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Cover crops, compost, and conversion to grassland to increase soil C and N stock in intensive agrosystems

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Abstract Organic fertilization or conversion to grassland may increase soil organic carbon (SOC) and soil total nitrogen (STN). However, responses of net SOC and STN accumulation are sometimes inconsistent and little is known about temporal patterns when those strategies are stopped. We (a) assessed the effects of rye (Secale cereale L.) and hairy vetch (Vicia villosa Roth) cover crops (CCs) on SOC and STN during a 4-year ''enriching'' period (EP), and a following 2-year ''depleting'' period (DP); (b) compared these strategies with compost application and conversion to permanent fescue (Festuca arundinacea Schreb.); and (c) determined the responses of maize (Zea mays L.), soybean (Glycine max L. Merr.), and sunflower (*Helianthus annuus* L.) yields. Crop yield increased only in maize with compost and hairy vetch, which downsizes the role of these strategies to support productivity. SOC and STN increased with 4-year compost $(+ 10.2$ Mg C ha⁻¹; + 0.5 Mg N ha⁻¹), rye (+ 8.8 Mg C ha⁻¹; $+ 0.6$ Mg N ha⁻¹), and hairy vetch ($+ 6.9$ Mg C ha^{-1} ; + 0.6 Mg N ha^{-1}). Afterwards, SOC stock loss during 2-year DP tended to be higher than annual C input where there were CCs, and accounted for about 70% of annual C input where there was compost. High SOC loss highlights the weak effect of CCs for longterm SOC stabilization. Conversely, STN increased even during DP, which indicates a more lasting effect. Green manuring with CCs may be relevant for enhancing SOC and STN, although the beneficial effects are short-lived. Conversion to grassland remains the reference strategy.

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Graphic abstract

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Introduction

Carbon (C) and nitrogen (N) are important components of soil organic carbon (SOC), which is known to enhance soil quality and provide nutrients for plant growth (Franzluebbers and Stuedemann [2009;](#page-17-0) Tabaglio et al. [2009\)](#page-18-0). Different kinds of land use systems (e.g. cropland, grassland) and soil-crop management (e.g. tillage, crop rotation, cover cropping, residue management, fertilization) may either positively or negatively impact soil C and N cycling (Fuentes et al. [2014\)](#page-17-0) by affecting fresh organic matter inputs into the soil and SOM losses through the mineralization process (Kong et al. [2005](#page-17-0); Mazzoncini et al. [2011\)](#page-17-0).

The adoption of conservation practices in intensive cropland, such as reduced tillage, improved fertilizer management, cover cropping, and perennial cultivation, has been widely suggested in order to increase soil organic C (SOC) and soil total N (STN) concentration (Perego et al. [2019](#page-17-0)). However, most soils in Europe are still mouldboard ploughed (FAO [2017](#page-16-0)), which is known to negatively affect SOC (Nyamadzawo et al. [2009\)](#page-17-0). In addition, long, bare fallow periods are known to further reduce SOC and the biological activity of soils (Restovich et al. [2012\)](#page-17-0).

Introducing cover crops (CCs) may be helpful tools to elicit soil quality improvement (Calderon et al. [2016\)](#page-16-0) and provide additional amounts of available N for plants (Mazzoncini et al. [2011](#page-17-0)), especially if the use of leguminous CCs is adopted (Kramberger et al. [2014\)](#page-17-0). Similarly, manure-derived organic fertilizers enhance soil quality and are major sources of nutrients for the plants (Bedada et al. [2014](#page-16-0)). Combining organic (i.e. CC residues and/or manure-derived fertilizers) and chemical fertilizer has been widely indicated as a recommended fertilization strategy (Wei et al. [2016](#page-18-0)), thus ensuring a longer beneficial effect for plants and reducing fertilization costs for farmers (Mazzoncini et al. [2011](#page-17-0)).

Converting lands from cultivation of crops to wellmanaged perennial grassland constitutes a potential Cand N-enriching strategy (Singer et al. [2009](#page-17-0)), especially in view of minimized soil disturbance. Tall fescue (Festuca arundinacea Schreb.) is a widely used, perennial, cool-season forage grass

(Annicchiarico and Proietti [2011\)](#page-16-0). Fescue is highly productive when moisture is available during the summer, and survives hot conditions during summer, making it an excellent choice for irrigated plain areas in the Po Valley (northern Italy). In addition, previous studies conducted under similar conditions have reported that fescue-based grasslands may be considered reference SOC and STN accumulation systems (Franzluebbers and Stuedemann [2009](#page-17-0)).

To date, the cultivation of CCs has been largely promoted by the Common Agricultural Policy of the European Union (CAP, Rural Development Programme 2014–2020), since it has also been repeatedly indicated as a climate-change mitigation strategy (Eagle and Olander, [2012](#page-16-0)). However, to our knowledge only a few studies in Europe have previously compared (e.g. Mazzoncini et al. [2011;](#page-17-0) Bongiorno et al. [2019](#page-16-0); Bolinder et al. [2020](#page-16-0)), in intensive arable land with temperate climate conditions, the effect on SOC and STN provided by green manuring of CC residues with that of widely investigated organic fertilizers (i.e. manure-derived compost) and/or reference systems for SOC and STN accumulation (i.e. fescue grassland). Furthermore, since CCs' performance varies from one region to another depending on climate, crop rotation, and soil conditions, one needs to identify which species provide satisfactory biomass production for green manuring for specific areas. Last but not least, little is known about the consequences of an ''enriching'' period (EP), when C- and N-enhancing strategies (e.g. incorporation of CCs and compost) have been applied, on the temporal patterns of SOC and STN during a "depleting" period (DP), when (if) those strategies are stopped.

The main objectives of this study were: (a) to investigate the effects on SOC sequestration and STN accumulation created by green manuring with residues of a gramineous (rye, Secale cereale L.) and a leguminous (hairy vetch, Secale cereale L.) CC, during a 4-year EP and a 2-year DP; and (b) to compare these effects with those derived from organic fertilization with manure-derived compost and cropland conversion to grassland. An additional objective was (c) to determine the responses of crop yield and of crop residue returned to the soil (biomass return) to all cropland fertilization strategies (i.e. control, rye CC, hairy vetch CC, and compost) for maize, soybean, and sunflower crops, during EP and DP.

We hypothesized that green manuring with CC residues might replace compost application in order to mitigate the loss of SOC and STN (which is typically the case in mouldboard-ploughed soils of the Po Valley), thus maintaining and stabilizing yield over time.

Materials and methods

Site and soil characteristics

A 6-year (2009–2015) field study was carried out at a commercial farm located in Meleti (45.124350 N; 9.831542 E; altitude 55 m), Lombardy region, Northern-Italy. The site is characterised by a temperate climate, with an average annual temperature of 14.1 \degree C and annual rainfall of 885 mm (30-year) average). Rainfall and air temperature during the trial period (see Supplementary Material; Figure S.1) were obtained from an automated meteorological station positioned in the experimental field.

The soil is well drained, non-saline, and classified according to Soil Taxonomy (Soil Survey Staff [2014\)](#page-18-0) as fine, mixed (non-acid) mesic, aquic Ustifluvents, with a sandy loam texture (sand 531 g kg^{-1} , silt 349 g kg⁻¹, clay 120 g kg⁻¹). The initial physicalchemical properties (September 2009) of the top soil layer (0.0–0.3 m) were as follows: pH_{H2O} 7.3; organic C 9.3 g kg^{-1} ; bulk density 1.3 Mg m⁻³; available P (Olsen) 48 mg kg^{-1} ; exchangeable K (NH₄⁺ Ac) 197 mg kg^{-1} ; total N (Kjeldahl) 1.1 g kg^{-1} ; and cation exchange capacity 20 cmol⁺ kg^{-1} .

Experimental design, treatments and crop management

The experiment was laid out in a randomized complete block (RCB) design, with four blocks and five treatments: one representing current cropland (CL) management with mineral fertilizer distribution only as a control, three as improved CL managements with organic plus mineral application, and the last one standing for conversion to grassland (GL) management. In detail, the five treatments were as follows: (T1) mineral fertilizer; (T2) mineral fertilizer $+$ green manuring with rye (Secale cereale L.) CC; (T3) mineral fertilizer $+$ green manuring with hairy vetch (Vicia villosa Roth.) CC; (T4) mineral

fertilizer $+$ application of manure-derived compost; (T5) permanent tall fescue (Festuca arundinacea L.). Plot dimensions were 10 m long by 5 m wide (50 m^2) , with buffer rows 1 m wide between plots. All plots had been cultivated with the mouldboard ploughing technique under maize monoculture for silage prior to starting the experiment.

Improved CL managements (treatments T2–T4) were selected as representing current recommended fertilization strategies in the Po Valley to enhance SOC and STN. The comparison among T2–T4 and the control (T1) was made up of: (a) the initial 4-year ''enriching'' period (EP, 2009–2013) when fertilization strategies to enhance SOC and STN were applied and their annual effect on C and N dynamics compared with the control studied; (b) the final 2-year "depleting'' period (DP, 2014–2015) when these strategies were stopped and the residual effects on C and N cycling monitored. Since T5 (tall fescue) represented conversion to permanent GL management, it was maintained until the end of the experiment for the purpose of assessing SOC and STN response to contrasting land use.

Under CL management (T1–T4), the main crop sequence was maize (Zea mays L.) in 2010 and 2013; sunflower (Helianthus annuus L.) in 2011 and 2014; and soybean (Glycine max L. Merr.) in 2012 and 2015. Starting from 2010, all crops under T1–T4 were cultivated for grain production and crop residues were left onto the soil surface. Sunflower, maize, and soybean were harvested from the end of September to the beginning of November according to crop and year. Nitrogen fertilizers were distributed during the growing seasons of the main crops across all periods $(EP + DP)$. In detail, these were the N fertilization rates: 220 kg N ha^{-1} for maize in 2010 and 2013; and 150 kg N ha^{-1} for sunflower in 2011 and 2014. No chemical fertilizer was applied to soybean in 2012 and 2015. Contribution to the N supply of green manuring with rye and hairy vetch, as well as with compost, was taken into account in computing the rate of N fertilization for the main crops in order to obtain the same amount of efficient N for all CL treatments (see Supplementary Material; Table S.1).

During EP, a suitable seedbed for CCs was prepared through disk harrowing immediately after harvesting the main crop. Winter CCs were sown each year in T2 and T3 between the end of September and the beginning of October. Hairy vetch was drilled at

85 kg ha⁻¹; rye at 110 kg ha⁻¹; both of them at 0.15m spacing between rows. No fertilizer, herbicide, or pesticide was applied to CCs. Cover crops were mechanically terminated in spring using a chopper machine and subsequently incorporated into the soil by mouldboard ploughing at 0.3 m depth. Also, T1 was ploughed to 0.3 m in spring. The manure-derived compost was distributed in T4 each year in spring and incorporated into the soil by mouldboard ploughing at the same depth. Dry matter of compost varied from 20 to 36% in different years. Similarly, C concentration ranged from 42 to 48%. Since the chemical composition (and C and N concentration) of compost varied slightly from year to year during EP, the amount applied each year was corrected to obtain the same rate of organic C input $(1.85 \text{ Mg ha}^{-1})$. This resulted in a slightly different N application in different year, with a maximum reduction of 9% in 2012 compared to 2010, which was taken into account in computing the distribution rate of chemical N fertilizers.

Spring main crops were planted in April–May of each year (maize and sunflower in the middle of April; soybean was sown at the beginning of May). During DP, for all the CL treatments (T1–T4) neither compost nor CC residues were applied; only the main crop residues were incorporated by ploughing to 0.3 m. After spring ploughing, a suitable seedbed for main crops under T1–T4 was obtained through rotary harrowing $(0.1–0.15 \text{ m depth})$.

In T5, tall fescue was sown at a rate of 60 kg ha^{-1} at the beginning of the experiment (September 2009) and maintained until October 2015. During the whole experimental period, fescue received only mineral N fertilizer at a rate of 200 kg N ha^{-1} in 2010 and at a rate of $300 \text{ kg N} \text{ ha}^{-1}$ from 2011 to 2015 (see Supplementary Material; Table S.1).

All main operations during the field experiment are summarised in Table S.1 (see Supplementary Material).

Biomass sampling and analyses

Total aboveground biomass of the main crop (maize, sunflower and soybean) and winter CCs (rye and vetch) was measured every year right before harvest and termination, respectively. Biomass samples were collected from two randomly chosen 4 m^2 areas within each plot for the main crops, and 2 m^2 areas for the CCs. In the case of main crops, grain and crop residues were manually separated. Once in the lab, samples were dried at 65 °C until constant weight and ground at a 1 mm size. Subsequently, C and N concentrations were determined by the Dumas combustion method (900 \degree C) with the varioMax C:N (VarioMax C:NS, Elementar, Germany) Element Analyser. The aboveground C and N inputs to the soil were calculated by multiplying the weight of biomass excluding the grain (in the case of main crops) by its C and N concentration. The C:N ratios of the residues from the different crops were also calculated by dividing the C concentration of the biomass by the N concentration.

Soil sampling and processing

Soil sampling was carried out at the beginning of the experiment (2009) and at the end of each year (from 2010 to 2015) immediately after harvesting the main crops. Within each plot, six soil sub-samples at 0.0–0.3 m soil depth were collected using a coring device with a 15-mm diameter auger and mixed. Samples were then air dried, ground at 2 mm, and analysed. Soil organic C was calculated with the Walkley and Black method (Nelson and Sommers [1996\)](#page-17-0). Soil total N analyses were performed according to the Kjeldahl method.

To calculate SOC and STN stock, soil bulk density (0.0–0.3 m depth) was measured each year by collecting three undisturbed soil cores within each plot at 50% of field capacity, in order to reduce deformation of soil cores during sampling. Soil cores were oven dried (105 \degree C for 24 h) and weighed; the soil bulk density was calculated as the ratio of the weight of oven-dried soil to the bulk volume of the soil.

Calculations and statistical analyses

The soil organic carbon (SOC) and total nitrogen stock $(Mg ha^{-1})$ at 0.0–0.3 m depth was calculated as follows.

SOC stock $(Mg ha^{-1}) = (SOC/100) \times bulk$ density (Mg m⁻³) \times depth (m) \times 10 000 (m² ha⁻¹). The C sequestration in soil was calculated for EP and DP, using the following formula (Benbi and Senapati 2010): (a) for EP, C sequestration (Mg C ha⁻¹) soil) = $SOC_{(2013)} - SOC_{(2009)}$, where $SOC_{(2013)}$ indicates the SOC stocks at the end of EP and $SOC_{(2009)}$ indicates SOC stocks at the beginning of the experiment; (b) for DP, C sequestration $(Mg C ha^{-1})$ soil) = $SOC_{(2015)} - SOC_{(2013)}$, where $SOC_{(2015)}$ indicates the SOC stocks at the end of DP and $SOC_{(2013)}$ indicates SOC stocks at the beginning of DP (the end of EP). Likewise, STN stock and accumulation $(Mg N ha^{-1})$ on the soil were determined.

The cumulative C and N input applied was quantified for each treatment and year, by summing the total C and N contribution from (a) main crop residues (maize, sunflower, and soybean); (b) winter CC residues (rye and vetch), or compost; and (c) mineral fertilizers (in the case of N only).

Nitrogen use efficiency (NUE) was calculated as follows: $NUE = Y/N$; where Y is the yield of the main crop (kg ha^{-1}) and N is the total amount of N applied $(kg ha^{-1})$. The total amount of N applied was calculated as the sum of (a) the applied N with chemical fertilizers; (b) the N applied with CCs and compost (in the respective treatments); and (c) the N applied with main crop residues of the previous year.

Analyses of variance (ANOVA) with linear model were performed (using the "agricolae" package of R Studio 3.3.3) to determine for every year the effect of CL management (T1–T4) on grain yield and N uptake of the main crops, as well as on biomass return (plus C and N inputs, and C:N ratio of biomass) both with main crops and CCs. Similarly, data on the average annual (and total) C and N input to the soil, as well as on SOC sequestration and STN accumulation rates separately during EP and DP, were analysed using linear ANOVA. Data on the evolution of SOC and STN stock as affected by experimental treatments over time were subjected to variance analysis (ANOVA) with a mixed-effect model using the "nlme" package (Pinheiro et al. [2015](#page-17-0)) of Rstudio 3.3.3. ''Years'' were included in the model as a fixed factor, while block effect was considered as random. Repeated measures were used to test tillage effects over time. When normality of variances was not confirmed using the Shapiro–Wilk test, the data were log-transformed before analysis. Mean values were separated with the Tukey HSD (honestly significant difference) test $(\alpha = 0.05)$ using the "Estimated Marginal Means, aka Least-Squares Means'' package, version 1.2.4.

Results

Grain yield, N uptake and use efficiency in cropland treatments

The CL management significantly affected maize yield in 2010 and 2013 (Table [1](#page-6-0)). In both years, T3 and T4 increased maize yield compared to T1 and T2. Crop yield was not statistically affected by CL treatments for both sunflower and soybean (Table [1\)](#page-6-0).

N uptake in the grain followed the same pattern as the grain yield (Table [1\)](#page-6-0): generally, the highest amount of N uptake in grain was observed in treatments with the highest grain production. Specifically, the N uptake in the grain was significantly affected by fertilization treatment only in the case of maize (2010 and 2013) and the highest values were recorded in T3 and T4.

Overall, NUE was significantly affected by CL management (with the exception of sunflower in 2014) (Table [1](#page-6-0)). The highest NUE values were generally obtained in T1, while the lowest in T3.

Biomass return, carbon and nitrogen inputs to the soil

The amount of biomass incorporated into the soil with main crop residues (biomass return) ranged from 4.0 to 9.3 Mg ha^{-1} year^{-1} over the 6-year period, depending on year and main crop (Table [2](#page-7-0)). On average, the highest biomass return occurred with maize in 2010, while the lowest was with soybean in 2015. Fertilization treatment significantly affected the biomass return only in 2013 when maize had a significantly higher biomass return in T3 than in T2, while T1 and T4 did not statistically differ from the highest (T3) and the lowest (T2).

The amount of C input to the soil with main crop residues $(Mg C ha^{-1})$ ranged from 1.4 to 4.6 Mg C ha^{-1} year⁻¹, depending on crop and fertilization treatment (Table [2](#page-7-0)). The highest C-input occurred with maize in 2010 and 2013, while the lowest was observed with soybean in 2012 and 2015. Fertilization treatment statistically affected C input due to maize residues in 2010 and 2013 (T4 was the highest in 2010 together with T1, and in 2013 with T3) and to soybean residues in 2015 (when T3 led to the highest C input).

N input derived from incorporation of main crop residues ranged from 29 to 107 kg N ha⁻¹ year⁻¹ (Table [2](#page-7-0)). The highest values were found with sunflower in 2011; the lowest with soybean in 2015. Each year, the fertilization treatment had a significant effect on N inputs due to main crop residues. In particular, the highest values were recorded under T1 and T3 in 2010 and 2011, under T3 in 2012 and 2015, and under T3 and T4 in 2013 and 2014.

The C:N ratio of main crop residues presented a high variation depending on the crop cultivated: on average, the C:N ratios of crop residues were 66 (2010) and 51 (2013) with maize; 37 (2011) and 40 (2014) with sunflower; and 48 (both 2012 and 2015) with soybean (Table [2](#page-7-0)).

The biomass return due to CCs (during the EP only) fluctuated considerably from year to year, and was on average 40% higher in rye than in vetch (Table [2](#page-7-0)). Related values of C input to soil also differed significantly among species and from year to year. On average, CC-derived C input was between 36 and 47% higher in rye than in vetch. CC-derived N input ranged from 47 to 191 kg N ha⁻¹ year⁻¹ during the experiment, and was almost twice as high with vetch than with rye. The C:N ratio of CC biomass was on average 30 for rye and 10 for vetch.

Soil organic C and total N stock during EP and DP

SOC and STN stock in the 0.0–0.3 m soil layer significantly increased with time under all CL treatments and GL management during EP (Figs. [1,](#page-9-0) [2](#page-9-0)). Nevertheless, significant differences among treatments during EP were observed from the third year of the experiment (2012) onwards only for SOC (Fig. [1](#page-9-0)), but not for STN (Fig. [2\)](#page-9-0). Specifically, T4 and T5 had a significantly higher SOC stock than both T2 and T3 in 2012, while T1 did not statistically differ from the other treatments. In 2013, SOC stock was the highest in T5, followed by T4, T2, T3, and, last, T1.

During DP, a general pattern of SOC depletion was observed in both CL managements with CCs (T2 and T3) (Fig. [1\)](#page-9-0), but not under T1, T4, and T5. This was not the case for STN stock, which significantly increased over time under all treatments also in DP (Fig. [2](#page-9-0)). Differences among treatments occurred in each DP year for both SOC and STN stock. Specifically, T5 had always the highest SOC stock values, followed in order by $T4 > T2 > T3 = T1$ in 2014 and

Table 1 Grain yield (Mg ha⁻¹), N uptake (in the grain for T1–T4, in the hay for T5; kg N ha⁻¹), and nitrogen use efficiency (NUE; kg biomass kg⁻¹ N) as affected by treatments in all years (2010–2015) and both peri

Period	Year	Main crop	Treatment	Grain yield $(Mg ha^{-1})$	N uptake in the grain $(kg N ha^{-1})$	NUE (kg biomass kg^{-1} N)
EP	2010	Maize	T1-Control	11.78ab	143.30ab	62.00a
			T2-Rye	11.11b	131.51b	46.88b
			T3-H. vetch	12.93a	165.35a	45.21b
			T4-Compost	13.58a	164.64a	46.19b
			Significance	**	**	***
			Average	12.35	151.20	
		Grassland	T5-Fescue	9.08	210.61	30.27
	2011	Sunflower	T1-Control	4.44	136.34	24.94a
			$T2 - Rye$	4.53	125.92	19.78ab
			T3-H. vetch	4.23	127.51	14.84b
			T4-Compost	4.60	134.51	16.97ab
			Significance	n.s.	n.s.	\ast
			Average	4.45	131.07	
		Grassland	T5-Fescue	12.31	280.63	41.03
	2012	Soybean	T1-Control	4.51	294.52	42.15a
			$T2 - Rye$	4.59	303.36	23.18bc
			T3-H. vetch	4.14	273.81	14.38c
			T4-Compost	4.55	289.00	26.61b
			Significance	n.s.	n.s.	***
			Average	4.45	290.17	
		Grassland	T5-Fescue	15.25	374.96	50.83
	2013	Maize	T1-Control	10.53bc	137.13b	45.98a
			$T2 - Rye$	9.50c	128.84b	32.99b
			T3-H. vetch	11.68ab	150.23ab	34.25b
			T4-Compost	13.05a	167.37a	35.66b
			Significance	\ast	*	$***$
			Average	11.19	145.89	
		Grassland	T5-Fescue	11.44	226.19	38.13
DP	2014	Sunflower	T1-Control	3.98	103.49	17.61
			$T2 - Rye$	4.21	109.56	19.05
			T3-H. vetch	4.29	118.67	17.51
			T4-Compost	4.09	115.24	16.56
			Significance	n.s.	n.s.	n.s.
			Average	4.14	111.74	
		Grassland	T5-Fescue	7.41	167.21	24.70
	2015	Soybean	T1-Control	2.79	181.86	53.65b
			T2-Rye	2.81	184.91	58.54a
			T3-H. vetch	3.25	214.41	54.17b
			T4-Compost	2.96	187.34	49.33c
			Significance	n.s.	n.s.	\ast
			Average	2.95		
		Grassland	T5-Fescue	5.14	192.13	17.13

*, **, ***Significance at 0.05, 0.01, and 0.001, respectively. Mean values are reported. Different letters indicate differences among the means

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Table 2 continued

*, **, ***Significance at 0.05, 0.01, and 0.001, respectively. Mean values are reported. Different letters indicate differences among the means *, **, ***Significance at 0.05, 0.01, and 0.001, respectively. Mean values are reported. Different letters indicate differences among the means

Fig. 1 Evolution of soil organic carbon (SOC) stock $(Mg C ha^{-1})$ as affected by the interaction between treatment and year during the experiment. Capital letters indicate

differences among years within the same treatment; lowercase letters indicate differences among treatments within the same year. Mean values \pm SE

Fig. 2 Evolution of soil total nitrogen (STN) stock $(Mg N ha^{-1})$ as affected by the interaction between treatment and year during the experiment. Capital letters indicate

2015. T5 had also the highest STN stock values in 2014 and 2015. However, this was only statistically higher than T1 and T2 in 2014, and then T1 in 2015.

Carbon inputs and SOC sequestration rate

During EP, the average annual C input under T1–T4 showed significant differences among the different CL treatments (Table [3](#page-10-0)). Specifically, annual C input was

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differences among years within the same treatment; lowercase letters indicate differences among treatments within the same year. Mean values ± SE

significantly higher in T4 than in T1, while T2 and T3 did not statistically differ from the highest (T4) and the lowest (T1). Conversely, no difference in annual C input was found among treatments during DP (Table [3](#page-10-0)). The total amount of C input during EP was 13.9 under T1 and 19.9 Mg C ha^{-1} on average under T2–T4. Corresponding values during DP were 1.9 Mg C ha⁻¹ and 2.2 Mg C ha⁻¹.

Table 3 Annual (and total) C input to the soil $(Mg ha^{-1})$ and SOC sequestration rate $(Mg ha^{-1} year^{-1})$ as affected by cropland management with (T2–T4) and without organic fertilization, as well as permanent fescue (T5), in both periods (EP, DP) of the experiment

*, **, ***Significance at 0.05, 0.01, and 0.001, respectively. Mean values are reported. Different letters indicate differences among the means

SOC sequestration rates during EP followed the same pattern of C input in CL treatments, with a strong positive linear correlation $(R^2 = 0.941$; data not shown) as follows: T4 $(+2.5 \text{ Mg C ha}^{-1} \text{ year}^{-1})$ had double the SOC sequestration rate than T1 $(+1.3 \text{ Mg C ha}^{-1} \text{ year}^{-1})$, while T2 and T3 again were not statistically different from T4 and T1 (Table 3). Of all CL treatments, T5 showed the highest SOC sequestration rate $(+ 2.9 \text{ Mg C ha}^{-1} \text{ year}^{-1}).$

During DP, negative values of SOC sequestration were recorded under all CL treatments (Table 3). Specifically, this corresponded to a loss of 2.6, 2.3, 1.4, and 0.9 Mg C ha⁻¹ year⁻¹ for T3, T2, T4, and T1, respectively (Table 3). Conversely, T5 still had positive SOC sequestration $(+ 0.9 \text{ Mg C ha}^{-1} \text{ year}^{-1})$, although at a lower rate than during DP.

Nitrogen inputs and STN accumulation rate

As for C input, annual N input was significantly affected by CL treatment during EP, but not during DP (Table [4](#page-11-0)). In detail, annual N input was significantly higher in T3 and T4 than in T1, while not statistically different in T2 compared with the others. Regarding GL management, T5 did not differ from the average T2–T4 annual N input during EP, and had a higher annual N input than T2–T4 and T1 during DP. The total amount of N input during EP was 0.9 Mg N ha^{-1} under T1; 1.2 Mg N ha⁻¹ on average under T2-T4; and 1.1 Mg N ha^{-1} under T5. Corresponding values of total N input during DP were $0.2 \text{ Mg N} \text{ ha}^{-1}$; 0.3 Mg N ha⁻¹; and 0.6 Mg N ha⁻¹.

STN accumulation was significantly affected by treatment during EP and DP (Table [4\)](#page-11-0). During both periods, STN accumulation was significantly higher in T2 and T3 than in T1, while T4 showed intermediate values compared with the former and the latter. STN

Treatment	Enriching period					Depleting period			
	$(Mg \text{ ha}^{-1})$	Annual (and 4-year) N input	STN accumulation $(Mg \text{ ha}^{-1} \text{ year}^{-1})$		$(Mg ha^{-1})$	Annual (and 2-year) N input	STN accumulation $(Mg \text{ ha}^{-1} \text{ year}^{-1})$		
0.22(0.86)b $T1-$ Control			0.09 _b		0.13(0.26)		0.22 _b		
$T2 - Rye$ 0.28 (1.10) ab		0.14a				0.13(0.26)	0.37a		
Т3—Н. vetch	0.34(1.38)a	0.15a			0.14(0.28)		0.36a		
$T4-$ Compost	0.31(1.24)a		0.12ab		0.13(0.27)		0.31ab		
Significance	$\ast\ast$		\ast		n.s.		\ast		
Average	0.29(1.14)		0.13		0.13(0.27)		0.32		
Management		Enriching period				Depleting period			
		Annual (and 4-year) N input (Mg ha^{-1})		STN accumulation $(Mg \text{ ha}^{-1} \text{ year}^{-1})$		Annual (and 2-year) N input (Mg ha^{-1})	STN accumulation $(Mg \text{ ha}^{-1} \text{ year}^{-1})$		
T1 - Mineral fertilizer		0.22(0.86)b		0.09c		0.13(0.26)b	0.22 _b		
$T2 \div T4$ - Mineral plus organic fertilizer		0.31(1.24)a		0.14 _b		0.13(0.27)b	0.34a		
T5-Fescue		0.28(1.10)a		0.17a		0.30(0.60)a	0.29ab		
Significance		*		*		\ast	\ast		
Average		0.26(1.12)		0.13		0.18(0.35)	0.28		

Table 4 Annual (and total) N input to the soil $(Mg ha^{-1})$ and STN accumulation rate $(Mg ha^{-1} year^{-1})$ as affected by cropland management with (T2–T4) and without organic

fertilization, as well as permanent fescue (T5), in both periods (EP, DP) of the experiment

*, **, ***Significance at 0.05, 0.01, and 0.001, respectively. Mean values are reported. Different letters indicate differences among the means

accumulation rate under T5 $(+ 0.2 \text{ Mg ha}^{-1} \text{ year}^{-1})$ was higher than average under T2–T4 $(+0.2 \text{ Mg ha}^{-1} \text{ year}^{-1})$ during EP, but not different from those under T2–T4 and T1 during DP. On average, the STN accumulation (0.1 Mg ha^{-1} year⁻¹) in EP was approximately two times lower than in DP $(0.3 \text{ Mg ha}^{-1} \text{ year}^{-1})$ (Table 4).

Discussion

Grain yield, N uptake and use efficiency as affected by fertilization strategies

Our results suggest that organic fertilization had a beneficial effect on grain yield only for maize (2010 and 2013). This indicates that the major contribution was related to the N supply and reduces the role of these strategies in increasing the yield of other crops.

The highest maize yield was obtained with compost $(13.3 \text{ Mg ha}^{-1} \text{ in } 2010 \text{ and } 13.1 \text{ Mg ha}^{-1} \text{ in } 2013).$ Applying compost before planting maize increases and modulates nutrient availability, thus better matching plant N requirements over time, which leads to vigorous growth and enhanced yield and NUE (Agegnehu et al. [2016](#page-16-0)). In addition, combining organic and mineral fertilizers might increase yield in irrigated conditions (Martinez et al. [2017\)](#page-17-0).

Consistently, in the present study maize yield also increased with green manure from vetch CC. For instance, the CL treatment with vetch CC had a 14% and a 19% higher maize yield than with rye CC, in 2010 and 2013, respectively. This is in substantial agreement with a previous meta-analysis from 37 experiments (Miguez and Bollero [2005\)](#page-17-0) showing that green manure from legume CCs rather than grass CCs may increase maize yield by 21–24%. This could be explained by the fact that the incorporation of N-rich biomass leads to a gradual release of N into the soil, thus increasing plant N uptake over time (Mugwe et al. [2009\)](#page-17-0).

The beneficial effect on grain yield and N uptake in maize resulting from the application of compost or the incorporation of legume CC residues in combination with mineral fertilizer had been previously reported (Abbasi et al. [2010](#page-16-0)). Accordingly, the application of compost (T4) and/or the incorporation of vetch residues (T3) combined with mineral fertilizer in our study resulted in higher N uptake in both years than that derived by incorporating rye residues (T2) and mineral fertilizer only (T1).

In this study, the grain yield of maize was the lowest under rye treatment (T2) in both years (2010 and 2013). Possible explanations for low maize yield after grass CCs are as follows: first, the mineral N pool is partially exhausted by the grass CC and the soil N supply often mismatches plant N uptake, which in turn hampers maize growth (Martinez et al. [2017](#page-17-0)); second, the high C:N ratio of grass CC residues (C:N ratio $> 20-30$) stimulates N immobilization by soil microorganisms, thus further decreasing available N for maize (Mugwe et al. [2009](#page-17-0)).

In this study, nitrogen use efficiency was also affected by the different treatments applied. The highest values were recorded in T1 during EP, while this pattern disappeared during DP. Lower NUE under T2–T4 than under T1 during DP was due to the fact that large amounts of N in T2–T4 $(19–61%)$ were applied as fresh organic matter, which implies a lower availability for plants of those N pools in the very short period. Nevertheless, such a ''lack of efficiency'' in terms of plant nutrition in the short term is balanced by the role of residue-derived N inputs in supporting soil C and N accumulation. In addition, previous studies reported that combining chemical and organic N inputs is crucial to boost simultaneously both yield and ecosystem services (Overman and Scholtz [1999](#page-17-0); Maris et al. [2018](#page-17-0); Boselli et al. [2020](#page-16-0)). Lastly, higher NUE values under CCs in the DP (even if not always significant) suggest that the residual effects of previous green manuring play a significant role to matched N demands of crops, and that long-term fertilization strategies should be entertained in order to maximize efficiency of N use.

Enhancement of SOC stock during the ''enriching'' period

Switching from silage to grain maize production as laid out at the beginning of the experiment increased the amount of organic matter periodically left onto the soil surface, which in turn increased the SOC stock under all CL treatments over time. This was also the case for mineral fertilizer treatment (T1) in which the SOC stock increased from 36.0 to 41.1 Mg ha^{-1} during EP. Banger et al. ([2009\)](#page-16-0) reported similar results for grain production in intensive maize cropping systems, as a consequence of additional C input of main crop residues (Kundu et al. [2007](#page-17-0)). Chemical fertilizers may also indirectly enhance SOC stock (Banger et al. [2009\)](#page-16-0) by increasing aboveground biomass of plants and biomass return to the soil (Mazzoncini et al. [2011\)](#page-17-0).

However, a larger SOC stock is generally known to occur with, rather than without, organic fertilizers (Rochette and Gregorich [1998](#page-17-0); Varela et al. [2014](#page-18-0)). Also, the higher the fresh organic matter input, the higher the potential for SOC increase (Kong et al. [2005\)](#page-17-0). A strong positive linear correlation $(R² = 0.941)$ between annual C input to the soil and SOC sequestration during EP in our study corroborates earlier results in both short- and long-term experiments (Rasmussen and Parton [1994](#page-17-0); Tabaglio et al. [2009\)](#page-18-0) and confirms the major importance of fresh organic matter as a driver affecting changes in SOC stock (Kong et al. [2005\)](#page-17-0). The linear pattern of this correlation produced under our experiment's conditions, also suggests a linear phase of C accrual and further SOC sequestration potential (Rochette and Gregorich [1998](#page-17-0)). In our study, the efficiency of CL treatments to sequester C (which varied from 36 to 47% of the amount of C applied yearly) was indeed similar to what reported in Kong et al. [\(2005](#page-17-0)), which found, for C non-saturated soils, values ranging from 30 to 52%.

Within CL treatments with mineral plus organic fertilization (T2–T4), the highest SOC sequestration value was observed under T4, suggesting that compost was the most valuable source of stable SOC. This was probably led by two driving factors: (a) the highest annual C input (in 3 out of the 4 years of EP) and (b) the enhancing effect of compost-derived fresh organic matter on the stabilization of soil aggregates and physical protection of SOC (Das et al. [2017](#page-16-0)).

Higher values of SOC stock under compost than under winter CCs were indeed reported to be the consequence of a higher humidification coefficient of compost (Drinkwater et al. [1998](#page-16-0)). In addition, Das et al. [\(2017](#page-16-0)) found that compost has large quantities of soluble C with a slower turnover than that of crop residue, further enhancing SOC accumulation.

In our experiment, within CC treatments, higher values of SOC sequestration with green manure from rye CC (T2) rather than from hairy vetch CC (T3) may be attributed both to the higher C input from gramineous species (on average, rye supplied twice as much C as vetch) and to the lower quality of their residues (higher levels of cellulose, hemicellulose, C:N and lignin/N rates) which reduced the microbial activity driving mineralization of CC residues (Bronick and Lal [2005\)](#page-16-0).

Apart from the amount and properties of organic matter input into the soil by ploughing, the results of our study suggest that SOC stock was also determined by the rate of organic matter mineralization. Although no direct input of fresh organic matter occurred with fescue management, SOC stock increased during EP and was the most effective treatment for SOC sequestration at the end of this period. This was in agreement with earlier results by Ghosh et al. ([2016\)](#page-17-0) and Vijai et al. ([2018\)](#page-18-0) which reported higher SOC stock with GL management than with cropland management. Tillage of soil was indeed reported to increase SOC turnover by breaking soil aggregates and reducing the residence time of C pools into the soil (Deng et al. [2016\)](#page-16-0). Conversely, the lack of soil disturbance under GL probably reduced SOC mineralization and promoted C inclusion within stable aggregates. In addition, the degradation of the plant litter which is formed under grassland was reported to be a major source of stable SOC (Vijai et al. [2018](#page-18-0)).

Such a major potential of GL to increase SOC stock under our experiment's conditions, compared with CL control, was in agreement with Franzluebbers and Stuedemann ([2009\)](#page-17-0) who reported increased SOC sequestration rates ranging from 0.6 to 3.0 Mg C ha⁻¹ year⁻¹. Our results also indicate that selecting GL systems resulted in an extra SOC sequestration of 0.9 Mg C ha⁻¹ year⁻¹ as compared with the average value of improved CL management. This is in agreement with what estimated by Freibauer et al. [\(2004](#page-17-0)) and indicates that GL management remains the reference management system to increase SOC sequestration under our climatic conditions.

It must also be said that coarse-textured soils with low organic matter (such as the soil in this field study) are often not carbon-saturated and have great potential for C uptake immediately after an increased input of organic materials (e.g. organic fertilizer, residues; Virto et al. [2012](#page-18-0)). For this reason, in our study, the sandy loam soil texture and the quite low initial SOC might have generally promoted this rapid upward response of SOC to land management. Results similar to those of our study have been found by Six et al. [\(2000](#page-18-0)) who observed that soils with a low initial SOC level and sand silt texture had a greater potential for carbon sequestration and total N increase.

Evolution of SOC stock during the ''depleting'' period

When only main crop residues were incorporated and both winter CCs and compost ceased to be applied, SOC stock started to decrease under all improved CL treatments (T2–T4). Losses in SOC stock under CCs (T2–T3) tended to be even higher than the annual C input. On the other hand, the SOC lost under compost (T4) accounted for about 70% of the annual C input. Since the rate of C input was similar under all treatments (T2–T4) during DP, variables other than C input-related ones could have played a major role in SOC dynamics during this phase of our experiment.

Overall, ceasing to incorporate exogenous organic C (CC residues and compost) increased the SOC decomposition rate, as a result of reduced C input (Kong et al. [2005](#page-17-0)), intense tillage operations (Coppens et al. [2006\)](#page-16-0), and aggregates destabilization (Six et al. [1998\)](#page-18-0). Moreover, those C inputs derived from main crop residues probably further promoted microbial activity, which accelerates the mineralization of unstable SOC fractions and C losses due to the priming effect (Majumder and Kuzyakov [2010\)](#page-17-0).

A higher loss of SOC stock in T2 and T3 than in T4 during DP was probably due to a higher decomposition rate into the soil of CC-derived plant material in comparison with that derived from mature compost (Fronning et al. [2008\)](#page-17-0). As previously reported, compost-derived organic compounds are known to stabilize SOC within soil aggregates (Rasse et al. [2005](#page-17-0)), thus better maintaining the SOC stock over time even in intensively cultivated cropping systems.

Within CC treatments, the treatment with hairy vetch as CC during EP (T3) increased SOC losses during DP compared with the treatment with rye CC (T2). This was probably due to the fact that the SOC increase under T3 was driven by an enhancing effect on C pools that were less stable than those enhanced under T2, mainly due to different properties of the biomass incorporated into the soil (as supported by the different C:N ratios of hairy vetch and rye residues in our study). As observed by Bayer et al. [\(2009](#page-16-0)), residues of gramineous CCs indeed enhance the accumulation of more recalcitrant materials than do leguminous CCs, increasing stable SOC pools and leading to a more stable SOC sequestration. As a consequence, intensive tillage operations have a greater depleting effect on leguminous-derived than on gramineous-derived accumulated SOC.

Enhancement of STN stock during the ''enriching'' period

In the present study, CL treatments did not affect STN stock patterns differently and all CL treatments increased STN stock during EP. Total soil N stock began to consistently increase under all treatments in 2012, 3 years after the treatments were first applied. This corroborated the following earlier findings: (a) directly correlated patterns of SOC and STN stocks when fresh organic matter is incorporated into the soil every year (Plaza-Bonilla et al. [2016](#page-17-0)) and (b) a delayed response of STN compared with SOC (Higashi et al. [2014](#page-17-0)).

Our findings showed that the increase of STN stock under all CL treatments resulted into an STN accumulation rate ranging from 0.1 to 0.2 Mg N ha⁻¹ year⁻¹ during EP. The highest rate of STN accumulation was observed under both winter CC treatments (T2, T3) compared with CL control (T1) and compost treatment (T4). The importance of introducing winter CCs for maintaining or increasing STN in Italy was also previously suggested by Mazzoncini et al. [\(2011](#page-17-0)), although under different climatic conditions. Higher STN accumulation with CCs as found under our conditions, could be explained by two factors: (a) the continuous incorporation of above- and below-ground biomass from CCs (from 2010 to 2013) is an important source of organic N; and (b) CCs had a short time for post-emergence growth before the winter stasis, which is known to promote high ratios of root to shoot production (McDaniel et al. [2014\)](#page-17-0) and increase soil N accumulation (Sainju et al. [2003;](#page-17-0) McDaniel et al. [2014\)](#page-17-0).

Beside CL management treatments, the permanent fescue (T5) method had a higher STN accumulation rate than the average of improved CL treatments (T2– T4) and of CL control (T1) during EP. Since the same was observed for SOC, this highlights the importance of reducing soil disturbance so to allow the enhancing of STN accumulation. Tillage indeed increased the rates of microbial decomposition and N transformation, resulting in a decreased STN accumulation (Yan et al. [2012\)](#page-18-0). Conversely, undisturbed soil conditions under GL reduced SOM mineralization, thereby increasing STN stock and STN accumulation (Fiorini et al. [2020a\)](#page-16-0). Similar results showing a lower STN stock and STN accumulation in cultivated soil than in GL have also been reported by others (Ghosh et al. [2016;](#page-17-0) Vijai et al. [2018\)](#page-18-0).

Evolution of STN stock during the ''depleting'' period

Although fertilization strategies under CL treatments were stopped during DP and lower values of annual N inputs were recorded, STN stock continued to increase gradually under all CL treatments, and STN accumulation in DP $(0.3 \text{ Mg N ha}^{-1} \text{ year}^{-1})$ was almost twice that in EP (0.1 Mg N ha⁻¹ year⁻¹ on average). This was probably caused by (a) the delay in STN stock response to exogenous N input, which was corroborated by the fact that STN accumulation in the last 2 years of EP $(0.28 \text{ Mg N} \text{ ha}^{-1} \text{ year}^{-1})$ was similar to that during DP; and (b) the mineralization processes of unstable soil organic matter in DP, which probably contributed to make N sources available to soil microorganisms (Dick [1984\)](#page-16-0) and provided the basis for N immobilization and STN accumulation during DP (Sainju et al. [2003\)](#page-17-0).

In addition, crop residues with high C:N incorporated in the soil in 2013 (maize residue with $C:N = 51$ on average) and in 2014 (sunflower residue with $C:N = 40$ on average) probably promoted N immobilization (Maris et al. [2018\)](#page-17-0), further increasing STN accumulation. Last but not least, soybean, which was cultivated in 2015, has a high nitrogen-fixation potential due to root nodules, which convert atmospheric N_2 in NH_4^+ by nitrogenase from nitrogenfixing bacteria. This additional NH_4^+ was probably

supplied to the soil environment and contributed to increase STN accumulation.

These results show the importance of studying STN stock changes resulting from different agricultural management practices and the effects of land use in medium-term experiments. Clearly, more studies may be needed to understand how medium-term or longterm cropping affects STN stock under different fertilization practices and land use systems.

Recommendations for intensive agro-ecosystems in temperate regions

Replenishment of organic matter derived from plant, animal, and microbial biomass in all stages of decomposition is critical for ensuring long-term soil fertility. Often, crop residues are not returned to the soil due to competing utilization on farms, and this is a major constraint for developing circular and sustainable agro-ecosystems (Doan et al. [2015](#page-16-0)).

In the present study, the main crop biomass return $(s$ tems $+$ leaves) represented an important source of C and N inputs to the soil, which had a significant influence on SOC and STN. In addition, CC residues were incorporated into the soil to further enhance SOC and STN, thus supporting soil fertility and crop yield. However, the cover crop biomass and quality highly depend on years and CC species. Higher biomass production, with higher C:N ratio values, and significantly higher C input suggest a higher potential for SOC sequestration under rye than under vetch in the long term. Conversely, the incorporation of vetch residue with a C:N ratio (10) lower than that of rye (C:N ratio ranging from 23 to 39) stimulates N mineralization and additional N supply for the subsequent main crop, thus it represents a good option for fertilizing high-N demand crops.

Furthermore, it seems particularly important to synchronize the decomposition rate of CC residues with the N uptake of main crops (Ruis and Blanco-Canqui [2017](#page-17-0)). If a legume CC is used, N fertilizers should be applied to the next main crop taking into account the expected rate and timing of available N deriving from the mineralization of their N-rich CC residues. On the other hand, when rye is used as a CC, which is known to be a good N scavenger, reducing the risk of $NO₃$ -leaching (Fiorini et al. $2020b$), care should be taken to avoid a possible pre-emptive competition with the subsequent main crop that might suffer from a temporary exhaustion of soil-available N, especially in the very early stages of growth. However, vetch can increase the risk of $NO₃$ -leaching unless appropriate timing for CC termination is applied. Last but not least, one ought to take into account additional operating costs (depending on cover crop type and agricultural scenario) in order to arrive at optimal management.

Therefore, understanding the effects of CCs on N cycling is a major issue in developing appropriate N management strategies in intensive agro-ecosystems. The management of cover crops should be improved, and one should take into account the consequences of incorporating CC residues (e.g. N mineralization vs. N immobilization) for enhancing C and N cycling towards circular and sustainable agro-ecosystems.

Conclusions

The results of this study highlight the benefits of green manuring with winter cover crops for improving soil fertility (i.e. SOC and STN), while only hairy vetch showed a relevant role for promoting yield production, and only in maize (grain yield and N uptake). However, when only main crop residue was incorporated and both cover crops and compost ceased to be applied, the SOC declined rapidly and there was a high rate of SOC loss in all cropland treatments.

Conversion to grassland had the highest positive impact on SOC and STN stock and remains the reference management system for increasing SOC and STN accumulation in our climatic conditions. This might be due to lack of soil disturbance, which is known to reduce mineralization and decomposition of soil organic matter.

This study underlines the importance of identifying which cropping strategy enhances or depletes SOC and STN, in order to calibrate environmentally and economically sustainable farming systems. To achieve this goal, grassland management and compost application are best practices. If these are not feasible options at the farm level, cover crops may play a major role in maintaining soil fertility and crop productivity by recycling N and increasing the amount of C input. Since this effect is short-lived, green manuring with cover crop residue should be considered every 1–2 years. Consequently, in intensive irrigated summer-crop rotations or monocultures, the main crop should be paired with the concomitant inclusion of winter cover crops.

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Compliance with ethical standards

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

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