshold.

Relationship between yields, residue-derived C/N inputs, soil aggregate stability parameters, soil biological activity (earthworms), and C/N sequestration parameters was assessed by performing two principal component analyses (PCA) with type II scaling separately for C and N parameters.

We used R 4.0.3. (R Core Team, 2020) with nlme (Pinheiro et al., 2013), multcomp (Hothorn et al., 2007), and FactoMineR (Lê et al., 2008) packages for the linear model, mixed model, HSD test and PCA, respectively.

3. Results

3.1. Crop grain yield

Standardized grain yield of main crops was significantly affected by treatments in 2014, 2015, and 2016 (Fig. 1). In detail, CT had higher *Z-score* than NT-R in 2014 with maize, while no differences were found between NT-V and other treatments. NT-R increased *Z-score* compared with CT and NT-V in 2015 with soybean (+14% and +17%,

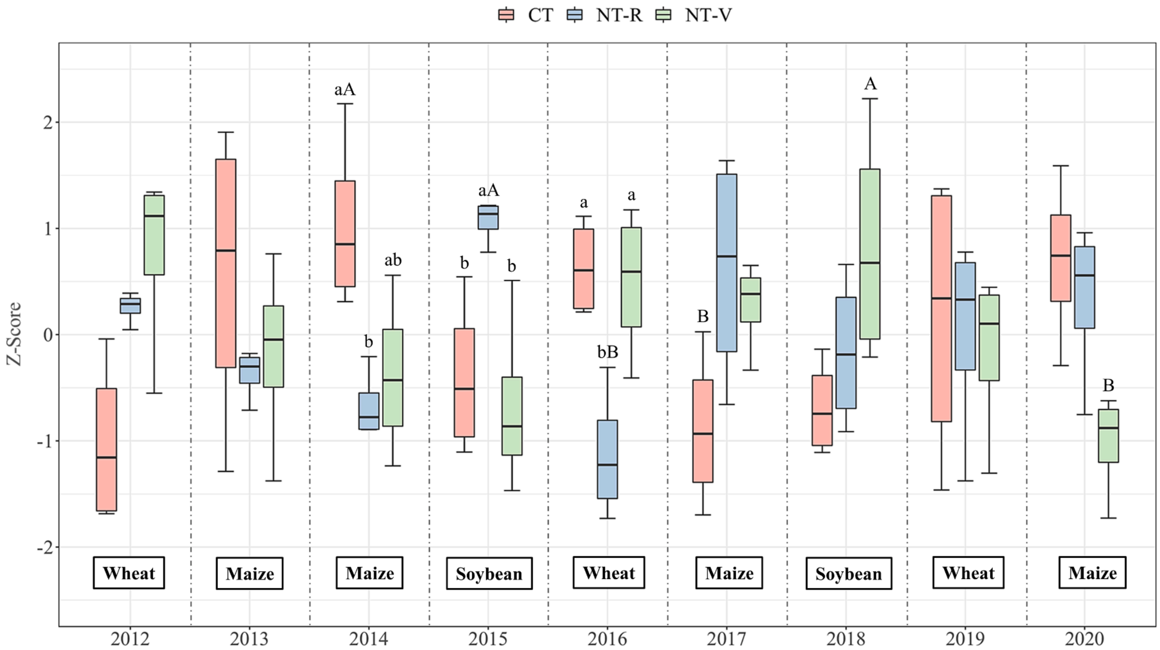

Fig. 1. Box-plots of crop yield, reported as *Z-scores*, as affected by soil management (conventional tillage [CT], no-till + rye [NT-R], and no-till + hairy vetch [NT-V]) from 2012 to 2020. The bottom and top of each box represent the lower and upper quartiles respectively, the line inside each box shows the median and whiskers indicate minimal and maximum observations. Capital letters indicate differences among years within the same soil management; lowercase letters indicate differences among soil management within the same year (p-value < 0.05).

respectively). In 2016, winter wheat grain yield showed higher *Z-score* under CT and NT-V than under NT-R. Each treatment was affected by year (Fig. 1): grain yield of CT was higher in 2014 than in 2012 and 2017; grain yield of NT-R was lower in 2016 than in 2015; and grain yield under NT-V was higher in 2018 than in 2020.

3.2. Biomass and residue-derived C/N input to the soil

Annual biomass input to the soil from main crop residues was never significantly affected by treatments (Table 1). However, 9-yr cumulative biomass input was higher under NT-V than under NT-R, whereas no difference was recorded between CT and NT treatments (Table 1). Similarly, only cumulative C input from main crop residues was affected by treatments, and NT-V increased cumulative C input compared with NT-R, while no differences were found between CT and NT treatments (Table 1). N input from main crops was higher under NT-V than under CT in 2016 (+42%) and with NT-R in 2018 (+27%) (Table 1). C:N ratio was affected by treatments in 2015, 2016, 2018, 2019, and as mean value (Table 1). In detail, NT-R increase C:N ratio compared with NT-V and CT in 2015 and in 2018, while both NT-R and NT-V reduced C:N ratio compared with CT in 2016, in 2019, and as mean value.

Biomass input of CCs was almost never affected by different CCs type except for 2020, when NT-V doubled biomass input compared with NT-R (Table 1). Same pattern was found for C input from CCs; indeed, NT-R reduced C input in 2020 (Table 1). N input from CCs was affected by treatments in 2013, 2018, 2020, and as cumulative rate (Table 1). NT-V increased N input from CCs by almost 50% in 2013, 63% in 2018, 365% in 2020, and 92% as 9-yr cumulative rate (Table 1). Finally, C:N ratio was consistently reduced by NT-V, ranging from 9 to 18, compared with NT-R (−46% as mean value) (Table 1).

3.3. Aggregate size distribution

Aggregate amounts were significantly affected by treatments in the 0–5 cm and in the 5–15 cm soil layers, whereas no difference was found in the 15–30 cm layer (Table 2). Indeed, large macroaggregates were significantly lower under CT than under NT-R and NT-V in the topmost layer (−84% and −82%, respectively). Consequently, all other aggregates fractions in the 0–5 cm layer (small macroaggregates, microaggregates and silt + clay) were reduced by NT treatments compared with CT. Conversely, CT increased large macroaggregates by 18% compared with NT-R in the 5–15 cm soil layer, but reduced them by 17% compared with NT-V. Either small macroaggregates, microaggregates and silt + clay were higher under NT-R than under NT-V in the 5–15 cm layer, whereas no difference occurred between CT and NT treatments.

Treatments significantly affected fractions within macroaggregates amount in the 0–5 cm and in the 5–15 cm soil layers. NT-R increased cPOM, microaggregates within macroaggregates, and silt + clay within macroaggregates compared with CT in the topmost layer, while no difference was found between NT-R and NT-V. Only microaggregates whitin macroaggregates were higher under NT-V than under CT in the 0–5 cm layer (+20%). Regarding the 5–15 cm soil layer, treatments affected only silt + clay within macroaggregates, which were increased under NT-V (289 g kg^{-1} soil) compared with NT-R and CT (234 g kg^{-1} soil both). NT-V had higher cPOM than NT-R and CT in the deepest soil layer, while NT-R even reduced cPOM compared with CT. MWD was higher under both NT treatments than under CT in the 0–5 cm soil layer, whereas NT-V increased MWD compared with NT-R and CT in the 5–15 cm layer.

3.4. SOC and aggregates-associated C

After 9 years of trial, soil organic C concentration was increased under NT-R and NT-V in both 0–5 and 5–15 cm soil layers, whereas no difference was found between treatments in the 15–30 cm soil layer (Table 3).

Similarly, C concentration in aggregates fractions was never affected by different treatments in the 15–30 cm soil layer (Table 3). CT reduced C content of large macroaggregates and small macroaggregates in the 0–5 cm soil layer compared with NT-R (−91% and −33%, respectively) and NT-V (−91% and −30%, respectively). In the 5–15 cm soil layer, C content associated with large macroaggregates was higher under NT-V (4.87 g kg^{-1} soil) than under NT-R (3.08 g kg^{-1} soil) and CT (3.18 g kg^{-1} soil). On the other hand, NT-R increased C content of m and

Table 1
Biomass input (Mg ha^{-1}), residue-derived carbon (C) input, residue-derived nitrogen (N) input, and C:N ratio of both main crops and cover crops as affected by soil management/CCs type. Conventional tillage (CT), no-till + rye (NT-R), and no-till + vetch (NT-V). Lowercase letters indicate differences among treatments within the same year.

Year	Main crop	Treatment	Main crop input				Cover crop input			
			Biomass input Mg ha^{-1}	C input Mg ha^{-1}	Biomass N kg ha^{-1}	C:N ratio	Biomass input Mg ha^{-1}	C input Mg ha^{-1}	Biomass N kg ha^{-1}	C:N ratio
2012	Wheat	CT	7.62	3.22	48.2	67.9	-	-	-	-
		NT-R	7.47	3.14	45.7	72.5	-	-	-	-
		NT-V	7.74	3.30	52.1	65.9	-	-	-	-
		p-value	n.s.	n.s.	n.s.	n.s.	-	-	-	-
2013	Maize	CT	16.3	6.98	111	62.8	-	-	-	-
		NT-R	15.4	6.51	107	60.8	3.13	1.35	55.9 b	24 a
		NT-V	16.8	7.13	126	56.6	4.28	1.82	103.4 a	18 b
		p-value	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.007	< 0.001
2014	Maize	CT	11.5	4.95	68.7	72.1	-	-	-	-
		NT-R	11.0	4.65	75.2	62.4	-	-	-	-
		NT-V	11.5	4.88	70.6	69.9	-	-	-	-
		p-value	n.s.	n.s.	n.s.	n.s.	-	-	-	-
2015	Soybean	CT	3.85	1.58	38.2	41.3 b	-	-	-	-
		NT-R	3.46	1.46	29.3	50.0 a	3.12	1.34	47.7	28 a
		NT-V	3.31	1.39	31.7	43.9 b	2.09	0.89	67.7	13 b
		p-value	n.s.	n.s.		0.008	n.s.	n.s.	n.s.	< 0.001
2016	Wheat	CT	7.48	3.17	36.1 b	87.8 a	-	-	-	-
		NT-R	7.16	3.03	44.3 ab	68.3 b	-	-	-	-
		NT-V	7.88	3.36	51.1 a	66.0 b	-	-	-	-
		p-value	n.s.	n.s.	0.012	0.003	-	-	-	-
2017	Maize	CT	9.56	4.10	82.9	51.8	-	-	-	-
		NT-R	9.76	4.11	111.2	37.1	2.34	1.01	70.3	14 a
		NT-V	9.42	4.00	94.8	42.4	1.97	0.84	91.4	9 b
		p-value	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		< 0.001
2018	Soybean	CT	3.86	1.58	38.3 a	41.3 b	-	-	-	-
		NT-R	3.25	1.37	27.5 b	50.0 a	2.83	1.21	52.5 b	23 a
		NT-V	3.67	1.54	34.8 a	44.1 b	1.92	0.81	85.4 a	10 b
		p-value	n.s.	n.s.	0.003	0.006	n.s.	n.s.	0.046	< 0.001
2019	Wheat	CT	9.66	4.09	46.9	87.8 a	-	-	-	-
		NT-R	8.84	3.72	53.6	69.5 b	-	-	-	-
		NT-V	10.28	4.39	66.3	66.0 b	-	-	-	-
		p-value	n.s.	n.s.	n.s.	0.002	-	-	-	-
2020	Maize	CT	10.9	4.68	81.4	62.1	-	-	-	-
		NT-R	11.6	4.88	102.8	50.9	1.98 b	0.85 b	32.43 b	26 a
		NT-V	12.0	5.10	103.7	53.1	3.93 a	1.68 a	150.7 a	11 b
		p-value	n.s.	n.s.	n.s.	n.s.	0.005	0.005	< 0.001	< 0.001
Cumulative		CT	80.8 ab	34.3 ab	552	62.4 a	-	-	-	-
		NT-R	77.4 b	32.7 b	592	55.2 b	13.4	5.76	259 b	22* a
		NT-V	82.4 a	35.0 a	630	55.6 b	14.2	6.04	499 a	12* b
		p-value	0.033	0.032	n.s.	0.034	n.s.	n.s.	< 0.001	< 0.001

* Mean values

silt + clay compared with NT-V and CT in the 5–15 cm soil layer.

C associated with fractions within macroaggregates in the 0–5 cm soil layer was generally higher under NT-R and NT-V than under CT, although no difference was found between NT-V and CT for silt + clay within macroaggregates. In detail, NT-V and NT-R increased approximately fourfold C associated with cPOM and doubled C associated with microaggregates within macroaggregates. Conversely, in the 5–15 cm soil layer C concentration was affected by treatments exclusively for silt + clay within macroaggregates: NT-V increased C concentration compared with CT (+48%), while no difference occurred between NT-R and other treatments. NT-R had lower C associated with cPOM in the 15–30 cm soil layer compared with NT-V and CT, while the latter reduced C associated with silt + clay within macroaggregates compared with NT-V by 29%.

3.5. *STN and aggregates-associated N*

Soil total N was increased by NT-R and NT-V compared with CT in both 0–5 and 5–15 cm soil layers, while treatments did not affect STN in the deepest layer (Table 4). Regarding the topmost soil layer (0–5 cm), N associated with aggregates was generally higher under NT-V and NT-R than under CT. In detail, both NT-R and NT-V enhanced N content in large macroaggregates and in small macroaggregates compared with CT, while m and silt + clay were not affected by treatments. NT-V enhanced N content of large macroaggregates by 69% compared with both CT and NT-R in the 5–15 cm soil layer, while N associated with microaggregates was higher under NT-R (0.205 g kg^{-1} soil) than under CT (0.166 g kg^{-1} soil). Treatments did not affected N associated with aggregates in the 15–30 cm soil layer. N content of aggregates within macroaggregates was higher under NT-R and NT-V than under CT for all fractions (cPOM,

Table 2
Aggregates soil distribution (g kg^{-1}; sand-free) acquired from wet sieving of whole soil and from macroaggregates in different soil layers (0–5 cm; 5–15 cm; 15–30 cm) and mean weight diameter (MWD; mm) as affected by soil management/CCs type after 9 years: conventional tillage (CT); no-till + rye (NT-R); no-till + vetch (NT-V). Lowercase letters indicate differences among treatments within the same soil layer. *P*-values are also reported for each soil layer and aggregate fraction.

Depth	Treatment	Aggregate fractions (g kg^{-1} soil)				Aggregate fractions within macroaggregates (g kg^{-1} soil)			MWD (mm)
		LM[1]	sM[2]	m[3]	s + c[4]	cPOM[5]	mM[6]	s + cM[7]	
0–5 cm	CT	51 b	620 a	226 a	103 a	37 b	406 b	229 b	0.88 b
	NT-R	314 a	492 b	120 b	73 b	50 a	486 a	271 a	2.33 a
	NT-V	291 a	505 b	130 b	73 b	47 ab	485 a	265 ab	2.21 a
	p-value	< 0.001	0.022	0.004	0.004	0.015	0.008	0.035	< 0.001
5–15 cm	CT	273 b	516 ab	140 ab	71 ab	35	521	234 b	2.10 b
	NT-R	231 c	535 a	153 a	81 a	39	493	234 b	1.87 b
	NT-V	331 a	490 b	118 b	61 b	38	494	289 a	2.43 a
	p-value	< 0.001	0.048	0.039	0.032	n.s.	n.s.	0.025	< 0.001
15–30 cm	CT	326	477	123	74	39 b	524	240	2.39
	NT-R	365	456	115	64	26 c	546	250	2.60
	NT-V	352	462	128	58	49 a	507	257	2.53
	p-value	n.s.	n.s.	n.s.	n.s.	< 0.001	n.s.	n.s.	n.s.

[1] LM: macroaggregates with a large size (>2 mm).
[2] sM: macroaggregates with a small size (2 mm - 250 μm).
[3] m: microaggregates (250–53 μm).
[4] s + c: silt and clay (< 53 μm).
[5] cPOM: coarse particulate organic matter within macroaggregates (>250 μm).
[6] mM: microaggregates within macroaggregates (250–53 μm).
[7] s + cM: silt and clay within macroaggregates (< 53 μm).

Table 3
Soil organic C (SOC) concentration of the whole soil and associated with aggregate-size fractions in different soil layers (0–5 cm; 5–15 cm; 15–30 cm) as affected by soil management/CCs type after 9 years: conventional tillage (CT); no-till + rye (NT-R); no-till + vetch (NT-V). Lowercase letters indicate differences among treatments within the same soil layer. *P*-values are also reported for each soil layer and aggregate fraction.

Depth	Treatment	SOC[1] (g C kg^{-1} soil)	Carbon concentration in aggregates (g C kg^{-1} soil)				Redistribution of C within macroaggregates (g C kg^{-1} soil)		
			LM[2]	sM[3]	m[4]	s + c[5]	cPOM[6]	mM[7]	s + cM[8]
0–5 cm	CT	11.8 b	0.61 b	7.22 b	2.44	1.54	0.48 b	4.8 b	3.03 b
	NT-R	21.0 a	6.76 a	10.82 a	2.00	1.43	2.48 a	10.6 a	4.85 a
	NT-V	20.4 a	6.71 a	10.29 a	2.17	1.19	2.17 a	10.2 a	4.48 ab
	p-value	< 0.001	0.003	< 0.001	n.s.	n.s.	< 0.001	< 0.001	0.024
5–15 cm	CT	11.6 b	3.18 b	6.12	1.48 b	0.82 b	0.43	6.2	3.1 b
	NT-R	13.1 a	3.08 b	7.17	1.81 a	1.04 a	0.66	6.7	3.4 ab
	NT-V	14.3 a	4.87 a	7.00	1.57 b	0.88 b	0.57	7.2	4.6 a
	p-value	0.003	< 0.001	n.s.	0.009	0.010	n.s.	n.s.	0.041
15–30 cm	CT	11.5	3.73	5.59	1.28	0.94	0.50 a	6.2	2.9 b
	NT-R	11.6	4.09	5.33	1.30	0.83	0.26 b	6.5	3.1 ab
	NT-V	11.7	4.18	5.39	1.43	0.74	0.48 a	5.9	4.1 a
	p-value	n.s.	n.s.	n.s.	n.s.	n.s.	0.006	n.s.	0.038

[1] SOC: soil organic carbon concentration.
[2] LM: macroaggregates with a large size (>2 mm).
[3] sM: macroaggregates with a small size (2 mm - 250 μm).
[4] m: microaggregates (250–53 μm).
[5] s + c: silt and clay (< 53 μm).
[6] cPOM: coarse particulate organic matter within macroaggregates (>250 μm).
[7] mM: microaggregates within macroaggregates (250–53 μm).
[8] s + cM: silt and clay within macroaggregates (< 53 μm).

microaggregates within macroaggregates and silt + clay within macroaggregates) in the 0–5 cm soil layer. In the 5–15 soil layer, cPOM and microaggregates within macroaggregates were not affected by treatments and NT-V enhanced N associated with silt + clay within macroaggregates compared with CT (+38%) and NT-R (+28%). In the deepest soil layer, NT-R reduced N content of cPOM compared with other treatments, while no differences were observed for microaggregates within macroaggregates and silt + clay within macroaggregates.

3.6. C and N sequestration parameters

No-till treatments increased C sequestration rate and efficiency compared with CT, with no significant difference between them (Fig. 2). In detail, almost 0.6 Mg ha^{-1} of C were sequestered each year in the 0–30 cm soil layer of NT-V and slightly more than 0.4 Mg ha^{-1} for NT-R. Conversely, CT reduced C stock by about 0.5 Mg ha^{-1} y^{-1}. NT-V and NT-R stored the 13% and the 10%, respectively, of annual C input provided as residues, both significantly higher than CT (−0.13%).

N sequestration rate follows the same pattern as for C (Fig. 2). Indeed, we did not find significant differences between the two NT treatments; but, NT-R and NT-V both increased N sequestration rate of 0–30 cm soil layer compared with CT. Specifically, N sequestration rate was 88 kg ha^{-1} y^{-1} for NT-R and 146 kg ha^{-1} y^{-1} for NT-V, while CT lost more than 50 kg ha^{-1} each year.

Table 4
Soil total N (STN) content of the whole soil and associated with aggregate-size fractions in different soil layers (0–5 cm; 5–15 cm; 15–30 cm) as affected by soil management/CCs type after 9 years: conventional tillage (CT); no-till + rye (NT-R); no-till + vetch (NT-V). Lowercase letters indicate differences among treatments within the same soil layer. *P*-values are also reported for each soil layer and aggregate fraction.

Depth	Treatment	STN[1] (g N kg^{-1} soil)	Nitrogen content in aggregates (g N kg^{-1} soil)				Redistribution of N within macroaggregates (g N kg^{-1} soil)		
			LM[2]	sM[3]	m[4]	s + c[5]	cPOM[6]	mM[7]	s + cM[8]
0–5 cm	CT	1.09 b	0.050 b	0.664 b	0.260	0.121	0.034 b	0.446 b	0.247 b
	NT-R	1.76 a	0.561 a	0.883 a	0.209	0.109	0.156 a	0.807 a	0.451 a
	NT-V	1.89 a	0.583 a	0.899 a	0.250	0.159	0.142 a	0.903 a	0.435 a
	p-value	< 0.001	0.002	0.002	n.s.	n.s.	< 0.001	< 0.001	0.002
5–15 cm	CT	1.11 b	0.289 b	0.542	0.166 b	0.111	0.028	0.621	0.308 b
	NT-R	1.31 a	0.289 b	0.674	0.205 a	0.142	0.040	0.633	0.333 b
	NT-V	1.46 a	0.488 a	0.677	0.189 ab	0.107	0.038	0.734	0.425 a
	p-value	0.002	< 0.001	n.s.	0.021	n.s.	n.s.	n.s.	0.004
15–30 cm	CT	1.12	0.339	0.516	0.147	0.117	0.038 a	0.619	0.287
	NT-R	1.16	0.397	0.490	0.159	0.112	0.018 b	0.718	0.356
	NT-V	1.22	0.424	0.519	0.184	0.097	0.037 a	0.524	0.284
	p-value	n.s.	n.s.	n.s.	n.s.	n.s.	0.022	n.s.	n.s.

[1] STN: soil total nitrogen content.
[2] LM: macroaggregates with a large size (>2 mm).
[3] sM: macroaggregates with a small size (2 mm - 250 μm).
[4] m: microaggregates (250–53 μm).
[5] s + c: silt and clay (< 53 μm).
[6] cPOM: coarse particulate organic matter within macroaggregates (>250 μm).
[7] mM: microaggregates within macroaggregates (250–53 μm).
[8] s + cM: silt and clay within macroaggregates (< 53 μm).

3.7. Relationships between variables

The first two principal components of PCA (Dim1 and Dim2) related to C parameters accounted for the 64.6% of the total variance (Fig. 3a). C input from main crops, crop yields and C associated with silt + clay fraction were the variables which contributed the least to the total variation. Conversely all other variables were found to greatly contribute to total variability (more than 6%). C sequestration parameters were positively related with C input from CCs and with C associated with large aggregate fractions (large macroaggregates and small macroaggregates) as well as with fractions within macroaggregates (cPOM, microaggregates within macroaggregates, and silt + clay within macroaggregates). In addition, C sequestration rate and efficiency were negatively related with C:N ratio and – partially – positively related to earthworms abundance and MWD.

The first two principal components of PCA (Dim1 and Dim2) related to N parameters explained the 69.3% of the total variation (Fig. 3b). Similarly to C, crop yields were the worst variables in terms of contribution to total variation (less than 4%). N sequestration rate was positively correlated with N associated with cPOM and large macroaggregates, and partially with MWD and N input from CCs. In addition, a weak positive correlation was found between N sequestration rate and N associated with microaggregates within macroaggregates and with small macroaggregates, as well as earthworms. Similarly to previous PCA, C:N appears to be negatively correlated with sequestration parameters.

4. Discussion

4.1. Crop yield, biomass return, C and N inputs to the soil as affected by soil management

Grain yield of main crops was sometimes moderately affected by NT and cover crops, but without a specific pattern among treatments during the 9-years experiment. As reported previously by Boselli et al. (2020), conversion to NT plus CCs did not drastically reduce the yield of wheat, maize, and soybean compared to CT during the transition period (first 5 years of experiment). In detail, yield was higher under CT than under NT-R in 2014 with maize, but not than under NT-V. A similar pattern was observed in 2016 with winter wheat, while NT-R outyielded both NT-V and CT in 2015 with soybean. Lower crop yield under NT has been previously reported by several studies, which ascribed the reduced performances of NT to soil waterlogging, cooler soil temperatures, and/or soil nutrient deficiencies (Alvarez and Steinbach, 2009; Van den Putte et al., 2010; Ogle et al., 2012). The reduced grain yield of maize in 2014 and wheat in 2016 under our NT-R may be due to the above-mentioned reasons. In fact, retaining cover crop residues with high C:N ratio on the soil surface may lead to lower soil temperature, due to higher residue persistence over the soil surface (Hadas et al., 2004), and to increased soil N-immobilization (Malhi et al., 2001; Burgess et al., 2002; Jin et al., 2008), thus limiting early plant development. However, since in our study N-fertilizer was applied to wheat and maize in order to address N-deficiencies, the relevance of N-immobilization for crop yield reduction under NT may be limited. Soil compaction may have concurred in reducing yield performances of NT. In fact, higher bulk density of NT soil compared with CT was previously reported by Fiorini et al. (2018) in the same field. Consequently, the combination of these multiple factors may have limited maize and wheat performances. Results on crop yield in 2014 and 2016 are in agreement with those reported in the global meta-analysis conducted by Pittelkow et al. (2015), which found an overall 5.1% reduction in crop yield due to NT across almost 700 studies.

Conversely, the higher grain yield of soybean under NT-R than under CT and NT-V in 2015 may be explained by two main factors: (i) the longer persistence of rye residues on the soil surface due to high C:N ratio, which may have helped to preserve soil moisture during early stages of plant growth; and (ii) the reduced weed pressure and competition thanks to both physical effect of the rye mulch and release of allelopathic compounds. These explanations are further supported by previous studies, which reported allelopathic weed control of gramineous cover crops (Schulz et al., 2013; Tabaglio et al., 2013) and increased soybean grain yield after NT + gramineous CCs adoption because of reduced weed pressure and competition (Williams et al., 2000). In addition, since legumes main crops – such as soybean – can meet a large part of their N demand through biological N-fixation (Liu et al., 2011), the negative effects of N-immobilization into the soil on

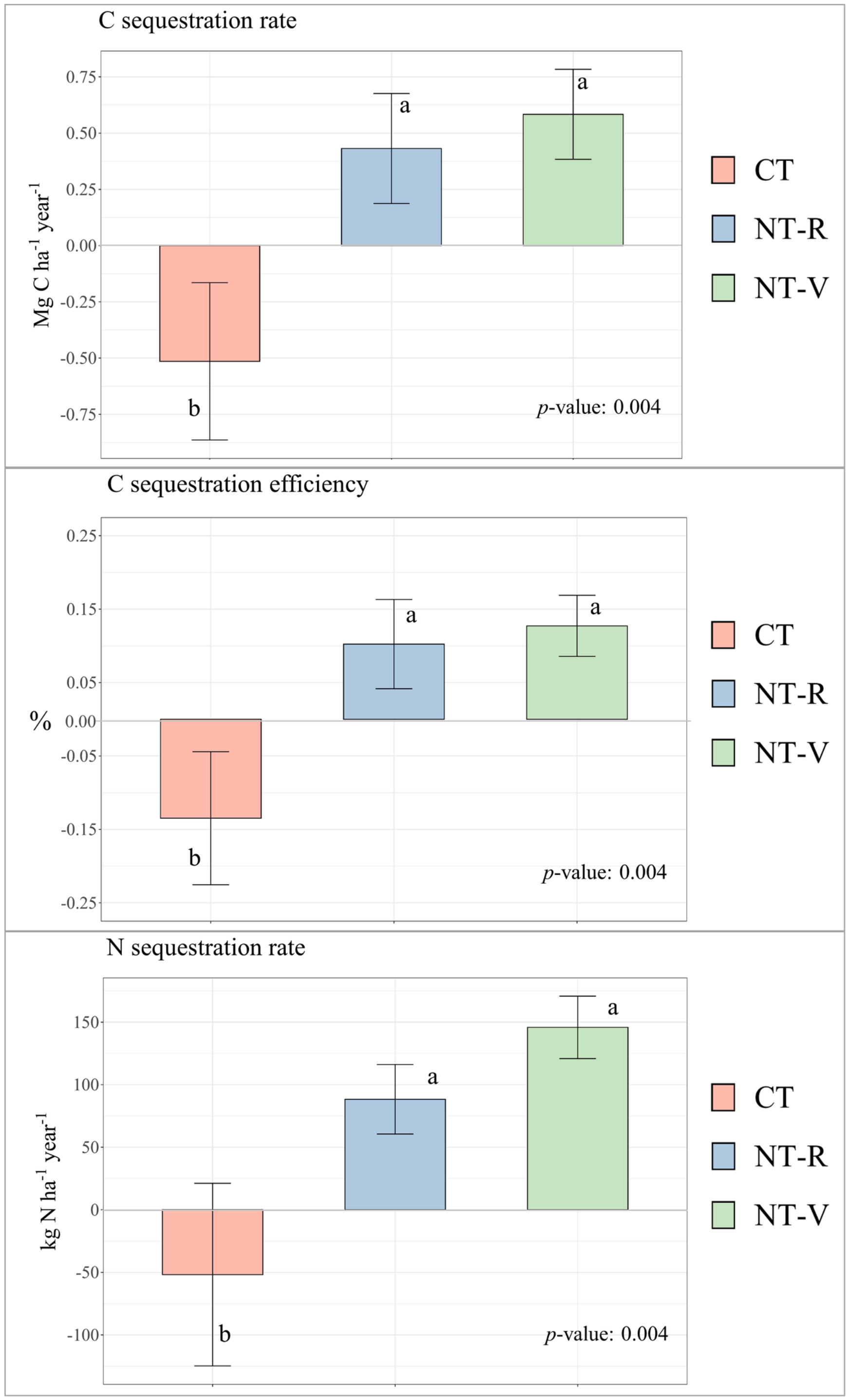

Fig. 2. C and N sequestration rates and C sequestration efficiency as affected by soil management (conventional tillage [CT], no-till + rye [NT-R], and no-till + hairy vetch [NT-V]) after 9 years of experiment. C sequestration efficiency has been calculated as the ratio (%) of average annual C sequestration to average annual C input. Mean values ± standard deviation. Letters indicate differences among soil management. *p*-values are reported.

plant growth and performances were avoided.

Grain yield was then more homogeneous between treatments in the last four years of our trial. This suggests that building fertility and restoring soil functions over time through NT and CCs synergic effect may be considered pivotal for maintaining crop yield in the long-term and offset potential negative effects of tillage ceasing.

Although annual biomass and residue-derived C inputs of main crops were never affected by different soil management, cumulative rates were higher under NT-V compared with NT-R, while CT was in the between. As previously described for grain yield, relatively high C:N ratio of rye residues may have caused a slightly decrease in the annual biomass production of main crops (Ruffo et al., 2004). This is supported by our findings on the higher C:N ratio of rye residue (22, on average) compared with that of vetch (12, on average), which may have promoted N immobilization into the soil in the early growth stages of main crops and reduced soil temperature. In fact, residues with high C:N ratio are known to be more persistent on the soil surface because of N-immobilization (Bengtsson et al., 2003; Fiorini et al., 2020a, 2020b),

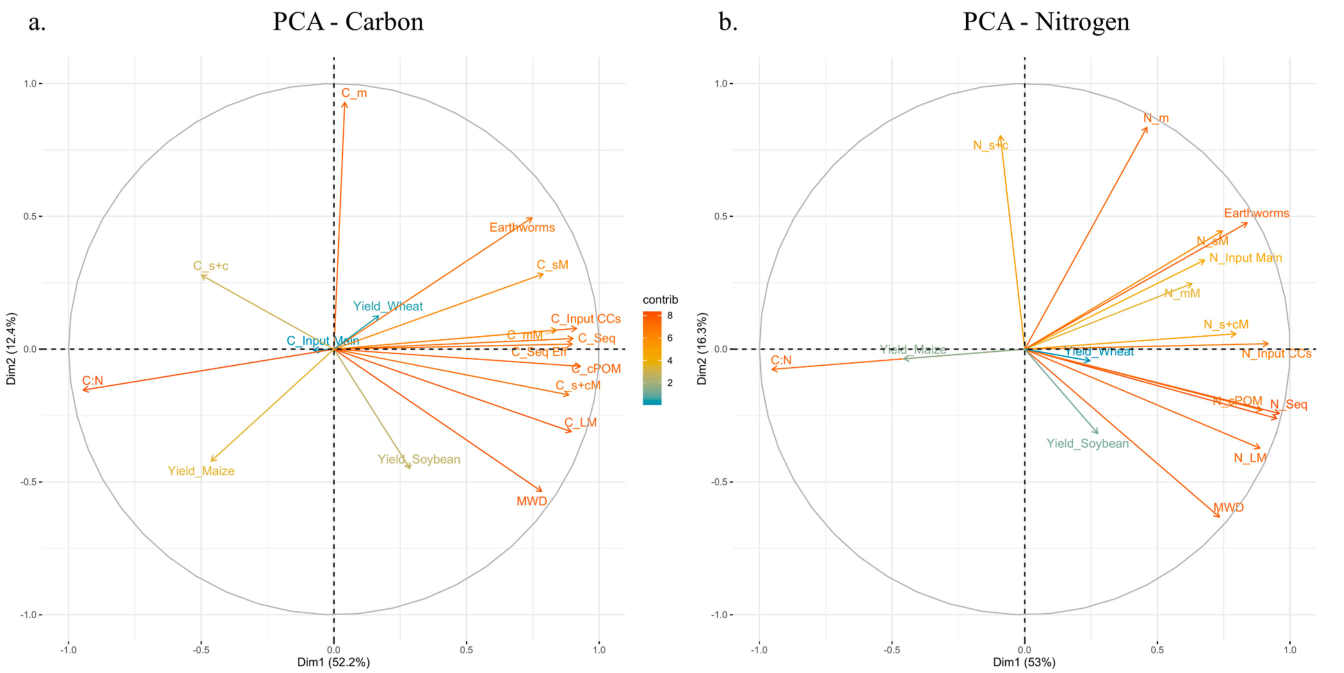

Fig. 3. Principal Component Analyses of C- (a) and N-related parameters (b). The contributions of variables in accounting for the variability in a given principal component are expressed in percentage.

which limits mineralization rate of residues (Frankenberger and Abdelmagid, 1985), thus lowering soil temperature (Teasdale and Mohler, 1993) and possibly delaying plant emergence as well as slowing early growth (Wang et al., 2012). On the contrary, vetch residue neither reduced nor increased biomass and C input from main crop compared with CT, despite having a low C:N ratio and providing almost 500 kg N ha^{-1} in nine years. This was because of the relatively high N fertilization rate to maize and winter wheat in our agro-ecosystem (> 200 kg N ha^{-1} yr^{-1}). Indeed previous studies reported increased main crop performances after hairy vetch termination mainly with reduced (or zero) application of N-fertilizer rates (Miguez and Bollero, 2005; Marcillo and Miguez, 2017; Pott et al., 2021), which was not the case in our study.

Regarding biomass input from CCs, vetch provided 14.2 Mg ha^{-1} of biomass input as cumulative rate, while NT-R 13.4 Mg ha^{-1}. These results are in contrast with our initial hypothesis and with a recent review performed by Ruis et al. (2019) reporting generally higher biomass production of rye compared with hairy vetch in temperate ecoregions. However, our findings could be justified by an early seeding timing and a late termination of the cover crops. In fact, Liebman et al. (2018) found higher biomass production (+3 Mg ha^{-1}) of hairy vetch when terminated in mid-May rather than in late April. This is because hairy vetch growth is particularly enhanced in spring when air temperature reaches 20 °C (Zachariassen and Power, 1991), while rye is well known to be adaptable to harsh temperatures (Stoskopf, 1985). Nonetheless, residue-derived N input was found affected by treatments: in fact, vetch provided about twice nitrogen compared with NT-R (499 kg N ha^{-1} vs 259 kg N ha^{-1}, respectively). Higher N concentration in vetch residue is related to the biological N fixation associated with N-fixing root symbioses of legumes (Larue and Patterson, 1981).

4.2. Soil aggregates, aggregate-associated C and N, and responses of SOC and STN

Reducing soil disturbance and enhancing biomass input to the soil with adoption of NT plus CCs may facilitate the formation and the preservation of water-stable macroaggregates (Bhattacharyya et al., 2012). Consistently with our hypothesis, NT treatments enhanced aggregate formation and stabilization in the 0–5 cm soil layer, as demonstrated by our results on MWD. In this case, the major contribution to the increased MWD under NT-V and NT-R was given by the increase in large macroaggregates, which were almost fivefold higher than under CT. Conversely, small macroaggregates, free microaggregates, and silt + clay fractions were found higher under CT than under NT treatments, indicating that intensive tillage operations promote large macroaggregate breakdown into smaller fractions. Moreover, hairy vetch and rye residues under NT treatments provided additional fresh organic material to the soil compared with CT. The decomposition of such material by soil micro- and macro-fauna is related to the release of polysaccharides and organic acids, which are the binding agents involved in aggregate formation and stabilization (Tisdall and Oades, 1982; Decaëns, 2000). Previous studies highlighted that avoiding soil disturbance and returning fresh organic material to the soil promote the macroaggregates stabilization by delaying aggregate breakdown and increasing aggregate formation rate (Six et al., 2000a; Álvaro-Fuentes et al., 2009). This findings are in agreement with previous results obtained by Sheehy et al. (2015) in *Vertic Cambisol* and in *Eutric Regosol*, reporting higher large macroaggregates under NT than under CT and increased small macroaggregates amount under CT in 3 out of 4 study sites. Our results corroborated also previous outcomes by Pareja-Sánchez et al. (2017) in a semiarid Mediterranean climate and *Typic Xerofluvent*, which observed higher amount of water-stable small macroaggregates under CT than under NT in 2 sampling dates out of 4, whereas no differences were recorded in the remaining dates. In addition, increased proportion of microaggregates and silt + clay due to macroaggregate breakdown after tillage operations have been also widely reported in literature (Six et al., 1999, 2000a; Denef et al., 2001; Bossuyt et al., 2002; Grunwald et al., 2016; Gao et al., 2019).

Here for the first time, we show that combining hairy vetch as CC with NT increases soil aggregate stability down to 5–15 cm soil layer, even compared with rye plus NT. In fact, MWD and large macroaggregates were higher under NT-V than under CT and NT-R. These results may be ascribed to the higher main crop residue (and C input from such residue as well) provided to the soil under NT-V and to the combination between no-till and different root architecture of hairy vetch and rye. Under NT, CCs residues are retained on the soil surface in addition to residues from main crops and, therefore, downward movement of organic material to deeper soil layers is mainly limited to macrofauna activity (e.g. earthworms). This means that SOM is increased especially in the topmost soil layer (up to 5–10 cm depth) under NT as observed in several studies (Kern and Johnson, 1993; Koch and Stockfisch, 2006; He et al., 2011). However, despite grasses generally having greater fibrous root mass and volume than legumes (Haynes, 1980), most of the latter have prominent taproots allowing them to explore deeper soil layers (Sheaffer, 1989). Therefore, the increased macroaggregate proportion in the shallow soil layer of both NT treatments is probably promoted by the aboveground residues of CCs left on the soil surface, whereas root residues of hairy vetch left into the soil after termination may have provided fresh organic material for large macroaggregates formation and stabilization in the 5–15 cm soil layer. No-till + CCs benefits on aggregate stabilization were not observed in the deepest soil layer (15–30 cm). This is in agreement with previous findings reported by a long-term study conducted by Sithole et al. (2019), in which the authors did not find differences in large macroaggregates amount between CT and NT at 20–30 cm depths. Fractions within macroaggregates were found to differ especially in the 0–5 cm soil layer. Coarse particulate organic matter was increased by NT-R and microaggregates within macroaggregates were higher under both NT treatments than under CT. This confirm that soil disturbance increase macroaggregate turnover rate, inhibiting the formation of microaggregates within macroaggregates, which are known to be key elements to long-term C sequestration (Six et al., 1998; Denef et al., 2004).

Continuous no-till and cover cropping enhanced SOC and STN in both 0–5 and 5–15 cm soil layers, while not in the 15–30 cm one. The effect of NT and CCs was particularly pronounced in the 0–5 cm layer, in which SOC was increased by 78% under NT-R and by 73% under NT-V compared with CT. Our findings are widely supported by previous research reporting higher SOM content in untilled soils than in conventional tilled soils (Arshad et al., 1990; Six et al., 2000a; Plaza-Bonilla et al., 2010; Sapkota et al., 2012). In our study, the greatest accumulation of SOC and STN in the 0–5 cm layer under NT treatments is explained firstly by the larger input of fresh organic residues (because of cover cropping), as supported by the positive correlation between C/N input from CCs and C/N sequestration parameters, and secondly by the higher protection from mineralization of SOM within soil aggregates. SOM is protected within macroaggregates because of two main mechanisms: (1) labile SOM (e.g. cPOM) is short-term physically protected (until macroaggregate disruption) by creating physical barriers between microorganisms and enzymes and their substrates (Beare et al., 1994; Six et al., 2002); (2) stable SOM is long-term physically and chemically protected in microaggregates within macroaggregates of which formation is promoted by macroaggregate stability and organic substances availability (Six et al., 2000a; Bandyopadhyay et al., 2010). This is supported by our results on redistribution of C and N within macroaggregates in the 0–5 cm soil layer: both cPOM- and microaggregates within macroaggregates-associated C and N were higher under NT treatments than under CT, thus corroborating higher protection for labile (cPOM-associated) and more stable (microaggregates within macroaggregates-associated) SOM. Moreover, the contribution of both protected labile and stable SOM to stock C into the soil is supported by the strong correlation between C stored in fractions within macroaggregates (cPOM and microaggregates) and C sequestration parameters.

In the 5–15 cm soil layer, despite NT treatments having higher SOC and STN than CT, results on aggregate-associated C and N were less affected by treatments. In contrast to our hypothesis, no difference in terms of SOC and STN was found between the two NT+CCs treatments in all soil layers, but NT-V increased large macroaggregate-associated C and N whereas NT-R had more C stored in microaggregates and in silt + clay fraction. This was probably again due to different root architecture of hairy vetch and rye (as previously discussed for aggregate fractions) and to the higher main crop residue provided to the soil under NT-V, which favoured the cementation of organic substances and soil particles to form macroaggregates (Decaëns, 2000). Last but not least, legume cover crops were reported to increase the amount and lability of residue inputs, thus favouring organomineral associations into the soil and further enhancing C protection within aggregates (Veloso et al., 2019).

4.3. *C and N sequestration potential of no-till plus cover crops*

Consistently with our initial hypothesis, NT plus CCs increased C and N sequestration rates and C sequestration efficiency. On average NT treatments sequestered around 0.5 Mg C ha^{-1} y^{-1} and 115 kg N ha^{-1} y^{-1}, emphasising the importance of conservation practices for climate change mitigation and soil fertility restoration. Indeed, CT lost around 0.5 Mg C ha^{-1} y^{-1} and 50 kg N ha^{-1} y^{-1}. The negative performance of CT in terms of C sequestration potential is emphasized by the results on C sequestration efficiency: CT, in fact, lost around 14% of the total annual C input. On the contrary, NT treatments sequestered on average 11% of the total annual C input. Such highly positive results on C and N sequestration potential and C sequestration efficiency of NT may be explained by the larger return of crop residues and by the enhanced aggregate formation and stabilization, thus limiting losses through mineralization (Six et al., 2000a). This is supported by our results showing strong correlation between C and N within macroaggregates, and associated with cPOM and microaggregates within macroaggregates as well, and sequestration parameters. Our results significantly argue outcomes from a recent meta-analysis performed by Powlson et al. (2014), in which the authors reported limited benefit of no-till for climate change mitigation mainly due to specific issues: (i) the increase of SOC in the topmost soil layer under NT would be counteracted by a decrease in the deeper layer (20–40 cm), meaning no net increase in SOC stock; (ii) the most of studies reporting positive effect under NT have focused on SOC concentration rather than on SOC stock, thus not considering the changes in soil bulk density. In addition, the authors reported a value of 0.3 Mg C ha^{-1} y^{-1} as a standard accumulation rate for NT in their study; yet they considered it very optimistic and overestimated. In our study, soil accumulated almost 0.6 Mg C ha^{-1} y^{-1} under NT-V and around 0.4 Mg C ha^{-1} y^{-1} under NT-R in the 0–30 cm soil layer, thus confirming the potential of conservation practices for C sequestration in fine-textured soils and temperate climates. However, we agree that more long-term studies are needed to fully assess the prolonged effect of NT and cover crops on climate change mitigation and, possibly, assessing the effect of periodic tillage events (e.g., subsoiling), which are occasionally used as practice to reduce possible NT unfavourable effect (Conant et al., 2007; Powlson et al., 2012). Also, in contrast to our hypothesis, no differences were found between rye and hairy vetch in terms of C sequestration rate. This may be explained by the same amount of biomass and C input provided by both cover crops (Poeplau and Don, 2015), which is supported by the positive correlation between C sequestration parameters and C input from CCs. Thus, cover crop biomass production rate (rather than biomass quality) and retention onto the soil as residue was the main driver of soil C and N sequestration in our study.

4.4. *Implications for efficient C farming via no-till plus cover crops adoption*

Based on findings reported here, conservation practices – such as no-till + CCs – may be adopted to sustain yield performances (even during the transition period), while providing more C and N inputs to the soil (and C and N sequestration) compared with conventional tillage practices in fine-textured soils of temperate areas. However, particular attention should be paid to sowing time of main crops (due to decreased soil temperatures), to soil compaction during the transition period, and with high N-demanding main crops due to N-immobilization into the soil, leading to possible N-deficiency during the initial stages of crop growth and yield losses. The latter could be particularly the case when adopting a grass (as rye, in our experiment) as previous CC may promote

N immobilization further than what expected in N-limited environments. When N-fertilization rate is relatively high (>200 kg N ha^{-1} y^{-1}, as for intensive agro-ecosystems with high-yielding crops), additional N inputs from legume CCs mismatching plant absorption capacity may results in significantly higher N losses as nitrous oxide (N_2O) emissions (Fiorini et al., 2020b). Indeed, C sequestration is not the only driver for assessing the contribution to climate change mitigation, and N_2O is recognized as a potent greenhouse gas with a global warming potential 273 times greater than that of CO_2 on a 100-year time horizon (de Haas and Andrews, 2022). Thus, further studies focusing on different levels of N-fertilization are needed to assess optimal balance between CC-derived N inputs and N-fertilizer rates, thus aiming to avoid surplus and losses.

Fiorini et al. (2020a), (2020b) already reported with data from the same field that NT vs. CT may concretely reduce N_2O emissions from intensive agro-ecosystems, while maintaining yield. Nevertheless, CC type was considered as a main driver of N_2O emissions in this study, since rye reduced N_2O compared with hairy vetch by 20–36%, because of the lower availability of soil mineral N (which is likely to have reduced also losses via leaching). This may partially explain also the lack of differences between rye and vetch in terms of N sequestration rate and efficiency in the present study. Indeed, a part of the N input provided by hairy vetch residues (which was higher under NT-V than under NT-R) may have been lost via NO_3^- leaching and/or N_2O emissions. Therefore, exceeding N inputs results neither into increased yield performance, nor into increased N storage into the soil.

Overall, both rye and hairy vetch combined with NT showed promising results for climate change mitigation and soil fertility restoration, with the potential of maintain crop yield even in intensively managed agro-ecosystems. Yet, we recommend that N fertilizers should be applied considering the expected rate and timing of available N from mineralization of different types of CC residues.

In conclusion, the adoption of no-till plus selected cover crops may be a viable C farming strategy to meet the ambitious goals of EU Green Deal and of Sustainable Development by FAO, promoting CO_2 sequestration into agricultural soils, sustaining food production while lowering fertilizer demand.

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CRediT authorship contribution statement

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

Conflicts of interest

The authors declare that they have no conflicts of interest.

Data Availability

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

References

Adetunji, A.T., Ncube, B., Mulidzi, R., Lewu, F.B., 2020. Management impact and benefit of cover crops on soil quality: a review. Soil Tillage Res. 204, 104717 https://doi.org/10.1016/j.still.2020.104717.

Alvarez, R., Steinbach, H.S., 2009. A review of the effects of tillage systems on some soil physical properties, water content, nitrate availability and crops yield in the Argentine Pampas. Soil Tillage Res. 104, 1–15. https://doi.org/10.1016/J.STILL.2009.02.005.

Álvaro-Fuentes, J., Cantero-Martínez, C., López, M.V., Paustian, K., Denef, K., Stewart, C.E., Arrúe, J.L., 2009. Soil aggregation and soil organic carbon stabilization: effects of management in semiarid mediterranean agroecosystems. Soil Sci. Soc. Am. J. 73, 1519–1529. https://doi.org/10.2136/SSSAJ2008.0333.

Arshad, M.A., Schnitzer, M., Angers, D.A., Ripmeester, J.A., 1990. Effects of till vs no-till on the quality of soil organic matter. Soil Biol. Biochem. 22, 595–599. https://doi.org/10.1016/0038-0717(90)90003-I.

Bandyopadhyay, P.K., Saha, S., Mani, P.K., Mandal, B., 2010. Effect of organic inputs on aggregate associated organic carbon concentration under long-term rice–wheat cropping system. Geoderma 154, 379–386. https://doi.org/10.1016/J.GEODERMA.2009.11.011.

Beare, M.H., Hendrix, P.F., Cabrera, M.L., Coleman, D.C., 1994. Aggregate-protected and unprotected organic matter pools in conventional- and no-tillage soils. Soil Sci. Soc. Am. J. 58, 787–795. https://doi.org/10.2136/SSSAJ1994.03615995005800030021X.

Bengtsson, G., Bengtson, P., Månsson, K.F., 2003. Gross nitrogen mineralization-, immobilization-, and nitrification rates as a function of soil C/N ratio and microbial activity. Soil Biol. Biochem. 35, 143–154. https://doi.org/10.1016/S0038-0717(02)00248-1.

Bhattacharyya, R., Tuti, M.D., Kundu, S., Bisht, J.K., Bhatt, J.C., 2012. Conservation tillage impacts on soil aggregation and carbon pools in a sandy clay loam soil of the Indian Himalayas. Soil Sci. Soc. Am. J. 76, 617–627. https://doi.org/10.2136/SSSAJ2011.0320.

Boselli, R., Fiorini, A., Santelli, S., Ardenti, F., Capra, F., Maris, S.C., Tabaglio, V., 2020. Cover crops during transition to no-till maintain yield and enhance soil fertility in intensive agro-ecosystems. F. Crop. Res. 255. https://doi.org/10.1016/j.fcr.2020.107871.

Bossuyt, H., Six, J., Hendrix, P.F., 2002. Aggregate-protected carbon in no-tillage and conventional tillage agroecosystems using carbon-14 labeled plant residue. Soil Sci. Soc. Am. J. 66, 1965–1973. https://doi.org/10.2136/SSSAJ2002.1965.

Burgess, M.S., Mehuys, G.R., Madramootoo, C.A., Campus, M., H9x, C., 2002. Nitrogen dynamics of decomposing corn residue components under three tillage systems. Soil Sci. Soc. Am. J. 66, 1350–1358. https://doi.org/10.2136/SSSAJ2002.1350.

Cardoso, E.J.B., Vasconcellos, R.L.F., Bini, D., Miyauchi, M.Y.H., et al., 2013. Soil health: looking for suitable indicators. What should be considered to assess the effects of use and management on soil health. Sci. Agric. 70, 274–289.

Conant, R.T., Easter, M., Paustian, K., Swan, A., Williams, S., 2007. Impacts of periodic tillage on soil C stocks: a synthesis. Soil Tillage Res. 95, 1–10. https://doi.org/10.1016/J.STILL.2006.12.006.

Dalby, P.R., Baker, G.H., Smith, S.E., 1996. "Filter paper method" to remove soil from earthworm intestines and to standardise the water content of earthworm tissue. Soil Biol. Biochem. 28, 685–687. https://doi.org/10.1016/0038-0717(95)00157-3.

Decaëns, T., 2000. Degradation dynamics of surface earthworm casts in grasslands of the eastern plains of Colombia. Biol. Fertil. Soils 32, 149–156.

Denef, K., Six, J., Bossuyt, H., Frey, S.D., Elliott, E.T., Merckx, R., Paustian, K., 2001. Influence of dry–wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biol. Biochem. 33, 1599–1611. https://doi.org/10.1016/S0038-0717(01)00076-1.

Denef, K., Six, J., Merckx, R., Paustian, K., 2004. Carbon sequestration in microaggregates of No-tillage soils with different clay mineralogy. Soil Sci. Soc. Am. J. 68, 1935–1944. https://doi.org/10.2136/SSSAJ2004.1935.

Du, Z., Angers, D.A., Ren, T., Zhang, Q., Li, G., 2017. The effect of no-till on organic C storage in Chinese soils should not be overemphasized: a meta-analysis. Agric. Ecosyst. Environ. 236, 1–11. https://doi.org/10.1016/J.AGEE.2016.11.007.

Dynarski, K.A., Bossio, D.A., Scow, K.M., 2020. Dynamic stability of soil carbon: reassessing the "permanence" of soil carbon sequestration. Front. Environ. Sci. 8, 218. https://doi.org/10.3389/FENVS.2020.514701/BIBTEX.

Elliott, E.T., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Sci. Soc. Am. J. 50, 627–633. https://doi.org/10.2136/SSSAJ1986.03615995005000030017X.

Elliott, E.T., Palm, C.A., Reuss, D.E., Monz, C.A., 1991. Organic matter contained in soil aggregates from a tropical chronosequence: correction for sand and light fraction. Agric. Ecosyst. Environ. 34, 443–451. https://doi.org/10.1016/0167-8809(91)90127-J.

European Commission, 2020. A Farm to Fork Strategy for a Fair, Healthy and Environmentally-friendly DG SANTE/Unit 'Food information and composition, food waste'

Fageria, N.K., Baligar, V.C., Bailey, B.A., 2007. Role of cover crops in improving soil and row crop productivity. Commun. Soil Sci. Plant Anal. 36, 2733–2757. https://doi.org/10.1080/00103620500303939.

Fiorini, A., Boselli, R., Amaducci, S., Tabaglio, V., 2018. Effects of no-till on root architecture and root-soil interactions in a three-year crop rotation. Eur. J. Agron. 99. https://doi.org/10.1016/j.eja.2018.07.009.

Fiorini, A., Boselli, R., Maris, S.C., Santelli, S., Ardenti, F., Capra, F., Tabaglio, V., 2020a. May conservation tillage enhance soil C and N accumulation without decreasing yield in intensive irrigated croplands? Results from an eight-year maize monoculture. Agric. Ecosyst. Environ. 296, 106926 https://doi.org/10.1016/J.AGEE.2020.106926.

Fiorini, A., Maris, S.C., Abalos, D., Amaducci, S., Tabaglio, V., 2020b. Combining no-till with rye (Secale cereale L.) cover crop mitigates nitrous oxide emissions without decreasing yield. Soil Tillage Res. 196, 104442 https://doi.org/10.1016/j.still.2019.104442.

Fiorini, A., Remelli, S., Boselli, R., Mantovi, P., Ardenti, F., Trevisan, M., Menta, C., Tabaglio, V., 2022. Driving crop yield, soil organic C pools, and soil biodiversity with

selected winter cover crops under no-till. Soil Tillage Res. 217, 105283 https://doi.org/10.1016/J.STILL.2021.105283.
Frankenberger, W.T., Abdelmagid, H.M., 1985. Kinetic parameters of nitrogen mineralization rates of leguminous crops incorporated into soil. Plant Soil 872 (87), 257–271. https://doi.org/10.1007/BF02181865.
Gao, L., Wang, B., Li, S., Wu, H., Wu, X., Liang, G., Gong, D., Zhang, X., Cai, D., Degré, A., 2019. Soil wet aggregate distribution and pore size distribution under different tillage systems after 16 years in the Loess Plateau of China. CATENA 173, 38–47. https://doi.org/10.1016/J.CATENA.2018.09.043.
Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M., Toulmin, C., 2010. Food security: the challenge of feeding 9 billion people. Science 80 (327), 812–818. https://doi.org/10.1126/SCIENCE.1185383.
Gruber, N., Galloway, J.N., 2008. An Earth-system perspective of the global nitrogen cycle. Nature 4517176 (451), 293–296. https://doi.org/10.1038/nature06592.
Grunwald, D., Kaiser, M., Ludwig, B., 2016. Effect of biochar and organic fertilizers on C mineralization and macro-aggregate dynamics under different incubation temperatures. Soil Tillage Res. 164, 11–17. https://doi.org/10.1016/J.STILL.2016.01.002.
de Haas, D., Andrews, J., 2022. Nitrous oxide emissions from wastewater treatment - revisiting the IPCC 2019 refinement guidelines. Environ. Chall. 8, 100557 https://doi.org/10.1016/J.ENVC.2022.100557.
Hadas, A., Kautsky, L., Goek, M., Kara, E.E., 2004. Rates of decomposition of plant residues and available nitrogen in soil, related to residue composition through simulation of carbon and nitrogen turnover. Soil Biol. Biochem. 36, 255–266. https://doi.org/10.1016/J.SOILBIO.2003.09.012.
Hansen, B., Thorling, L., Schullehner, J., Termansen, M., Dalgaard, T., 2017. Groundwater nitrate response to sustainable nitrogen management. Sci. Rep. 7. https://doi.org/10.1038/S41598-017-07147-2.
Haynes, R.J., 1980. Competitive aspects of the grass-legume association. Adv. Agron. 33, 227–261. https://doi.org/10.1016/S0065-2113(08)60168-6.
He, J., Li, H., Rasaily, R.G., Wang, Q., Cai, G., Su, Y., Qiao, X., Liu, L., 2011. Soil properties and crop yields after 11 years of no tillage farming in wheat–maize cropping system in North China Plain. Soil Tillage Res. 113, 48–54. https://doi.org/10.1016/J.STILL.2011.01.005.
Herrick, J.E., Wander, M.M., 2018. Relationships between soil organic carbon and soil quality in cropped and rangeland soils: the importance of distribution, composition, and soil biological activity. Soil Process. Carbon Cycle 405–425. https://doi.org/10.1201/9780203739273-28.
Hossain, A., Krupnik, T.J., Timsina, J., Mahboob, M.G., Chaki, A.K., Farooq, M., Bhatt, R., Fahad, S., Hasanuzzaman, M., 2020. Agricultural land degradation: processes and problems undermining future food security. Environ. Clim. Plant Veg. Growth 17–61. https://doi.org/10.1007/978-3-030-49732-3_2.
Hothorn, T., Bretz, F., Westfall, P., Heiberger, R., 2007. The multcomp Package. R Doc.
Jian, J., Du, X., Reiter, M.S., Stewart, R.D., 2020. A meta-analysis of global cropland soil carbon changes due to cover cropping. Soil Biol. Biochem. 143, 107735 https://doi.org/10.1016/J.SOILBIO.2020.107735.
Jin, K., Sleutel, S., De Neve, S., Gabriels, D., Cai, D., Jin, J., Hofman, G., 2008. Nitrogen and carbon mineralization of surface-applied and incorporated winter wheat and peanut residues. Biol. Fertil. Soils 44, 661–665. https://doi.org/10.1007/S00374-008-0267-5/FIGURES/1.
Kern, J.S., Johnson, M.G., 1993. Conservation tillage impacts on national soil and atmospheric carbon levels. Soil Sci. Soc. Am. J. 57, 200–210. https://doi.org/10.2136/SSSAJ1993.03615995005700010036X.
Koch, H.J., Stockfisch, N., 2006. Loss of soil organic matter upon ploughing under a loess soil after several years of conservation tillage. Soil Tillage Res 86, 73–83. https://doi.org/10.1016/J.STILL.2005.02.029.
Kong, A.Y.Y., Six, J., Bryant, D.C., Denison, R.F., Kessel, C. van, 2005. The relationship between carbon input, aggregation, and soil organic carbon stabilization in sustainable cropping systems. Soil Sci. Soc. Am. J. 69, 1078–1085. https://doi.org/10.2136/SSSAJ2004.0215.
Lal, R., 2004. Soil carbon sequestration impacts on global climate change and food security. Science 80 (304), 1623–1627. https://doi.org/10.1126/SCIENCE.1097396/SUPPL_FILE/LAL.SOM.PDF.
Lal, R., 2015. Sequestering carbon and increasing productivity by conservation agriculture. J. Soil Water Conserv 70, 55A–62A. https://doi.org/10.2489/JSWC.70.3.55A.
Larue, T.A., Patterson, T.G., 1981. How much nitrogen do legumes fix? Adv. Agron. 34, 15–38. https://doi.org/10.1016/S0065-2113(08)60883-4.
Lê, S., Josse, J., Husson, F., 2008. FactoMineR: an R package for multivariate analysis. J. Stat. Softw. 25, 1–18. https://doi.org/10.18637/JSS.V025.I01.
Liebman, A.M., Grossman, J., Brown, M., Wells, M.S., Reberg-Horton, S.C., Shi, W., 2018. Legume cover crops and tillage impact nitrogen dynamics in organic corn production. Agron. J. 110, 1046–1057. https://doi.org/10.2134/AGRONJ2017.08.0474.
Liu, Y., Wu, L., Baddeley, J.A., Watson, C.A., 2011. Models of biological nitrogen fixation of legumes. Sustain. Agric. Vol. 2, 883–905.
Malhi, S.S., Grant, C.A., Johnston, A.M., Gill, K.S., 2001. Nitrogen fertilization management for no-till cereal production in the Canadian Great Plains: a review. Soil Tillage Res 60, 101–122. https://doi.org/10.1016/S0167-1987(01)00176-3.
Marcillo, G.S., Miguez, F.E., 2017. Corn yield response to winter cover crops: an updated meta-analysis. J. Soil Water Conserv. 72, 226–239. https://doi.org/10.2489/JSWC.72.3.226.
McNutt, M., 2013. Climate change impacts. Science 80- (341), 435. https://doi.org/10.1126/SCIENCE.1243256/ASSET/293A1C33-F006-4627-AF78-F13A2A65236E/ASSETS/GRAPHIC/341_435_F2.JPEG.
Mengel, K., Kirkby, E.A., 2001. Principles of plant nutrition. 5th edidtion ed.
Miguez, F.E., Bollero, G.A., 2005. Review of corn yield response under winter cover cropping systems using meta-analytic methods. Crop Sci. 45, 2318–2329. https://doi.org/10.2135/CROPSCI2005.0014.
Ogle, S.M., Swan, A., Paustian, K., 2012. No-till management impacts on crop productivity, carbon input and soil carbon sequestration. Agric. Ecosyst. Environ. 149, 37–49. https://doi.org/10.1016/J.AGEE.2011.12.010.
Oliver, M.A., Gregory, P.J., 2015. Soil, food security and human health: a review. Eur. J. Soil Sci. 66, 257–276. https://doi.org/10.1111/EJSS.12216.
Pareja-Sánchez, E., Plaza-Bonilla, D., Ramos, M.C., Lampurlanés, J., Álvaro-Fuentes, J., Cantero-Martínez, C., 2017. Long-term no-till as a means to maintain soil surface structure in an agroecosystem transformed into irrigation. Soil Tillage Res. 174, 221–230. https://doi.org/10.1016/J.STILL.2017.07.012.
Perego, A., Rocca, A., Cattivelli, V., Tabaglio, V., Fiorini, A., Barbieri, S., Schillaci, C., Chiodini, M.E., Brenna, S., Acutis, M., 2019. Agro-environmental aspects of conservation agriculture compared to conventional systems: a 3-year experience on 20 farms in the Po valley (Northern Italy). Agric. Syst. 168, 73–87. https://doi.org/10.1016/j.agsy.2018.10.008.
Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Team, R.C., 2013. nlme: Linear and nonlinear mixed effects models. R. Packag. Version 3, 111.
Pittelkow, C.M., Linquist, B.A., Lundy, M.E., Liang, X., van Groenigen, K.J., Lee, J., van Gestel, N., Six, J., Venterea, R.T., van Kessel, C., 2015. When does no-till yield more? A global meta-analysis. F. Crop. Res. 183, 156–168. https://doi.org/10.1016/J.FCR.2015.07.020.
Plaza-Bonilla, D., Cantero-Martínez, C., Álvaro-Fuentes, J., 2010. Tillage effects on soil aggregation and soil organic carbon profile distribution under Mediterranean semi-arid conditions. Soil Use Manag. 26, 465–474. https://doi.org/10.1111/J.1475-2743.2010.00298.X.
Poeplau, C., Don, A., 2015. Carbon sequestration in agricultural soils via cultivation of cover crops – a meta-analysis. Agric. Ecosyst. Environ. 200, 33–41. https://doi.org/10.1016/J.AGEE.2014.10.024.
Pott, L.P., Amado, T.J.C., Schwalbert, R.A., Gebert, F.H., Reimche, G.B., Pes, L.Z., Ciampitti, I.A., 2021. Effect of hairy vetch cover crop on maize nitrogen supply and productivity at varying yield environments in Southern Brazil. Sci. Total Environ. 759, 144313 https://doi.org/10.1016/J.SCITOTENV.2020.144313.
Powlson, D.S., Bhogal, A., Chambers, B.J., Coleman, K., Macdonald, A.J., Goulding, K.W.T., Whitmore, A.P., 2012. The potential to increase soil carbon stocks through reduced tillage or organic material additions in England and Wales: a case study. Agric. Ecosyst. Environ. 146, 23–33. https://doi.org/10.1016/J.AGEE.2011.10.004.
Powlson, D.S., Stirling, C.M., Jat, M.L., Gerard, B.G., Palm, C.A., Sanchez, P.A., Cassman, K.G., 2014. Limited potential of no-till agriculture for climate change mitigation. Nat. Publ. Gr. 4. https://doi.org/10.1038/NCLIMATE2292.
R Core Team, 2020. R: A language and environment for statistical computing. R A Lang. Environ. Stat. Comput. R Found. Stat. Comput. Vienna, Austria.
Ruffo, M.L., Bullock, D.G., Bollero, G.A., 2004. Soybean yield as affected by biomass and nitrogen uptake of cereal rye in winter cover crop rotations. Agron. J. 96, 800–805. https://doi.org/10.2134/AGRONJ2004.0800.
Ruis, S.J., Blanco-Canqui, H., Creech, C.F., Koehler-Cole, K., Elmore, R.W., Francis, C.A., 2019. Cover crop biomass production in temperate agroecozones. Agron. J. 111, 1535–1551. https://doi.org/10.2134/AGRONJ2018.08.0535.
Sachs, J.D., 2012. From millennium development goals to sustainable development goals. Lancet 379, 2206–2211. https://doi.org/10.1016/S0140-6736(12)60685-0.
Sapkota, T.B., Mazzoncini, M., Bàrberi, P., Antichi, D., Silvestri, N., 2012. Fifteen years of no till increase soil organic matter, microbial biomass and arthropod diversity in cover crop-based arable cropping systems. Agron. Sustain. Dev. 32, 853–863. https://doi.org/10.1007/S13593-011-0079-0/TABLES/5.
Schulz, M., Marocco, A., Tabaglio, V., Macias, F.A., Molinillo, J.M.G., 2013. Benzoxazinoids in rye allelopathy - from discovery to application in sustainable weed control and organic farming. J. Chem. Ecol. 39, 154–174. https://doi.org/10.1007/S10886-013-0235-X/FIGURES/6.
Sheaffer, C.C., 1989. Effect of competition on legume persistence. Persistence Forage Legum 327–334.
Sheehy, J., Regina, K., Alakukku, L., Six, J., 2015. Impact of no-till and reduced tillage on aggregation and aggregate-associated carbon in Northern European agroecosystems. Soil Tillage Res. 150, 107–113. https://doi.org/10.1016/J.STILL.2015.01.015.
Shepherd, T.G., Stagnari, F., Pisante, M., Benites, J., 2008. Visual soil assessment, Organization.
Shukla, P.R., Skeg, J., Buendia, E.C., Masson-Delmotte, V., Pörtner, H.-O., Roberts, D.C., Zhai, P., Slade, R., Connors, S., van Diemen, S., 2019. Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems.
Singh, B., 2018. Are nitrogen fertilizers deleterious to soil health. Agron Vol. 8 (2018), 48. https://doi.org/10.3390/AGRONOMY8040048.
Sithole, N.J., Magwaza, L.S., Thibaud, G.R., 2019. Long-term impact of no-till conservation agriculture and N-fertilizer on soil aggregate stability, infiltration and distribution of C in different size fractions. Soil Tillage Res. 190, 147–156. https://doi.org/10.1016/J.STILL.2019.03.004.
Six, J., Elliott, E.T., Paustian, K., Doran, J.W., 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Sci. Soc. Am. J. 62, 1367–1377. https://doi.org/10.2136/SSSAJ1998.03615995006200050032X.
Six, J., Elliott, E.T., Paustian, K., 1999. Aggregate and soil organic matter dynamics under conventional and no-tillage systems. Soil Sci. Soc. Am. J. 63, 1350–1358. https://doi.org/10.2136/SSSAJ1999.6351350X.
Six, J., Elliott, E.T., Paustian, K., 2000a. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage

agriculture. Soil Biol. Biochem. 32, 2099–2103. https://doi.org/10.1016/S0038-0717(00)00179-6.
Six, J., Paustian, K., Elliott, E.T., Combrink, C., 2000b. Soil structure and organic matter I. Distribution of aggregate-size classes and aggregate-associated carbon. Soil Sci. Soc. Am. J. 64, 681–689. https://doi.org/10.2136/SSSAJ2000.642681X.
Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant Soil 241, 155–176. https://doi.org/10.1023/A:1016125726789.
Soil Survey Staff, 2014. Soil Survey Field and Laboratory Methods Manual. United States Dep. Agric. Nat. Resour. Conserv. Serv.
Stoskopf, N.C., 1985. Cereal grain crops. Reston Publishing Company inc.
Tabaglio, V., Marocco, A., Schulz, M., 2013. Allelopathic cover crop of rye for integrated weed control in sustainable agroecosystems. Ital. J. Agron. 8 https://doi.org/10.4081/IJA.2013.E5.
Teasdale, J.R., Mohler, C.L., 1993. Light transmittance, soil temperature, and soil moisture under residue of hairy vetch and rye. Agron. J. 85, 673–680. https://doi.org/10.2134/AGRONJ1993.00021962008500030029X.
Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. J. Soil Sci. 33, 141–163.
van Bavel, C.H.M., 1950. Mean weight-diameter of soil aggregates as a statistical index of aggregation. Soil Sci. Soc. Am. J. 14, 20–23. https://doi.org/10.2136/SSSAJ1950.036159950014000C0005X.
Van den Putte, A., Govers, G., Diels, J., Gillijns, K., Demuzere, M., 2010. Assessing the effect of soil tillage on crop growth: a meta-regression analysis on European crop yields under conservation agriculture. Eur. J. Agron. 33, 231–241. https://doi.org/10.1016/J.EJA.2010.05.008.
Veloso, M.G., Cecagno, D., Bayer, C., 2019. Legume cover crops under no-tillage favor organomineral association in microaggregates and soil C accumulation. Soil Tillage Res. 190, 139–146. https://doi.org/10.1016/J.STILL.2019.03.003.
Wang, X., Wu, H., Dai, K., Zhang, D., Feng, Z., Zhao, Q., Wu, X., Jin, K., Cai, D., Oenema, O., Hoogmoed, W.B., 2012. Tillage and crop residue effects on rainfed wheat and maize production in northern China. F. Crop. Res. 132, 106–116. https://doi.org/10.1016/j.fcr.2011.09.012.
Williams, M.M., Mortensen, D.A., Doran, J.W., 2000. No-tillage soybean performance in cover crops for weed management in the western Corn Belt. J. Soil Water Conserv. 55.
Zachariassen, J.A., Power, J.F., 1991. Growth rate and water use by legume species at three soil temperatures. Agron. J. 83, 408–413. https://doi.org/10.2134/AGRONJ1991.00021962008300020029X.