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Title

Residue incorporation and organic fertilisation improve carbon and nitrogen turnover and stabilisation in maize monocropping

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Abstract

Residue incorporation and organic fertilisation are recommended to increase soil organic matter (SOM) content, thus promoting the provision of multiple ecosystem services. However, the positive effect of crop residue on SOM is often considered rather low, thus requiring a deeper knowledge of their management. In addition, organic fertilisation is thought to be less efficient than mineral. In this context, it is important to understand the response of SOM pools to long-term crop residue incorporation and organic fertilisation and their effect on nutrient cycling and on feed production, in order to judge the sustainability of these practices. We carried out an in-depth multidisciplinary survey to investigate the effect of 28 years of residue incorporation combined with three different nitrogen (N) fertilisation strategies (manure, slurry and mineral) on density fractionated SOM pools differing for their turnover time, and on soil enzymatic activity in a monocropping maize system in NW Italy. Results showed that in the long-term crop production was not altered by organic fertilisation. Organic fertilisation in combination with residue incorporation led to the highest total organic C and total N content down to 90 cm, which was reflected in each SOM pool. In addition, regular applications of crop residue and manures markedly increased C- and N-degrading enzymes, thus enhancing the turnover rates of C and N. We conclude that combining crop residue with organic fertilisation enhanced the provision of regulating services and soil activity, and is a valid approach for maintaining sustainable production in agroecosystems.

Keywords

Ecosystem services, SOM fractionation, enzymatic activity, organic fertilisation, C sequestration.

1. Introduction

Agroecosystems constitute a source of ecosystem services (ES), while at the same time they highly depend on them to function (Power, 2010). Ecosystem services include a variety of functions generally classified into four categories of supporting, provisioning, regulating and cultural (Millennium Ecosystem Assessment (MA, 2005)), while the CICES framework (Haines-Young and Potschin, 2010) distinguishes only provisioning, regulating and cultural categories to avoid "double counting" between regulating and supporting services. The delivery of ESs by agroecosystems becomes more and more important as the global demand for crops is increasing. Global food production is largely dependent on intensive agricultural management (Tilman et al., 2002) but intensive management is not the only solution, as agriculture, while providing food and feed, has to adapt and contribute to mitigate climate change, but also face a rising public awareness of the environmental and socio-cultural impacts of land-use change towards intensification (Chen et al., 2020). In fact, the supply of ESs including food provision are essential to both human existence and life quality, but intensive agricultural practices also have inadvertent, detrimental effects on the environment and soil health, thus drawing attention to the need of more sustainable agricultural strategies (Tilman et al., 2002; Arriagada and Perrings, 2011).

Despite the negative effects of intensive agriculture are well known, monocropping systems are widespread all over the world (Plourde et al., 2013). The Po Plain, in Northern Italy, is a good example of an intensively managed area for both animal husbandry and cropping, threatened by a progressive decrease in soil and water quality (Sacco et al., 2003). Maize, which is the main crop in the area (Zavattaro et al., 2012), is often accused of menacing environmental quality to a greater extent than other crops, also because its strong response to fertilisers and irrigation encourages a wide use of these inputs (Kramer et al., 2002). In particular, mineral nitrogen (N) supply, which has represented a major contributor to the crop yield increase since the 1950 s all over the world (Robertson and Vitousek, 2009; Francioli et al., 2016), has shown negative effects on other ESs, by reducing soil biodiversity, ecosystem functions related to carbon (C) and N cycling (De Vries et al., 2013; Wagg et al., 2014), as well as fresh and groundwater quality (Zavattaro et al., 2012). To prevent such adverse processes and to improve soil health, organic fertilisers and crop residue incorporation are considered useful management options (Abiven et al., 2009).

Organic fertilisation is known to improve soil structure and fertility by augmenting nutrient status and soil organic matter (SOM) content, and stimulate soil life and activity (Liang et al., 2012). It is the key practice to close the nutrient cycles at a farm and sub-regional level, while reducing the recourse to fossil-fuel consuming mineral fertilisers (Alluvione et al., 2011; Hou et al., 2018). Crop

residue represent a source of organic C, which can favour SOM accumulation in soil (Kumar and Goh, 1999). This depends undoubtedly on the protection processes that this residue undergo when it is incorporated, including physical, chemical and biochemical stabilisation (von Lützow et al., 2007). As the production of cereal grain has increased globally, Jiang et al. (2014) estimated that worldwide more than five billion tons of crop residue is annually produced in croplands. Furthermore, combining organic fertilisation with crop residue incorporation is an effective approach for maintaining a sustainable production capacity (Zhang et al., 2020), recycle and retain nutrients from the farm (Singh and Rengel, 2007) and have a significant influence on the soil microbial and enzymatic activities (Zhang et al., 2020).

Recent frameworks suggest that dividing SOM into free and unprotected (*f*POM), physically protected (*o*POM) and mineral-associated organic matter (MAOM) pools can well describe SOM accrual, persistence and response to microbial decomposition (Cotrufo et al., 2015). Free POM (*f*POM) is more readily available to microorganisms and may experience a fast turnover, thus returning nutrients for next crops. However, it can persists in soil because of the presence of recalcitrant compounds, and/or microbial inhibition. Free POM can be stabilized through inherent chemical recalcitrance, inclusion in aggregates and protection by spatial inaccessibility as oPOM (Lavallee et al., 2020). Conversely, MAOM persists in soil much longer because of chemical bonding to minerals and protection into microaggregates (Witzgall et al., 2021). The POM vs MAOM framework has been recently proposed to support recommendations on SOM management to practitioners and policy makers (Lavallee et al., 2020) and help elucidate the drivers of SOM storage mechanisms in C-addressing farming practices.

In a cereal-based cropping system, residue incorporation, N fertilisation and their interaction can play a key role in co-targeting OM protection processes, thus meeting both C sequestration and soil health goals. On the other hand, organic fertilisers and plant residues stimulate soil microorganisms and enzymatic activity (EA), thus accelerating SOM turnover (Kumar and Goh, 1999; Nannipieri et al., 2012). Previous research demonstrated an increase in both microbial abundance and EA when organic compounds such as crop residue were incorporated (Zhao et al., 2016). However, most studies reported the effects of fertilisation and residue incorporation on the soil ecosystem in the short-term, while these are expected to notably differ from those in the long-term (Guan et al., 2020). Long-term repeated fertilisation and residue incorporation might have persistent impacts on SOM fractions, microbial abundance and EA (Poeplau et al., 2015; Zhang et al., 2020; Mooshammer et al., 2022), and this emphasizes the importance of long-term experiments in agricultural sciences. Furthermore, the importance of these practices on SOM has mostly been considered for topsoil, while data for subsoil are insufficient (Shahbaz et al., 2017). Compared with topsoil, no clear information is

available on the long-term management effects on subsoil SOM pools that are characterised by different processes regarding OM input and transformations (Rumpel and Kögel-Knabner, 2011). The present study tried to close both these knowledge gaps.

The underlying hypothesis of this work was that long-term incorporation of crop residue in arable cropping systems fertilised with manures would favour an accumulation of SOM both in fast degraded and persistent pools, concurrently stimulating microbial activity and SOM accrual. To address this hypothesis, a 28 years-old long-term experiment hosting two continuous maize production systems (silage and grain maize, with different amounts of crop residue returned to the soil) with four contrasting N fertilisation strategies (mineral, bovine slurry and farmyard manure, plus a 0 N control) was used. This, in order to operationally link and understand how POM and MAOM fractions contribute to three important management goals of agroecosystems, i.e. nutrient cycling, C sequestration (both classified as regulating ESs) and feed provision. With this aim, we carried out an in-depth multidisciplinary survey on the long-term effects of different fertilisation strategies and residue incorporation on the above-mentioned ecosystem services in maize monocropping systems.

2. Materials and methods

2.1. Experimental site

Measurements were conducted at the long-term experimental platform of Tetto Frati (44°53′N, 7°41′E; 232 m a.s.l.) of the University of Turin (NW Italy) that has been running since 1992. The climate in this region is temperate sub-continental, with an average annual rainfall of 766 mm and a mean annual temperature of +12°C from 1992 to 2019 (Figure 1). The soil is loamy-textured in the 0–40 cm Ap horizon (7.5% clay, 44.3% silt, 48.2% sand), with a mean pH 8.1, bulk density 1.42 g cm^{-3}, cation exchange capacity 9.1 $cmol_{(+)}\ kg^{-1}$, exchangeable potassium (K^+) 0.20 $cmol_{(+)}\ kg^{-1}$ and available phosphorus (P) 21.4 mg kg^{-1} (Grignani et al., 2007).

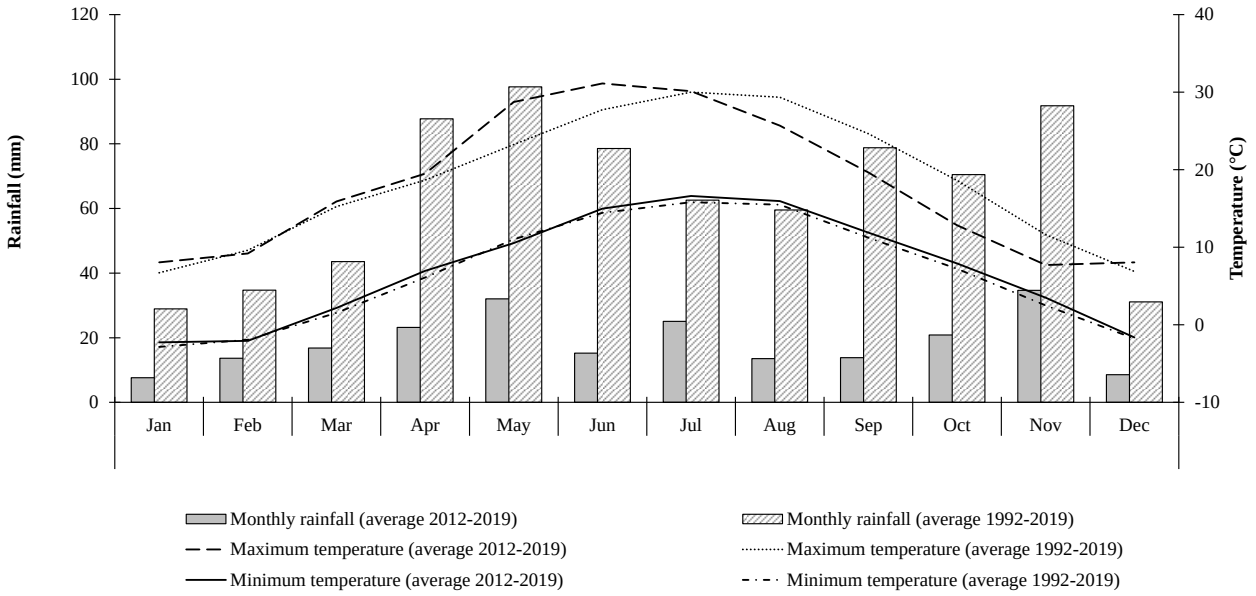

Figure 1 Average monthly temperature and monthly total rainfall during 2012–2019 and in the long-term (1992 - 2019). Data recorded by the weather station at Tetto Frati.

2.2. Experimental design and agronomic management

The experiment was laid out as three randomised blocks that compared, among others, two maize monocropping systems factorially combined with four contrasting N fertilisation regimes. The two crop systems, that differed only for the management of crop residue, were maize for silage (MS), where maize was fully harvested (no aboveground crop residues), and maize for grain (MG), where crop residue (stems, leaves, cobs and bracts) were incorporated into the soil. The four contrasting N fertilisation regimes were arranged as follows: 0N input (CTR); mineral N as urea at a dose of 250 kg N ha^{-1} (MIN); bovine slurry at a dose of 250 kg N ha^{-1} (SLU); farmyard manure at a dose of 250 kg N ha^{-1} (FYM). K and P were provided through mineral fertilisers (potassium chloride and triple superphosphate respectively) in mineral plots, in order to supply 300 kg K_2O ha^{-1} and 100 kg P_2O_5 ha^{-1} in MS and 180 kg K_2O e 50 kg P_2O_5 ha^{-1} in MG. Before 2012, the fertilisation regime was slightly different and generally higher. Additional information is reported in Grignani et al. (2007), Zavattaro et al. (2016) and Battisti et al (2022). In this work, we considered the period from 2012 to 2019 as reference for calculating mean aboveground biomass (AGB) production, as well as inputs of fertilisers and crop residues.

Organic fertilisers were sampled and analysed every year (Table 1). The dry matter content was measured after drying samples; C and total N contents were determined using a CN elemental analyser (FlashEA 1112, Thermoquest, Italy) according to MIPAF, 2000. Total P and K contents

were determined, after mineralisation at 450°C for 5 h, by spectroscopy under continuous-flow conditions and atomic absorption spectroscopy, respectively.

Table 1 Mean concentrations and ± standard errors of organic C, total N, K and P contents in bovine slurry (SLU) and farmyard manure (FYM) applied from 2012 to 2019. Values are expressed over the fresh weight.

Fertiliser	**C (kg Mg^{-1})**	**N (kg Mg^{-1})**	**C:N**	**K (kg Mg^{-1})**	**P (kg Mg^{-1})**
SLU	23.91 ± 2.14	2.38 ± 0.25	10.21 ± 0.55	1.90 ± 0.29	0.33 ± 0.05
FYM	64.28 ± 8.37	5.17 ± 0.27	12.41 ± 1.82	5.54 ± 1.35	1.41 ± 0.30

Maize was managed similarly in all plots. Soil was disk harrowed in autumn in order to incorporate the maize residues in the MG system. All fertilisers – farmyard manure, slurry and mineral - were distributed in spring (mid April) and quickly incorporated into the soil using a spading machine, few days before seeding. A mechanical maize seeder provided a crop density of about 8.3 plant m^{-2} of commercial maize hybrids belonging to the 500 or 600 FAO maturity class. Weed control was standardised among treatments spreading pre and post-emergence herbicides. Irrigation was adapted to weather conditions providing once or twice per year 40 mm with sprinkler method.

Every year at crop maturity AGB production (in the MS system), grain yield and crop residue (in the MG system) were measured on a 18 m^2 sampling area in each plot. The roots and stalk base residues, common to both systems, were not measured in this study. However, C, N, K and P contents were determined as described above, in order to calculate the amount of nutrients returned in MG system with the crop residues (Table 2).

Table 2 Mean amounts and ± standard error of C, N, K and P in maize residues that were incorporated in the soil every year from 2012 to 2019 in the MG system.

Fertilisation	**C kg ha^{-1}**	**N kg ha^{-1}**	**K kg ha^{-1}**	**P kg ha^{-1}**
CTR	3550.8 ± 114.4	36.9 ± 2.4	138.8 ± 6.2	24.3 ± 1.0
MIN	6540.7 ± 263.8	108.8 ± 7.9	230.8 ± 8.4	16.3 ± 1.2
SLU	6344.2 ± 161.9	95.8 ± 5.5	250.7 ± 6.0	27.8 ± 1.7
FYM	6622.0 ± 158.2	107.6 ± 7.0	260.5 ± 8.9	30.7 ± 1.7

2.3. Soil sampling and SOM, microbial and enzymatic analyses

Soil sampling was performed at the end of March 2020, before fertilisation. In each plot, three soil cores were extracted with an auger of 7 cm diameter and pooled, keeping six layers separated: 0-15 cm, 15-30 cm, 30-45 cm, 45-60 cm, 60-75 cm and 75-90 cm. Part of the samples were stored at -18ºC

for microbiological analysis. The remaining soil was air-dried, then gently milled and sieved at 2mm, for pH and density fractionation analysis. A small amount of dried soil samples was sieved to 1 mm for enzymatic analysis.

Soil pH was measured on a soil:water suspension (1:5 wt/vol). Total organic C and total N was determined in the dry soil samples by elemental analysis.

2.3.1. SOM fractionation

Soil organic matter density fractionation was performed as described by Cerli et al. (2012) and aimed at separating three organic fractions: a first light fraction separated without sonication, comprising organic material that is not strongly attached to minerals or occluded within aggregates (free particulate organic matter - *f*POM); a second light fraction consisting of material floating after sonication, hence organic matter released upon aggregates disruption *(*occluded particulate organic matter - *o*POM); and the remaining heavy (not floating) material, that contains organic matter strongly associated to minerals (mineral associated organic matter - MAOM). The procedure started with the addition of 125 ml of sodium polytungstate (NaPT) solution at a density of 1.6 g cm^{-3} to 25 g of soil into a pre-weighed 250-ml centrifuge bottle. The soil was well submerged in the solution to allow an easy separation of the floating debris. The suspension was gently mixed to ensure complete soil wetting, but avoiding the disruption of aggregates, then allowed to settle for approx. 30 minutes. Thereafter, the sedimentation of the heavier material was forced by centrifugation at 12,800 g for 20 min. Supernatant containing floating *f*POM was carefully decanted on a 0.7µm GF/F filter placed on a Buckner funnel with the vacuum pump on. The filtrate NaPT was collected from the vacuum flask, added into the centrifuge tube and used to re-suspend the remaining soil. The *f*POM residue was rinsed with deionised water until the electrical conductivity (EC) of the filtrate was <20 µS cm^{-1}, then flushed from the filter into pre-weighed plastic containers and dried in a ventilated oven at 40ºC.

After the remaining soil was re-suspended in the collected 125 ml NaPT solution, the sample was ultrasonically (Sonoplus HD 2200, Bandelin electronic GmbH & Co. KG, Berlin, Germany) treated applying an energy of 175 J mL^{-1}. The ultrasonic energy was previously determined to optimise the dispersion of the occluded debris without dispersing clay particles. The temperature of the samples during ultrasonication was kept under 40 °C using an ice bath, in order to avoid the thermal alteration of organic matter. The sample was allowed to stand for 1 h after ultrasonication and then centrifuged at 12,800 g for 20 min. The floating material, representing the *o*POM fraction, was then separated, rinsed with deionised water and dried as above described for the *f*POM fraction.

After the NaPT solution together with the *o*POM was removed, the remaining soil was washed by repeated addition of deionised water followed by centrifugation (30 min or more at 17,700 g to ensure complete sedimentation of the soil), until the conductivity of the solution was >50 μS cm^{-1}. The sediment, i.e. the MAOM fraction, was then transferred into dark 150-ml containers and dried as the *f*POM and *o*POM fractions. The bulk soil and the SOM fractions were homogenised using a mortar and a pestle and analysed by dry combustion with an elemental analyser to determine the C and N contents.

2.3.2. Microbiological analysis

Soil microbial biomass was determined using two different methods: as double-strand DNA content and by quantification of the abundance of soil Bacteria, Fungi and Archaea.

Double strand DNA (dsDNA) was used as proxy for the soil microbial biomass and was determined according to Fornasier et al. (2014). Briefly, 400 g of dry soil were transferred to sterile 2 mL Eppendorf tubes containing 0.4 mL of Ø 0.3 and mm 0.4 mL of Ø 0.6 mm glass beads. Then, after addition of 1 mL of 0.12 M sodium phosphate buffer at pH 8, Eppendorf tubes were subjected to bead-beating using a Retsch MM 400 beating mill set at 30 Hz for 2 min. Next, the tubes were centrifuged at 20,500 g for 5 min, and the supernatant containing the DNA (approx. 1 mL) was transferred into a clean sterile 2 mL micro-centrifuge tube. The crude and not purified DNA-extracts were immediately fluorometrically quantified for dsDNA, using PicoGreen (Life Technologies) reagent. Data were expressed as micrograms of dsDNA g^{-1} dry soil.

To determine the abundance of soil Bacteria, Fungi and Archaea, soil DNA was firstly extracted using the using the FastDNA™ SPIN Kit for Soil and the FastPrep® Instruments (MP Biomedicals) from a 500 mg of frozen soil sample, following the manufacturer's instructions. Quality, quantity, and integrity of the extracted DNA were estimated using a NanoDrop™ ND-1000 spectrophotometer (Thermo Fischer Scientific, Milan, Italy) and trough agarose gel electrophoresis. Bacteria, Fungi and Archaea abundances were assessed by quantitative real time PCR (qPCR) using a Chromo4™ Real Time PCR Detection System (Bio-Rad Laboratories). In the case of Bacteria and Archaea 16S rRNA genes were quantified using primer pairs 338F and 518R (Lane, 1991; Muyzer et al., 1993), and 340F and 1000R (Gantner et al., 2011) respectively. For Fungi the selected molecular marker was the 26S rRNA gene, amplified with primers NL1 and LS2 (O'Donnell, 1993). All the samples and standards were analysed in triplicate by applying the reaction conditions reported in Table S1. PCR specificity was verified by melting curve analysis. Standard curve R^2 value was always higher than 0.992, and the reaction efficiencies always higher than 80 %.

2.3.3. Enzymatic analysis

Sixteen enzymatic activities (EA) involved in key steps of C and N biogeochemical cycles were measured: α-glucosidase (alfaG), β-glucosidase (betaG), α-mannosidase (alfaMAN), β-mannosidase (betaMAN), α-galactosidase (alfaGAL), β-galactosidase (betaGAL), α-arabinase (alfaARAB), β-D-glucuronidase (uroni), β-1,4-xylanase (xylo) and β-1,4-glucanase (cell) involved in C cycle; N-acetyl-b-D-glucosaminidase (chit), leucine amino-peptidase (leu), trypsin-like protease (trip), serine protease (CBZ), and arginine aminopeptidase (arginine) involved in N cycle. Nonanoate esterase (nona) activity was measured as well, as being involved in the hydrolysis of esters. All enzymatic activities were measured in duplicate using a heteromolecular exchange procedure as described by Cowie et al., 2013, via bead-beating to disrupt microbial cells and soil aggregates on air-dried soil. As demonstrated by Mondini et al. (2004), air-dried soil can be used instead of fresh soil for enzymatic analyses. Briefly, 0.3 g of dry soil sieved at 1 mm were transferred to 2 mL microcentrifuge tubes together with 1.4 mL of 3% lysozyme containing solution, 0.4 mL of Ø 0.8 mm ceramic beads and 0.4 mL of Ø 0.1mm glass beads. Bead-beating was performed using a Retsch 400 beating mill (at 30 strokes s^{-1} for 3 min) further, samples were centrifuged at 20,000g for 5 min. The supernatant that contained the desorbed enzymes was dispensed into 384-well white microplates where the appropriate buffers had been added in order to determine the EA by fluorometry using 4- methyl-umbelliferyl (MUF) and 4-amido-7-methyl-coumarine (AMC) fluorogenic substrates. The readings were carried out with a Synergy HT microplate reader (Bio-Tek, Winooski, Vermont, United States). All measurements were expressed as nanomoles of MUF (or AMC) h^{-1} g^{-1} dry soil.

2.4. Statistical analysis

A generalised least squares (gls) model was used to investigate the difference between crop systems, fertilisation strategies, and their interaction (random effects) at a significance level of $P < 0.05$. This analysis was conducted for all variables and each soil layer, separately. Means were compared using Tukey's significant difference ($\alpha = 0.05$). Data transformations were performed to satisfy assumptions of normality and heteroskedasticity when needed. Analyses were performed using *nmle* (Pinheiro et al., 2015), *emmeans* (Lenth et al., 2020) and *multcomp* (Hothorn et al., 2015) R packages.

Distance-based redundancy analysis (dbRDA) based on Bray-Curtis distance was used to assess the overall differences in EA and soil microbial abundance. dbRDA was performed separately on each soil layer using vegan R package (Oksanen et al., 2013). The dbRDA statistical tool was chosen because it has nonlinear distance-metric options with robust multidimensional resolution to assess categorical variables (Legendre and Anderson, 1999). dbRDA was run on a four step basis: 1) Bray–Curtis dissimilarity (nonlinear) matrix was calculated on square root transformed data; 2) stepwise

multiple regression was performed to select the best model (AIC); 3) a principal coordinate analysis (PCoA) was calculated based on the distance matrix (999 permutations) to obtain dbRDA axis coordinates for fertilisation and crop systems to be plotted as multivariate centroids surrounded by 95 % confidence interval ellipsoids and coordinates of species (enzymes and microbial abundance) and environmental variables (soil variables) respectively as points and arrows; 4) one-way permutational Multivariate Analysis of Variance (PERMANOVA) based on Bray-Curtis matrix was conducted for 9999 permutations was used to test for fertilisation and crop systems effects on microbial abundance and EA. Planned contrasts of PERMANOVA, according to Bonferroni's Test ($P > 0.05$) were set as follows: fertilisation and crop system vs enzymes activities grouped by element cycle (C-, N-degrading and esterases).

3. Results

3.1. *Feed production*

The average grain and aboveground biomass production between 2012 and 2019 (Table 3) was influenced by the fertilisation type in both MS and MG crop systems ($P < 0.001$). As expected, the lowest production was found in the unfertilised CTR, 52% lower than the fertilised treatments. The three fertilisation techniques acted similarly in boosting the plant production in both MG and MS crop systems.

Table 3 Grain and aboveground biomass production and ± standard errors expressed in Mg DM ha^{-1} in the two crop systems (grain production in the MG system – maize for grain; AGB production in MS system – maize for silage) and fertilisations (FYM – farmyard manure, SLU – slurry, MIN – mineral fertilisation and CTR – 0N) between 2012 and 2019.

Fertilisation	**MG**				**MS**			
	Grain				**AGB**			
CTR	7.15	±	0.36	*b*	13.88	±	0.66	*b*
MIN	14.77	±	0.35	*a*	27.79	±	0.84	*a*
SLU	14.34	±	0.31	*a*	28.34	±	1.03	*a*
FYM	15.06	±	0.31	*a*	30.28	±	0.89	*a*
Fertilisation p(F)	*<.0001*				*<.0001*			

3.2. *Total organic Carbon (TOC) and Nitrogen (TN) along the soil profiles*

The greatest amount of soil TOC (Table 4) was found in the 0–15 cm layer, where most of residues were incorporated with autumn harrowing, and progressively decreased along the soil profile, with a

sharp discontinuity below 30 cm, corresponding to the maximum tillage depth. In particular, TOC in the 15–30, 30–45, 45–60, 60–75 and 75–90 cm layers was respectively 24 %, 46 %, 52 %, 56 %, and 59 % lower than that in the first layer (0–15 cm) in the MG system. The trend was similar in the MS system, where TOC at 75–90 cm was 54 % lower than that in the 0–15 cm layer. The decrease with depth was more marked in treatments that received more C, such as FYM, or even SLU, than in CRT or MIN. The crop system affected soil TOC at all depths except in the layer just below tillage (30–45 cm), and in the deepest layer. Higher values were recorded in MG, coherently with a larger supply of crop residue in the tilled layer. However, the difference held true also in the 60–75 cm layer, far below the incorporation depth of crop residue. Regardless the crop system, fertilisation also significantly affected TOC at all depths, except for the 30–45 cm layer, that showed inconsistent results for several parameters we analysed. This could be due to difficulties in sampling around the tillage depth when the soil surface was not perfectly even. TOC was significantly higher in FYM treatments and SLU compared to MIN and CTR in the tilled layer (0–30 cm), whereas in the deep layers (45–90 cm), the concentration of TOC in CTR was similar to that of the two manured treatments. No significant effects were revealed for fertilisation × crop system interaction across the soil profile.

Soil Total N (TN) concentration pattern was similar to TOC (Table 4), being equally affected by depth and management. MG was significantly higher than MS in the tilled 0–15 and 15–30 cm layers, and in the deepest 75–90 cm layer. A significant effect of fertilisation type was observed in all layers. FYM had the highest TN concentration and CTR the lowest one in the tilled layers (0–15 and 15–30 cm), while SLU and MIN usually had intermediate values.

The SOM pool composition was influenced by crop system, fertilisation, and depth as well, but not all SOM fractions were affected to the same extent (Table 5). Free POM showed no differences between MS and MG crop systems across the soil profile. Fertilisation had a significant effect only in the first layer, thus differentiating FYM with the highest *f*POM C content (1.29 g C kg^{-1}), SLU and MIN with intermediate values (1.06 and 0.72 g C kg^{-1}), and CTR with the lowest one (0.5 g C kg^{-1}). An interaction effect between fertilisation and crop system was observed in the 45–60 cm layer, where MG FYM showed the highest *f*POM C content, while MS MIN and MG SLU were the ones with the lowest. The organic C content in the physically (*o*POM) and chemically (MAOM) protected fractions were more related to both crop system and fertilisation in most layers, except for 30–45 and 75–90 cm depths. Both *o*POM C and MAOM C were higher in MG than in MS system and in FYM than in the other fertilisation treatments.

The N contents in the SOM fractions presented a pattern similar to C (Table 6). However, the fPOM N fraction was affected by fertilisation and crop system to a greater extent than the C content of the

same fraction. Fertilisation with FYM significantly increased *f*POM N in the 0–15, 15–30 and 45–60 cm layers. Fertilisation systematically influenced the distribution of N in oPOM and in MAOM fractions in all soil layers, down to 90 cm, leading to a greater N content in FYM treatment. Crop system significantly affected *o*POM and MAOM N mostly in the tilled layers (0–15 and 15–30 cm), thus revealing a slight increase in N content due to residue incorporation.

3.3. *Soil microbial abundance*

The microbial abundance was mainly affected by the interaction between crop system and fertilisation, as shown by the abundance of Bacteria, Fungi and Archaea assessed by qPCR (Fig. 2). In both 0–30 cm and 30–60 cm pooled layers, Bacteria and Fungi abundance reacted differently to fertilisation when residue was incorporated or removed (Fig. 2a, b). In the 0–30 cm pooled layer, when residue was incorporated FYM exhibited the highest bacterial abundance and CTR the lowest, while in the MS system there were no significant differences between the fertilised treatments and the CTR. The 30–60 cm pooled layer showed results similar to the 0–30 cm layer in the MG system, while in the MS crop system bacterial abundance was higher in MIN and lowest in FYM. Fungal abundance followed a trend similar to that of Bacteria in both pooled layers. In the 60–90 cm pooled layer no significant differences were highlighted. Archaea abundance did not evidence any significant effects between crop system and fertilisation at any of the three pooled depths (Fig. 2c). However, when the statistical analysis is performed for every individual depth, it reveals crop system and fertilisation significant effects (Table S2). Archaea abundance in MG FYM was found to be dominant over the other treatments in the 0–15 cm and 30–45 cm layers. MG CTR, MS CTR and MS SLU treatments had the lowest archaeal abundance in the first layer, and MG SLU, MS FYM, MS SLU and MS CTR in the third one. Interestingly, there was a significant effect of the crop system in the 75–90 cm layer, where MG showed a higher archaeal abundance than MS.

The trend of all three microbial groups was scarcely influenced by depth. Archaea decreased only by 4%, Bacteria by 6% and Fungi by 13%. Contrarily, microbial biomass (dsDNA) decreased abruptly with depth, showing 86% less microbial biomass in the 75-90 cm layer compared to the 0-15 cm layer (Figure 2d). In addition, substantial differences along the soil profile were found in the dsDNA data. Crop system had an effect on microbial biomass only in the 0-30 cm pooled layer, where MG was significantly higher than MS. The fertilisation effect was significant in both 0-30 cm and 30-60 cm pooled layers, highlighting FYM and SLU with the highest microbial biomass and MIN and CTR with the lowest. No significant effects were evidenced in the 60-90 cm layer.

Table 4 Total organic carbon (TOC) and total nitrogen (TN) concentrations in the two different crop systems (MG – maize for grain; MS – maize for silage) and different fertilisations (FYM – farmyard manure, SLU – slurry, MIN – mineral fertilisation and CTR – 0N), separated by depth. Lower case letters in italic in the average row indicate significant differences in the crop systems, at each depth. Lower case letters in the average columns indicate significant differences between fertilisations, at each depth. Capital letters are used to separate fertilisation and crop system means when the fertilisation × crop system interaction was significant, at each depth.

TOC (g kg^{-1})																		
Depth	**0-15**			**15-30**			**30-45**			**45-60**			**60-75**			**75-90**		
Treatment p(F)	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*
CTR	13.22	9.57	*11.40* c	10.76	8.90	*9.83* c	8.77	7.43	*8.10*	7.73	6.30	*7.02* a	7.53	6.43	*6.98* a	7.20	5.30	*6.25* ab
MIN	13.43	10.97	*12.20* bc	9.90	8.70	*9.30* c	7.53	7.47	*7.50*	5.60	4.80	*5.20* b	4.87	5.03	*4.95* b	4.60	4.70	*4.65* b
SLU	15.40	12.80	*14.10* ab	11.96	10.16	*11.07* b	8.27	7.80	*8.03*	7.20	6.73	*6.97* a	6.97	5.50	*6.23* ab	6.43	6.90	*6.67* a
FYM	19.13	16.20	*17.70* a	13.76	12.53	*13.15* a	8.40	8.27	*8.33*	8.80	6.97	*7.88* a	7.47	6.30	*6.88* a	7.13	6.03	*6.58* ab
Average	*15.30 a*	*12.40 b*		*11.60 a*	*10.10 b*		*8.24*	*7.74*		*7.33 a*	*6.20 b*		*6.71 a*	*5.82 b*		*6.34*	*5.73*	
Crop system p(F)	*0.002*			*0.000*			*n.s.*			*0.018*			*0.035*			*n.s.*		
Treatment p(F)	*0.000*			*<.0001*			*n.s.*			*0.003*			*0.005*			*0.033*		
*Treatment*Crop system p(F)*	*n.s.*			*n.s.*			*n.s.*			*n.s.*			*n.s.*			*n.s.*		
TN (g kg^{-1})																		
CTR	1.83	1.57	*1.70* c	1.57	1.43	*1.50* c	1.30	1.33	*1.32* ab	1.10 B	1.07 B	*1.08*	1.07 AB	1.07 AB	*1.07*	1.10	1.00	*1.05* a
MIN	1.93	1.70	*1.82* c	1.63	1.47	*1.55* bc	1.27	1.27	*1.27* b	0.97 B	0.97 B	*0.97*	0.80 C	0.90 BC	*0.85*	0.83	0.80	*0.82* b
SLU	2.13	1.97	*2.05* b	1.77	1.67	*1.72* ab	1.40	1.43	*1.42* a	1.10 B	1.20 AB	*1.15*	1.17 A	1.07 AB	*1.12*	1.13	1.07	*1.10* a
FYM	2.60	2.37	*2.48* a	2.00	1.93	*1.97* a	1.50	1.33	*1.42* a	1.40 A	1.10 B	*1.25*	1.27 A	1.03 ABC	*1.15*	1.30	1.07	*1.18* a
Average	*2.12 a*	*1.90 b*		*1.74 a*	*1.62 b*		*1.37*	*1.34*		*1.14*	*1.08*		*1.07*	*1.02*		*1.09 a*	*0.98 b*	
Crop system p(F)	*0.001*			*0.033*			*n.s.*			*n.s.*			*n.s.*			*0.024*		
Treatment p(F)	*<.0001*			*0.000*			*0.040*			*0.001*			*0.000*			*0.000*		
*Treatment*Crop system p(F)*	*n.s.*			*n.s.*			*n.s.*			*0.011*			*0.042*			*n.s.*		

Table 5 Organic carbon content in the different organic matter fractions (free particulate organic matter – *f*POM; occluded particulate organic matter – *o*POM; mineral associated organic matter - MAOM) in the two different crop systems (MG – maize for grain; MS – maize for silage) and different fertilisations (FYM – farmyard manure, SLU – slurry, MIN – mineral fertilisation and CTR – 0N), separated by depth. Lower case letters in italic in the average row indicate significant differences in the crop systems, at each depth. Lower case letters in the average columns indicate significant differences between fertilisations, at each depth. Capital letters are used to separate fertilisation and crop system means when the fertilisation × crop system interaction was significant, at each depth.

***f*POM (OC g kg^{-1})**																		
Depth	**0-15**			**15-30**			**30-45**			**45-60**			**60-75**			**75-90**		
Treatment p(F)	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*
CTR	0.60	0.41	*0.50* b	0.38	0.26	*0.32*	0.22	0.15	*0.19*	0.12 AB	0.12 AB	*0.12*	0.11	0.12	*0.12*	0.18	0.01	*0.13*
MIN	0.86	0.58	*0.72* ab	0.38	0.56	*0.47*	0.23	0.23	*0.23*	0.19 AB	0.10 B	*0.15*	0.17	0.22	*0.19*	0.10	0.11	*0.11*
SLU	0.92	1.20	*1.06* ab	0.66	0.49	*0.58*	0.22	0.25	*0.24*	0.09 B	0.25 AB	*0.17*	0.19	0.13	*0.16*	0.13	0.29	*0.21*
FYM	1.45	1.13	*1.29* a	0.72	0.58	*0.65*	0.38	0.23	*0.31*	0.47 A	0.17 AB	*0.32*	0.36	0.12	*0.24*	0.42	0.11	*0.27*
Average	*0.96*	*0.83*		*0.53*	*0.47*		*0.27*	*0.22*		*0.22*	*0.16*		*0.21*	*0.15*		*0.21*	*0.15*	
Crop system p(F)	*n.s.*			*n.s.*			*n.s.*			*n.s.*			*n.s.*			*n.s.*		
Treatment p(F)	*0.002*			*n.s.*			*n.s.*			*n.s.*			*n.s.*			*n.s.*		
*Treatment*Crop system p(F)*	*n.s.*			*n.s.*			*n.s.*			*0.040*			*n.s.*			*n.s.*		
***o*POM (OC g kg^{-1})**																		
Depth	**0-15**			**15-30**			**30-45**			**45-60**			**60-75**			**75-90**		
Treatment p(F)	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*
CTR	0.36	0.26	*0.31* c	0.31	0.31	*0.31* c	0.33	0.26	*0.30* b	0.27 B	0.12 B	*0.19*	0.20	0.17	*0.18* b	0.25	0.18	*0.21*
MIN	0.50	0.22	*0.36* c	0.47	0.46	*0.47* bc	0.44	0.24	*0.34* ab	0.15 B	0.15 B	*0.15*	0.13	0.23	*0.18* b	0.16	0.14	*0.15*
SLU	0.88	0.61	*0.74* b	0.85	0.50	*0.68* b	0.30	0.26	*0.28* b	0.17 B	0.23 B	*0.20*	0.28	0.18	*0.23* ab	0.17	0.27	*0.22*
FYM	2.65	1.64	*2.15* a	1.75	1.46	*1.60* a	0.54	0.63	*0.58* a	0.84 A	0.20 B	*0.52*	0.46	0.22	*0.34* a	0.61	0.23	*0.42*
Average	*1.10 a*	*0.68 b*		*0.85 a*	*0.68 b*		*0.40*	*0.35*		*0.36*	*0.18*		*0.27 a*	*0.20 b*		*0.30*	*0.20*	
Crop system p(F)	*0.003*			*0.002*			*n.s.*			*0.011*			*<.0001*			*n.s.*		
Treatment p(F)	*<.0001*			*<.0001*			*0.020*			*0.003*			*0.033*			*n.s.*		
*Treatment*Crop system p(F)*	*n.s.*			*n.s.*			*n.s.*			*0.005*			*n.s.*			*n.s.*		

MAOM (OC g kg^{-1})																		
Depth	**0-15**			**15-30**			**30-45**			**45-60**			**60-75**			**75-90**		
Treatment p(F)	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*
CTR	11.93	8.93	*10.43* b	10.04	8.30	*9.17* ab	8.21	6.98	*7.59*	7.37	6.06	*6.72* a	7.24	6.15	*6.70* a	6.80	5.08	*5.94* ab
MIN	11.76	9.97	*10.87* b	9.12	7.99	*8.56* b	6.92	7.01	*6.96*	5.26	4.56	*4.91* b	4.60	4.62	*4.61* b	4.37	4.47	*4.42* b
SLU	13.41	11.14	*12.27* b	10.55	9.16	*9.85* a	7.78	7.34	*7.56*	6.96	6.29	*6.63* a	6.54	5.24	*5.89* ab	6.15	6.37	*6.26* a
FYM	15.13	13.74	*14.40* a	11.48	10.92	*11.20* a	7.69	7.55	*7.62*	7.52	6.65	*7.09* a	6.78	6.00	*6.39* a	6.24	5.75	*6.00* ab
Average	*13.10 a*	*10.90 b*		*10.30 a*	*9.09 b*		*7.65*	*7.22*		*6.78 a*	*5.89 b*		*6.29 a*	*5.50 b*		*5.89*	*5.42*	
Crop system p(F)	*0.001*			*<.0001*			*n.s.*			*0.026*			*0.039*			*n.s.*		
Treatment p(F)	*<.0001*			*<.0001*			*n.s.*			*0.001*			*0.004*			*0.025*		
*Treatment*Crop system p(F)*	*n.s.*			*n.s.*			*n.s.*			*n.s.*			*n.s.*			*n.s.*		

Table 6 Total nitrogen content in the different organic matter fractions (free particulate organic matter – *f*POM; occluded particulate organic matter – *o*POM; mineral associated organic matter - MAOM) in the two different crop systems (MG – maize for grain; MS – maize for silage) and different fertilisations (FYM – farmyard manure, SLU – slurry, MIN – mineral fertilisation and CTR – 0N), separated by depth. Lower case letters in italic in the average row indicate significant differences in the crop systems, at each depth. Lower case letters in the average columns indicate significant differences between fertilisations, at each depth. Capital letters are used to separate fertilisation and crop system means when the fertilisation × crop system interaction was significant, at each depth.

***f*POM (N g kg^{-1})**																		
Depth	**0-15**			**15-30**			**30-45**			**45-60**			**60-75**			**75-90**		
Treatment p(F)	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*
CTR	0.039	0.025	*0.032* b	0.019	0.016	*0.018* b	0.013	0.009	*0.011*	0.006 B	0.006 AB	*0.006*	0.005	0.005	*0.005*	0.006	0.004	*0.005*
MIN	0.058	0.040	*0.049* b	0.024	0.032	*0.028* ab	0.013	0.017	*0.015*	0.011 AB	0.007 AB	*0.009*	0.009	0.009	*0.009*	0.005	0.009	*0.007*
SLU	0.059	0.077	*0.068* ab	0.036	0.031	*0.034* ab	0.011	0.014	*0.013*	0.004 B	0.013 AB	*0.009*	0.010	0.007	*0.009*	0.006	0.011	*0.008*
FYM	0.098	0.093	*0.096* a	0.048	0.046	*0.047* a	0.024	0.016	*0.020*	0.029 A	0.009 AB	*0.019*	0.022	0.007	*0.014*	0.025	0.007	*0.016*
Average	*0.063*	*0.059*		*0.032*	*0.031*		*0.02*	*0.01*		*0.01*	*0.01*		*0.011 a*	*0.007 b*		*0.010*	*0.008*	
Crop system p(F)	*n.s.*			*n.s.*			*n.s.*			*n.s.*			*0.040*			*n.s.*		
Treatment p(F)	*0.004*			*0.031*			*n.s.*			*0.031*			*n.s.*			*n.s.*		
*Treatment*Crop system p(F)*	*n.s.*			*n.s.*			*n.s.*			*0.016*			*n.s.*			*n.s.*		
***o*POM (N g kg^{-1})**																		
Depth	**0-15**			**15-30**			**30-45**			**45-60**			**60-75**			**75-90**		
Treatment p(F)	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*
CTR	0.027	0.018	*0.023* c	0.018	0.018	*0.018* b	0.020	0.014	*0.017* b	0.013 B	0.005 B	*0.009*	0.008	0.008	*0.008* b	0.011	0.007	*0.009* ab
MIN	0.037	0.017	*0.027* c	0.035	0.030	*0.032* b	0.027	0.016	*0.021* b	0.008 B	0.009 B	*0.008*	0.006	0.011	*0.009* ab	0.007	0.007	*0.007* b
SLU	0.066	0.046	*0.056* b	0.070	0.034	*0.052* b	0.019	0.019	*0.019* b	0.007 B	0.013 B	*0.010*	0.015	0.010	*0.013* ab	0.007	0.010	*0.009* ab
FYM	0.227	0.151	*0.189* a	0.145	0.092	*0.118* a	0.040	0.046	*0.043* a	0.058 A	0.010 B	*0.034*	0.032	0.011	*0.022* a	0.043	0.013	*0.028* a
Average	*0.089 a*	*0.058 b*		*0.067 a*	*0.043 b*		*0.026*	*0.024*		*0.022*	*0.009*		*0.015 a*	*0.010 b*		*0.017*	*0.009*	
Crop system p(F)	*0.009*			*0.045*			*n.s.*			*0.008*			*0.022*			*n.s.*		
Treatment p(F)	*<.0001*			*0.000*			*0.006*			*0.001*			*0.006*			*0.044*		
*Treatment*Crop system p(F)*	*n.s.*			*n.s.*			*n.s.*			*0.002*			*n.s.*			*n.s.*		

MAOM (N g kg^{-1})																		
Depth	**0-15**			**15-30**			**30-45**			**45-60**			**60-75**			**75-90**		
Treatment p(F)	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*	**MG**	**MS**	*Average*
CTR	1.77	1.52	*1.65* c	1.53	1.40	*1.46* b	1.27	1.31	*1.29*	1.08	1.06	*1.07* a	1.05 AB	1.05 AB	*1.05*	1.08	0.99	*1.04* a
MIN	1.84	1.64	*1.74* c	1.57	1.41	*1.49* b	1.23	1.23	*1.23*	0.95	0.95	*0.95* b	0.79 C	0.88 BC	*0.83*	0.82	0.78	*0.80* b
SLU	2.01	1.84	*1.93* b	1.66	1.60	*1.63* ab	1.37	1.40	*1.39*	1.09	1.17	*1.13* a	1.14 A	1.05 AB	*1.10*	1.12	1.05	*1.08* a
FYM	2.27	2.12	*2.20* a	1.81	1.80	*1.80* a	1.44	1.27	*1.35*	1.31	1.08	*1.20* a	1.21 A	1.02 AB	*1.11*	1.23	1.05	*1.14* a
Average	*1.97 a*	*1.78 b*		*1.64 a*	*1.55 b*		*1.33*	*1.30*		*1.11*	*1.07*		*1.05*	*1.00*		*1.06 a*	*0.97 b*	
Crop system p(F)	*0.000*			*0.049*			*n.s.*			*n.s.*			*n.s.*			*0.016*		
Treatment p(F)	*<.0001*			*0.001*			*n.s.*			*0.002*			*0.000*			*0.000*		
*Treatment*Crop system p(F)*	*n.s.*			*n.s.*			*n.s.*			*n.s.*			*0.049*			*n.s.*		

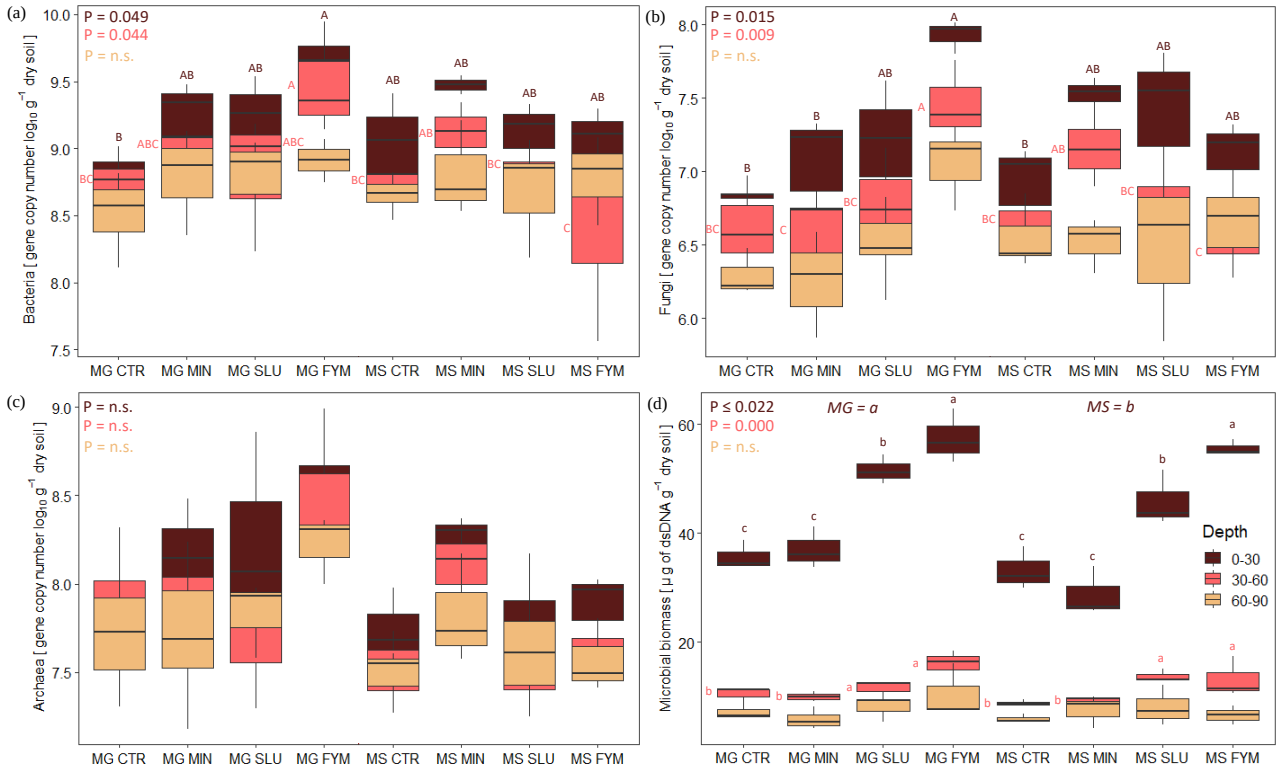

Figure 2 Boxplots showing (a) Bacteria 16S rRNA gene copy numbers; (b) Fungi 26S rRNA gene copy numbers; (c) Archaea 16S rRNA gene copy numbers; (d) Microbial biomass (µg of dsDNA g-1dry soil) in the different treatments. The coloured rectangle shows the first and third quartile, the segment shows the median, and whiskers indicate minimum and maximum values. Different colours are used for different soil layers, obtained by pooling sampled depths. Capital letters separate fertilisation and crop system means when the fertilisation × crop system interaction was significant. Lower case letters indicate significant differences between fertilisations. Lower case italic letters indicate significant differences in the crop systems. The statistical analysis was performed separately for each depth.

3.4. *Enzymatic activity*

The enzymatic activity was significantly influenced by both crop system (Fig. 3) and fertilisation (Fig. 4). Residue incorporation affected C- and N-degrading EAs differently in function of the fertilisation (Fig. 3). Small differences were evidenced between the incorporated (MG) and removed (MS) residue CTR treatments, where only few of the C- and N-degrading enzyme activities (betaGAL, betaMAN, arabin, xylo, CBZ and leu) were stimulated by residue additions. However, the effect was visible in both tilled layers (0–30 cm) and subsoil (30–90 cm). Crop residue incorporation in combination with MIN led to poor differences in C-degrading EAs as well, and they were mainly localized in the topsoil. No positive differences were visible in the N-degrading enzymes between MG MIN and MS MIN treatments. Compared to MS SLU, MG SLU increased only arabin and cell

EA in the 0–30 cm layers, and significantly decreased alfaGAL, CBZ and argi activities. Curiously, crop residue in addition to FYM fertilisation fostered both C- and N-degrading enzymes activities, mostly below the tilled layer (>30 cm).

Heatmap analysis revealed contrasting EA results when the fertilised treatments were compared to their unfertilised control, in both MG and MS systems (Fig. 4). Compared to MG CTR, MG MIN showed similar C-degrading and N-degrading enzyme activitiesContrariwise, compared to MS CTR, MS MIN presented a lower N-degrading EA. C- degrading EA in MS MIN in the tilled layer showed generally a lower activity compared to MS CTR, as well. However, below the tilled layer, betaGAL activity resulted significantly higher in MS MIN than in MS CTR. The activity of betaG, alfaGAL, betaGAL, arabin, uroni and xylo were significantly higher in the MG SLU treatment than its corresponding control, especially in the 15–30 cm layer. N-degrading EA in the MG SLU treatment did not differ significantly from that in the MG CTR treatment with the exception of chit, leu and argi in the 15–30 cm layer. In contrast, MS SLU significantly improved C- and N-degrading EAs compared to MS CTR up to 60 cm depth. MS FYM showed similar results to MS SLU, while MG FYM significantly increased C-degrading EA up to 90 cm depth, and chit, leu and argi (N-degrading enzymes) up to 75 cm depth.

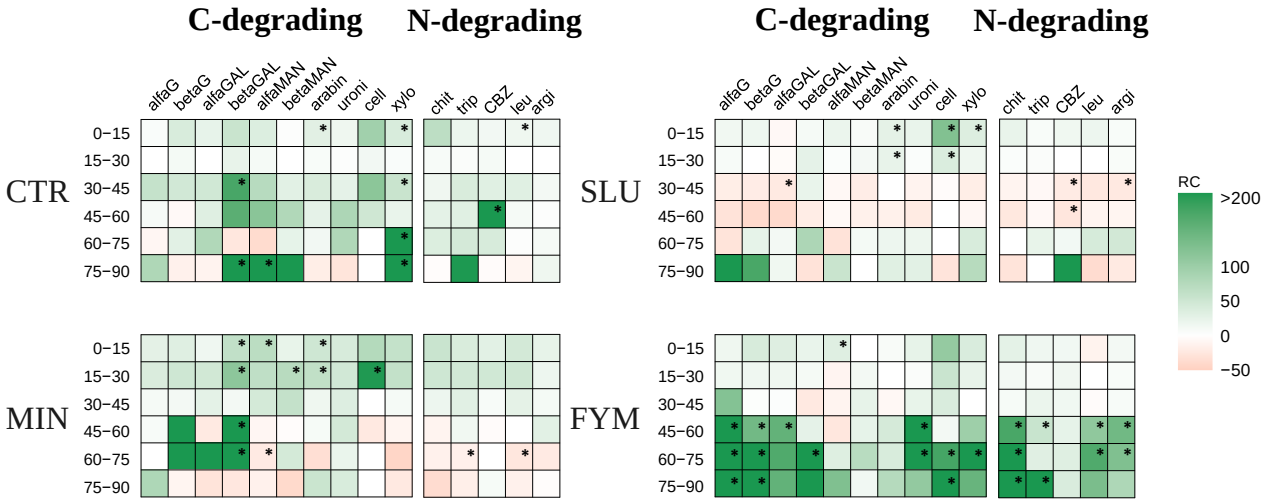

Figure 3 Heatmap of relative change percentages of specific enzyme activities (μmol h^{-1} g^{-1} dry soil) between MG (maize for grain with residues incorporated) and MS (maize for silage with residues removed) crop systems (RC: MG-MS/MG*100) for every fertilisation type (FYM – farmyard manure, SLU – slurry, MIN – mineral fertilisation and CTR – 0N). Stars indicate significant differences (Tukey's test, P: 0.05) between the two crop systems. Green nuances refer to a positive RC between MG and MS, while pink nuances to a negative one.

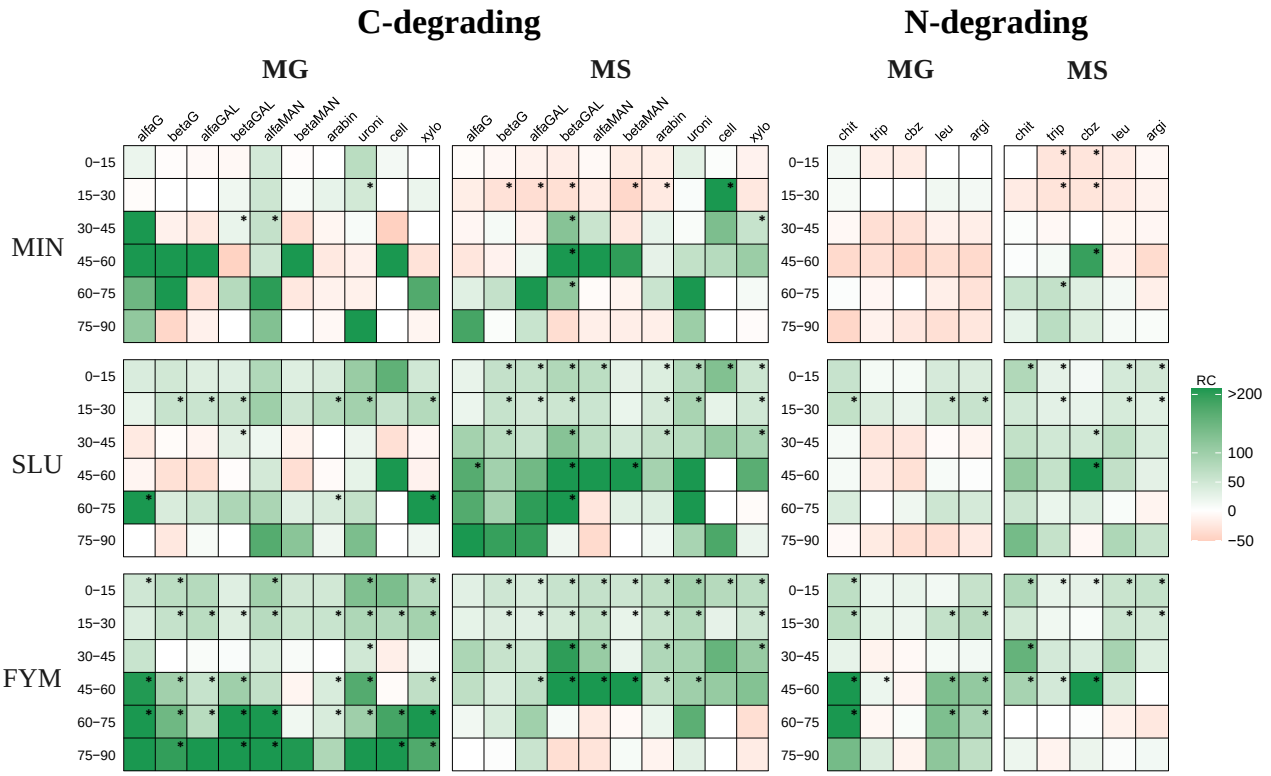

Figure 4 Heatmap of relative change percentages of specific enzyme activities (μmol h^{-1} g^{-1} dry soil) between the fertilised treatments (FYM – farmyard manure, SLU – slurry, MIN – mineral fertilisation) with respect to the 0N control (RC: fertilised-control/control*100) in the two crop systems (MG-maize for grain with residues incorporated, MS-maize for silage with residues removed). Stars indicate significant differences (Tukey's test, P: 0.05) between the fertilised treatments with respect to the control, in the two crop systems. Green nuances refer to a positive RC between MG and MS, while pink nuances to a negative one.

3.5. *dbRDA analysis*

Distance-based redundancy analysis (dbRDA) was used to assess the overall differences in microbial abundances and in EA, and to understand the influence of soil and management variables. In the 0–15 cm soil layer, dbRDA analysis proved that Bacteria, Fungi and Archaea abundance differed among treatments with separation along axis 1 (F: 57.3 and P = 0.001) and along axis 2 (F: 13.2 and P = 0.021) accounting for 82.2 % of the total variance (Fig. 5a). The horizontal differentiation in the dbRDA plot is a consequence of the higher microbial biomass in FYM and SLU than in MIN and CTR. The soil parameters that had the highest influence on microbial abundance distribution across the treatments were TN and mineral N input. In the 15–30 cm layer the microbial abundance differed among treatments with separation along axis 1 (F: 55.8 and P = 0.001) accounting for 80.4 % of the total variance (Fig. 5b). TOC and organic N input influenced Bacteria, Fungi, Archaea and microbial biomass trend, in this layer. The 30–45 cm layer showed that microbial abundance varied between

the treatments with separation along axis 1 (F: 13.7 and P = 0.001) and along axis 2 (F: 9.7 and P = 0.005), jointly accounting for 60.6 % of the total variance (Fig. 5c). The vertical differentiation denoted higher Bacteria, Fungi and Archaea abundance in the FYM and MIN treatments than in SLU and CTR. This trend was shaped by the organic K input. The differences in the last three layers were mostly driven by the N content in the various SOM fractions. In all cases, the significant differentiations along axis 1 (P = 0.001, P = 0.002, P = 0.001) in the dbRDA plots were generally determined by microbial biomass, and not by Bacteria, Fungi and Archaea abundance (Fig. 5d, e, f). Interestingly, pH (Table S5) had a significant effect on shaping the microbial abundances only in the 60–75 cm layer.

The EA in the 0–15 cm layer differed among crop systems and fertilisations with the separation along axis 1 (F: 101 and P = 0.001) accounting for 65.8 % of the total variance (Fig. 6a). The higher value of three N-degrading enzymes (leu, argi and chit), betaG and nona are the cause of the horizontal differentiation in the dbRDA plot between FYM - SLU and MIN - CTR. The dbRDA plot for the 15–30 cm layer showed a very similar pattern to the shallowest one (Fig. 6b), while in the 30–45 cm layer there were no significant differences neither along axis 1 (F: 10.3 and P = 0.007) nor axis 2 (F: 1.3 and P = 0.977) between treatments (Fig. 6c). The 45–60 cm layer highlighted significant differences along axis 1 (F: 17.5 and P = 0.005) that were mostly driven by more intense N-degrading enzymes (chit, CBZ, leu and argi), Cdegrading enzymes (betaG, alfa and betaGAL) and nona activities (Fig. 6d). A significant decrease of EA with depth was found in all treatments (data not shown). In the last two layers (60–75 cm and 75–90 cm), EA differed among crop systems and fertilisations, with the separation along axis 1 (P = 0.012 and P = 0.012) accounting for 58.2 % and 53.9 % of the total variance (Fig. 6e, f). Results from multivariate multiple regression on C- and N-degrading enzymes indicate that in the tilled layer the soil parameter that had the highest influence on EA distribution was the C content in the various SOM fractions, while below the tilled layer the N content in the various SOM fractions dominated.

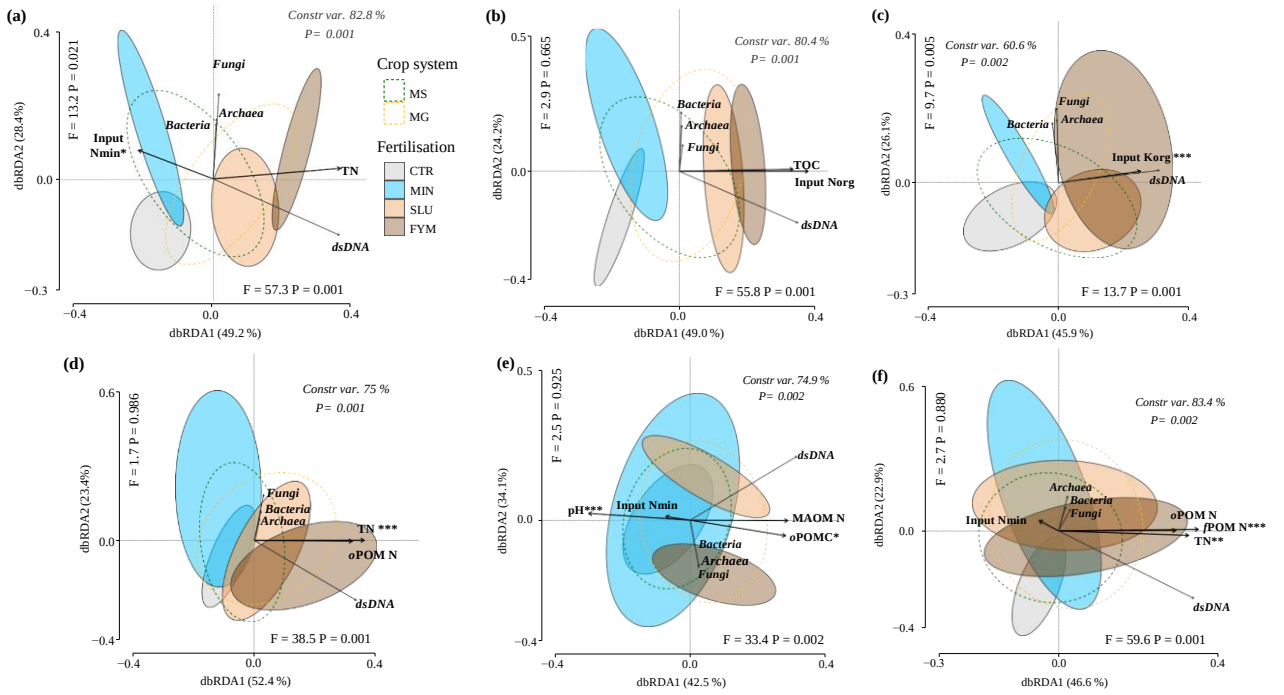

Figure 5 Distance-based Redundancy Analysis (dbRDA) plots showing shifts in microbial abundance in 0-15 cm layer (a); 15-30 cm layer (b); 30-45 cm layer (c); 45-60 cm layer (d); 60-75 cm layer (e); 75-90 cm layer (f). Statistics of the dbRDA models and explanatory soil variables, resulting from multivariate forward multiple regression, is reported in Table S3.

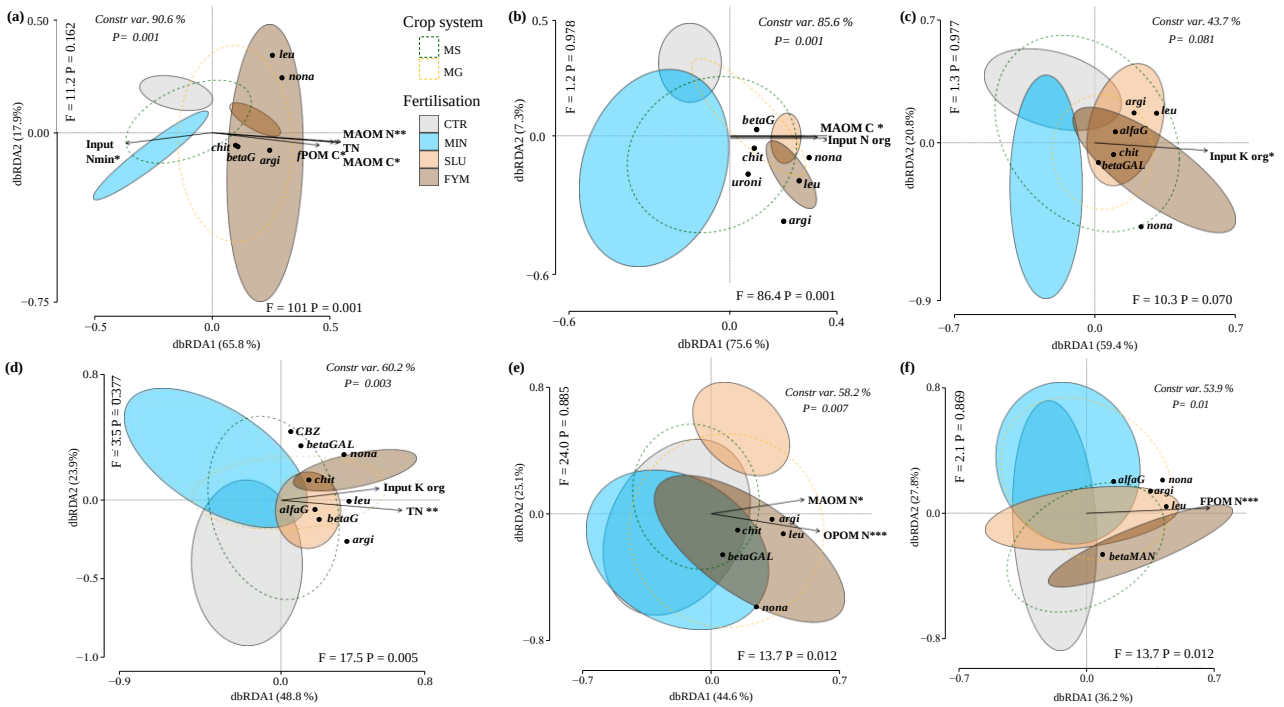

Figure 6 Distance-based Redundancy Analysis (dbRDA) plots showing shifts in specific enzyme activities in 0-15 cm soil layer (a); 15-30 cm layer (b); 30-45 cm layer (c); 45-60 cm layer (d); 60-75 cm layer (e); 75-90 layer (f). Statistics of the dbRDA models and explanatory soil variables, resulting from multivariate forward multiple regression, is reported in Tables S4.

4. Discussions

Feed production

All systems produced feed at a similar level, but they differed in providing other ESs. Prioritising non-feed ESs may in fact come at a cost of the provision of animal feed (Schils et al., 2022). While this trade-off is clear for reducing mineral N input, other management interventions show mixed or even synergic outcomes and thus should be implemented more frequently. We found that organic fertilisation is favourable for both regulating services of nutrient cycling and C sequestration and provision of animal feed (Li et al., 2020). The proposed shift from feed to non-feed ecosystem services will not come with a reduced production per hectare without any potential negative effects on farm income.

Soil organic matter storage and stability

Intensive agriculture with regular tillage, removal of crop residue and lack of organic amendments can lead to SOM depletion, degraded soil structure and poor nutrient cycling potential of agricultural soils (Spiegel et al., 2018). Among practices that reverse the negative trends on SOM depletion, both organic fertilisation and residue incorporation in maize monoculture represent valid techniques that potentiate the delivery of ESs connected to soil health (Sanderson et al., 2013). The present study showed in fact a general higher TOC concentration in the organic fertilised treatments (FYM and SLU) and in the residue incorporation crop system (MG). The distribution of organic fertilisers increased soil TOC in the tilled layers by 31 % and 16 % when FYM and SLU were applied compared to CTR, and 30 % and 15 %, respectively, if compared to MIN. This agrees with Zavattaro et al. (2017), who evidenced an increase in topsoil TOC by 32 % in long-term manured sites, in a review of 80 long-term experiments across Europe where manures and mineral fertilisers were compared at similar N rates. The mineral N fertilisation practice was instead not determinant for C storage in the topsoil at this site, consistently with previous findings (Bertora et al., 2009; Zavattaro et al., 2016). Where manures are not available, other organic sources should be used, and among these the incorporation of crop residue increases the soil organic C content. The long-term increase of 16 % observed in the MG tilled layers (0–30 cm) is in line with other investigations (Banger et al., 2010; Zhang et al., 2020), even though the accumulation was slow due to a fast mineralisation (Bertora et al., 2009).

Deep TOC, below the tilled layers (>30 cm), contributes significantly to total carbon pools (Dal Ferro et al., 2020). Significant differences between MG and MS crop systems were observed (+11% in the 30-90 cm pooled depth). Fertilisation affected TOC in the subsoil to a great extent as well, in the

522 order FYM>SLU=CTR>>MIN. The increased TOC contents in MG and in manured treatments in
523 deeper soil layers could be explained by two mechanisms. One is the translocation of dissolved
524 organic matter (DOM), that was recognised as a very important C source in subsoil horizons (Kaiser
525 and Guggenberger, 2000; Giannetta et al., 2022). The second is an increased root growth or deposition
526 (Chabbi et al., 2009), as root C was shown to be stabilised preferentially if compared to shoot C
527 (Rasse et al., 2005). However, an opposite trend was observed in the mineral-fertilised treatment.
528 Interestingly, while MIN was similar to CTR in the tilled layer, it led to a 27 % decrease of TOC in
529 the subsoil, probably because of a stimulation of mineralisation by translocated mineral N (Bertora
530 et al., 2009), or to higher root development and exudates in the N-depleted CTR (Shahbaz et al.,
531 2017).

532 Even if they represent a small proportion of TOC, free and occluded POM fractions are crucial
533 components of soil organic matter, as they support nutrient biogeochemical transformations and are
534 sensible indicators of changes in soil functioning and quality (Cotrufo et al., 2019). In the present
535 study, on average of all samples, *f*POM and *o*POM contributed to 4 % and 5 % of soil TOC, while
536 MAOM represented on average 91 %. Free POM is mostly derived from freshly added OM, still
537 undecomposed or only partly decomposed (Witzgall et al., 2021); hence, crop residue and organic
538 amendment incorporations are expected to increase soil fPOM. Nevertheless, our results showed no
539 difference in *f*POM C between the removal or incorporation of maize residue and only small
540 variations between fertiliser types. This can be due to a fast decomposition and transformation of
541 *f*POM C in soils, in particular in warm and humid conditions, with annual tillage and high soil pH (Li
542 et al., 2016b; Poeplau et al., 2017). However, the legacy of past residue and manure incorporations
543 that is not evidenced in the *f*POM fraction can be visible in the *o*POM pool, that includes
544 undecomposed *f*POM entrapped in soil aggregates (Six et al., 2000; Cotrufo et al., 2019; Moretti et
545 al., 2020; Witzgall et al., 2021). Our results showed that residue incorporation in MG led to a higher
546 *o*POM C both in tilled and subsoil layers. The addition of C-rich fertilisers also increased *o*POM in
547 the order FYM>SLU>MIN=CTR, all along the soil profile. In particular, FYM ensures a high
548 aggregate stability and long-term C protection within aggregates (Liu et al., 2013; Das et al., 2014).

549 The OM associated with mineral surfaces showed that both residue incorporation and organic
550 fertilisation significantly increased MAOM C, with a synergic effect of the two practices. The
551 mechanisms that explain this increase are various and differ for the two types of additions. According
552 to Samson et al. (2020), crop residue initially passes through a physical-biochemical phase that forms
553 the fPOM fraction, and are then gradually incorporated into the MAOM fraction through microbial
554 decomposition, while animal manures more directly end up as MAOM because they already contain
555 degraded organic compounds with a strong affinity with mineral surfaces. Other authors state instead

that the MAOM fraction mainly derives from the microbial-derived products released during POM degradation that diffuse into the neighbouring soil matrix (Cotrufo et al., 2013; Kravchenko et al., 2019).

A novelty of this study lies in showing that in the long term both residue incorporation and organic fertilisation increased MAOM C all along the soil profile, while most previous studies focused on the topsoil and short-term effects (Shahbaz et al., 2017). After 28 years, crop residue and organic fertilisation (FYM and SLU) increased both oPOM and MAOM C not only in the tilled layer but also in subsoil, with a synergic effect of the two practices. This can be attributed to bioturbation due to pedofauna present in the field that can transport the OM to deeper soil layers (Angst et al., 2019) even if the input of plant residue and manure is primary limited to the topsoil, or to the DOM vertical flux to the deeper layers (Kaiser and Kalbitz, 2012). While organic additions increased soil TOC and its fractions compared to MIN, the mineral fertilisation had a detrimental effect on the same variables, compared to CTR. In particular, MAOM C decreased with MIN application independently of the fate of crop residue, thus indicating that root-derived C was unable to increase, or even sustain, MAOM C under mineral N fertilisation (Shahbaz et al., 2017). Long-term soil TOC data also evidenced a continuous decrease in MIN at all depths with time (data not shown).

Soil TN showed similar trends to TOC, but results were even more surprising because the three types of fertilisation added the same amount of total N. The MG FYM and MG SLU treatments were those with the highest TN content, demonstrating that part of N that was not acquired by the crop was stored in the various OM fractions. Conversely, a trade-off was found in the case of MIN, that had a lower TN content, thus showing that mineral N supplied was not retained in the soil, not even when in combination with crop residue incorporation (Nardi et al., 2004; Bertora et al., 2009). The relatively high TN observed in CTR could be linked to the higher TOC content in this treatment (Sand´en et al., 2019). In the manured treatments, TN showed a trend similar to TOC down to 45 cm, highlighting a strong connection between the two elements. Downward this layer, conversely to TOC that revealed simple additive effects, TN presented significant interactions between crop system and fertilisation that did not allow us to detect clear trends, while the negative impact of MIN on TN was visible also in deeper horizons. Notwithstanding the different forms of N supplied with mineral and or organic fertilisation, soil organic N followed C dynamics and perfectly reflected its fractionation and distribution all along the soil profile.

The effect of residue incorporation was slightly visible on the *f*POM N fraction, and more evident in the *o*POM N fraction. Conversely, organic fertilisation significantly increased both *f*POM N and *o*POM N in the tilled layer. These results are in line with other studies (Li et al., 2015; Moretti et al.,

2020; Xu et al., 2020), who showed higher *f*POM and *o*POM N contents when compost or manure was applied, compared to mineral fertiliser alone. Despite lighter OM fractions remained confined to the uppermost soil layers, similar to the case of C, the effect of organic fertilisation on *o*POM N was evident down to a higher depth than that influenced by crop residue incorporation.

Both organic fertilisation and residue incorporation increased the N content in the MAOM fraction in the tilled layer, while MIN application substantially altered N stabilisation compared to the unfertilised CTR. In the subsoil, again organic fertilisation increased MAOM N to a greater depth than crop residue did. The synergic effect of crop residue, with a high C:N ratio, and FYM, probably favoured N translocation and immobilisation to the deeper layers (Cotrufo et al., 2015). The MAOM C: N ratio was generally greater in the subsoil (9.02 on average) than in the tilled layer (7.26 on average), pointing out that the source of MAOM was different in the two cases, as described regarding C. In particular, as free and occluded POM pools were generally scarce in the subsoil, it is unlikely that root and litter decomposition following microbial assimilation is the dominant pathway of MAOM formation in subsoil, while translocation of DOM and subsequent immobilisation could control SOM stabilisation (Mikutta et al., 2019).

Due to the high stability of MAOM, litters that increase this fraction can be considered of higher quality for C sequestering (Lavallee et al., 2020), and organic fertilisers showed to be more effective that residue incorporation. Moreover, because of the long mean residence times of SOM in subsoil, MAOM might be subjected to a more important aging process in subsoil than in topsoil (Mikutta et al., 2019). In this vision, organic fertilisation was effective in increasing MAOM to very deep soil horizons, and therefore to contribute to a larger extent to C sequestration.

Actors of SOM turnover/stability

The role of soil microorganisms as drivers of SOM cycling, storage and sequestration is not new, but is more and more acknowledged as crucial (Lehmann and Kleber, 2015). Microorganisms contribute to degradation and mineralisation of SOM substrates and to the genesis of new metabolites (Chenu et al., 2019). In this experiment, as a general trend, Bacteria were found to be the most abundant (8.92 16S rRNA gene copy numbers $\log_{10}$ g^{-1} dry soil), followed by Archaea (7.86 16S rRNA gene copy numbers $\log_{10}$ g^{-1} dry soil) and Fungi (6.85 26S rRNA gene copy numbers $\log_{10}$ g^{-1} dry soil), reflecting a situation common to other cropland soils (Szoboszlay et al., 2017). The scarce reduction of all groups with depth can be attributed to the vertical flow of SOM in the subsoil that represents a significant supply of nutrients for the endogenous microorganisms in deeper soil layers (Kindler et al., 2011, Sandén et al., 2019). The microbial abundance of Bacteria, Fungi and Archaea was only poorly affected by crop residue and organic fertiliser additions. There was a tendency of higher values

in FYM and in MG systems, coherently with Liu et al. (2022) who measured a higher amount and stability in time of microorganisms in C-amended systems. When analysed separately for each soil layer (Table S2), our results showed a stimulatory effect of residue incorporation and manure application on Archaea, thus suggesting they were dependent on organic inputs and promoted their decomposition (Wessén et al., 2010; Dong et al., 2021). However, despite differences among treatments were limited, not necessarily microbial functioning was the same in all treatments (Widdig et al., 2020), as found by Sandén et al. (2019) at the same site.

Management altered the microbial biomass (dsDNA) more strongly than microbial abundance. Since the microbial biomass includes either bacterial, archaeal, and fungal DNA, as well as a relevant proportion of non-microbial DNA such as plant and soil fauna (Gangneux et al., 2011), it is not surprising that it allows for a more comprehensive view of the changes occurring within the soil systems, highlighting quantitative variations that are not detectable observing the single microbial components. Organic fertilisation had a positive impact on dsDNA, as the highest values were observed in FYM and SLU. Farmyard manure and slurry provide C, N, and a variety of other elements to the soil, so there should not be nutrient limitations for microbes in these treatments. Contrarily, mineral fertilisation only supplies mineral N, especially in the MS crop system, which probably leads to a limitation of the available C in the soil (Zhou et al., 2017; Averill and Waring, 2018). Similarly, the low dsDNA content in CTR treatments can be associated to a nutrient limitation situation regarding both C and N.

The EA, an indicator of nutrient cycling (Nannipieri et al., 2012), was improved by organic fertilisation and to a lesser extent by crop residue incorporation especially in the 0–30 cm soil layers. FYM and SLU additions stimulated OM decomposition and mineralisation. By contrast, MIN and CTR had the lowest EA, both in C-cycle and N-cycle related degrading enzymes (Aira et al., 2007; Zhang et al., 2015). EA in manured treatments differed significantly from MIN and CTR in all C-degrading and in some N-degrading enzymes (chit, leu and argi) and nona activities. Residue management instead influenced activities of some of the C-degrading enzymes (betaGAL, alfaMAN, arabin, cell and xylo), that significantly increased in MG compared to MS (Li et al., 2016a; Guan et al., 2020; Zhang et al., 2020). Conversely, N-degrading EA were scarcely influenced by residue management, probably because of the low N content of maize stalks and leaves (Fig. 4 and Fig. 5). Enzymes involved in C and N reserves remobilisation (alfaG, argi), OM decomposition (betaG, uroni, xylo) and in microbial acquisition of N (leu) showed the greatest activity in the MG FYM, especially below the tilled layer.

Pooling together crop, management and soil information regarding treatments in a multivariate approach (Fig. 6), C-degrading enzyme betaG played an important role in differentiating treatments in the topsoil, while alfaG e betaGAL did so in the subsoil. One of the most interesting results of this study is that there was an interaction between crop residue and FYM in stimulating EAs, in particular in the subsoil horizons (Fig. 5). Apparently, the presence of crop residue in combination with FYM fostered SOM movements towards the subsoil.

Our findings corroborate evidences that SOM density fractionation is a useful technique to identify agricultural practices capable of increasing stable TOC, but also soil life activity in nutrient cycling. This study evidenced a correlation between EA and fPOM and oPOM fractions and C- and N-degrading enzymes ($P < 0.01$). *f*POM had a stronger relationship with C- and N-degrading enzymes ($R^2 = 0.775$ and $R^2 = 0.669$, respectively) than *o*POM ($R^2 = 0.482$ and $R^2 = 0.471$, respectively), as it is more readily available for degradation than that occluded within aggregates. However, the specific mechanisms leading to the relationships are still uncertain.

5. Conclusions

In the long-term, a combination of crop residue incorporation and farmyard manure was able to improve both regulating services - nutrient cycling and C sequestration - without negatively influencing feed provision. The positive effects of crop residue incorporation were evident, but more limited than those of organic fertilisation. Results showed that both FYM and SLU ensured greater C input and significantly increased the soil organic C and N contents, especially that in the more stable fraction (MAOM). However, differences between FYM and SLU were remarkable and significant, because SLU supplied about one third of the C of FYM, at equal N dose. The effects of using manures were visible in both topsoil and subsoil, thus indicating a translocation and stabilisation of OM to deep soil horizons. TOC and TN distribution in the soil profile, as well as their partitioning in the SOM fractions, indicated a higher retention of N when manures were applied, compared to mineral fertiliser. Although little differences were visible in the soil microbial abundance, the MIN treatment was apparently unable to promote a good SOM turnover all along the soil profile. FYM and SLU fertilisation, in particular if combined with maize residues, strongly stimulated C- and N-degrading enzymes, as a consequence of the higher TOC and TN contents in the various SOM pools. FYM combined with residue incorporation promoted the transfer of organic matter to deep soil horizons, thus fostering both soil life and C stabilisation in the subsoil.

From an agronomical point of view, our study also showed that wellmanaged livestock effluents can immobilise part of the N surplus in the various SOM fractions, in particular in the mineral-associated one, while in the mineral-fertilised treatments the N that was not taken up by the crop was not retained

687 in soil and was probably lost. Therefore, the N from manure value is superior to the N from mineral
688 fertiliser. Favouring organic fertilisation practices in spite of mineral ones can thus mitigate climate
689 change and sustain the delivery of regulating services as well as soil activity and health.

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