Effect of long-term compost fertilization on the distribution of organic carbon and nitrogen in soil aggregates

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ABSTRACT

Soil fertility is mainly related to soil total organic carbon (C) that should be preserved in order to optimize soil quality and functionality. Consequently, the use of organic amendments could be a possible strategy to increase soil C and nitrogen (N) storage particularly in orchard systems where the disturbance of soil is reduced thus making favorable conditions for C and N stabilization.

The aim of this study was to evaluate the distribution and stabilization of organic C and total N in aggregate fractions after a 14-years-period of compost application to a peach orchard. The trial was conducted in the southeastern Po valley, on a commercial nectarine orchard and the following treatments were compared in a complete randomized block design with four replicates: 1. unfertilized control; 2. mineral fertilization; 3. compost at a rate of 10 Mg dry weight ha^{-1} yr^{-1}. At the end of orchard lifetime, soil was sampled from the row at four depths (0–0.15, 0.16–0.25, 0.26–0.45, and 0.46–0.65 m) and physically fractionated to separate macroaggregates (> 250 μm), microaggregates (250–50 μm) and silt and clay (< 50 μm) that were analyzed for organic C, total N, $\delta^{13}C$, and $\delta^{15}N$.

Compost addition induced a significant increase of macroaggregates (66%), no changes in microaggregates and a decrease of silt and clay (22%) compared to control and mineral fertilization. With compost the accumulation of organic C and total N content in the macroaggregates was four-five times higher than the other two treatments in all the depths, therefore almost 50% of the soil organic C was in this fraction, compared to 20–24% in the control and mineral. In the micro-aggregates more C and N accumulated only in the two top layers, while no effect was observed in the silt and clay fraction. From macro-to microaggregates to the silt and clay fraction, the C/N ratio shifted from 9 to 7.5 to 6 on average indicating that the C and N stabilized in the finer fractions is mainly of microbial origin. The enrichment in C and N isotopic composition from macro-to micro to silt and clay is also indicative of the isotopic fractionation due to microbial metabolism and the consequent stabilization of microbial residues in the finer fractions. In the control and mineral, only receiving orchard litter, this change was observed in all the layers, on the contrary, with compost similar $\delta^{13}C$ and $\delta^{15}N$ values characterized the fractions in the top soil layer suggesting the occlusion of compost in all the fraction. With depth the macroaggregates maintained the same $\delta^{13}C$ and $\delta^{15}N$ values indicating consistent redistribution of compost in deep soil layers, while the finest fractions showed a progressive enrichment of $\delta^{13}C$ due to the presence of C fractionated during microbial metabolism and progressively stabilized. In conclusion, compost supply leads to positive effects on C and N accumulation and stabilization also in the deeper layers favoring the increase of long-term soil fertility and C storage.

1. Introduction

Peach is the second most produced temperate fruit crop in the world following apple; however, in terms of production, peach cultivation is slowly and progressively decreasing. The global peach production is currently largely dominated by China (FAOSTAT, 2020), while historically important countries, such as Italy and USA, has decreased, in the last decade (2002–2022), from about 1.6 to 1.1 and from 1.4 to 0.6 Mt, respectively (FAOSTAT, 2020). Although it is difficult to establish a precise cause, the competition with other fruit crops as well as the saturation of the European and American markets have played the major role. Moreover, peach suffers from soil sickness that is mainly caused by replanting and loss of soil fertility. Consequently, the use of organic matter in soil could be a possible strategy to reduce this condition and to induce farmers to cultivate peach.

Soil total organic carbon (TOC) in agricultural systems is one of the main drivers of soil quality and functionality, therefore, an adequate level of TOC must be preserved in order to achieve multiple agro-ecosystem services, including mitigation of carbon dioxide (CO_2) emissions to the atmosphere, reduction of soil erosion, and increase of soil nutrient reservoir (Paustian et al., 1997; Robertson and Swinton, 2005). The use of organic amendments (e.g., compost) is one of the agricultural practices able to increase soil C and nitrogen (N) storage in the short- and medium-term. Consequently, organic fertilization should be included in orchard management strategies to improve physical, biological, and chemical soil properties (Hargreaves et al., 2008) as well as C sequestration (Lal, 2004). However, to achieve these goals, the C and N added must be stabilized, preserving the organic compounds from microbial degradation and ensuring their long-term storage in soil (Marschner et al., 2008; Lutzow et al., 2006).

The stabilization of C and N in the soil can occur thanks to different mechanisms (Keil and Mayer, 2014; Sollins et al., 1996) that are categorized into three general types: (i) biochemical recalcitrance; (ii) physical protection; and (iii) chemical stabilization (Chen et al., 2020; Kida and Fujitake, 2020). The biochemical recalcitrance is the intrinsic stability of soil TOC; it is strictly linked to the chemical composition of the molecules and depends on soil chemical processes such as condensation reactions (Cadisch and Giller, 1997; Six et al., 2002). Physical protection (inaccessibility) occurs through TOC occlusion within aggregates or small pores, reducing the accessibility of microorganisms, enzymes, and oxygen diffusion (Plaza et al., 2013; Guidi et al., 2021). Finally, chemical stabilization develops through the interaction of TOC with mineral surfaces of silt and clay or metals (Christensen, 2001).

These stabilization mechanisms lead to the formation of soil organo-mineral aggregates characterized by a different stability degree of their associated C (Verchot et al., 2011) and N; an increase in TOC content is usually associated with increasing C and N-rich macroaggregates (Tisdall and Oades, 1982; Six et al., 2000; Gioacchini et al., 2016). Indeed, inside the macroaggregates plant C and N components, microbial metabolites, and mineral particles are coated together to form the microaggregates where C and N are protected through physical and chemical mechanisms and thus sequestered for a long time (Plaza et al., 2013; Six et al., 2000). Consequently, all those management practices that protect macroaggregate from disruption (e.g., reduced tillage) favour C accumulation in the microaggregates and in the silt and clay fraction inside the macroaggregates ensuring long-term C sequestration (King et al., 2019, Six et al., 2000, Christensen, 1996; Puget et al., 1995) and N stock enhancement (Matus, 2021).

One powerful tool used to evaluate the stabilization degree of C and N in the soil physical fractions is the measurement of their isotopic composition. Several metabolic processes in C and N cycles cause isotopic fractionation with the loss from the system of the lighter isotope and the preferential utilization of the heavier isotope (Ågren et al., 1996; Nadelhoffer and Fry, 1988). Therefore, a progressive enrichment in ^{13}C and ^{15}N of the C and N from the fresh residues to the soil organic matter (SOM) is observed (Fernandez et al., 2003); this shift in the isotopic composition generally increases with depth (Nadelhoffer and Fry, 1988; Wang et al., 2015). At the same time, heavier isotope enrichment occurs from the coarsest to the finest physical fractions, where the associated C and N are more microbially processed and more stabilized (Cadisch et al., 1996; Roscoe et al., 2001; Gunina and Kuzyakov, 2014).

The orchard agro-ecosystem combines a continuous input of C and N from root turnover and leaf litter with reduced tillage that slows down the mineralization of TOC and N, allowing their aggregation in the macro- and micro-aggregates. In this agricultural context, repeated compost application represents another C and N input that could be accumulated and stabilized through these mechanisms, either directly or indirectly, after being used as energy source and nutrient by soil microorganisms for their growth and activity.

Long-term experiments at Rothamsted Research, United Kingdom (Poulton et al., 2018) on three soil types, provided 114 treatment comparisons over 7–157 years and evidenced that in most plots soil TOC stock increased more than 7‰ per year (or 5‰ in soils with larger initial TOC content) in the 0–0.23 m soil layer. Moreover, in a synthesis on recent scientific manuscripts (Tiefenbacher et al., 2021), it was evidenced that mineral fertilization decreased the TOC stock at a rate of −198 kg C ha^{-1} y^{-1} while compost supply induced an average C sequestration potential of 714 kg C ha^{-1} y^{-1}. Furthermore, increases of 3.73 Mg C ha^{-1} y^{-1} were observed as a consequence of organic fertilizer application in orchards worldwide (Hu et al., 2022).

In a previous paper, Baldi et al. (2018) demonstrated that the yearly application of compost at a rate of 10 Mg ha^{-1} during the 14-years orchard lifetime promoted a stock of C in soil up to 54.8 Mg ha^{-1}, significantly higher than the 1.75 Mg ha^{-1} stocked with mineral fertilization.

Soil TOC increase was observed in the whole soil profile where compost was applied, demonstrating the potentiality of organic amendments in enhancing C storage in orchard ecosystem (Baldi et al., 2018). In detail, TOC increased, in the shallowest soil layer (0–0.15 m) from 13.1 g kg^{-1} in control plots to 42.3 mg kg^{-1} in compost at 10 Mg ha^{-1}; while in the deepest soil layer (0.45–0.65 m) from 6.93 to 11.3 mg kg^{-1} in control and compost respectively (Baldi et al., 2018). Similarly, the supply of compost also promoted the buildup of N pools in the soil (Toselli et al., 2019) and increased the N potentially available, which is able to guarantee high chemical fertility over time. However, the degree of stabilization of the C and N accumulated in soil over the 14 years of the experiment remains to be verified.

Therefore, this research investigates the distribution of the C and N accumulated over the 14 years of compost amendment into the different aggregate soil fractions and their stabilization degree by measuring C and N isotopic composition. Considering that the increase in δ^{13}C and δ^{15}N signature is indicative of the transformation and fluxes of C and N through microbial biomass that led to their stabilization, we hypothesized that: (i) repeated compost application stimulating the increase of the microbial biomass would lead to a δ^{13}C and δ^{15}N enrichment of the C and N associated to the different aggregate fractions; (ii) heavier isotope enrichment would occur in the finest aggregates and the deepest soil layer.

2. Material and methods

2.1. Experimental site and treatments

The trial was conducted in the south-eastern part of the Po valley (44°27′ N; 12°13′ E) on a Calcaric Cambisol (WRB, 2014) soil (main characteristics are reported in Table S1) of a nectarine [*Prunus persica*, Batsch var. *nucipersica* (Bockh.) Schn.] orchard of the variety Stark RedGold grafted on hybrid GF677 (*Prunus persica* x *Prunus amygdalus dulcis*). The trees, trained as in a "delayed-vasette" system, were planted with a distance of 5 m between row and 3.8 m between plants along the row for a total of 526 plants ha^{-1} (Baldi et al., 2018). Since its plantation (2001) the orchard was subjected to the following treatments:

1 Unfertilized control (Control);

2. mineral fertilization (Mineral), including phosphorous (P) and potassium (K) applied only at planting at 100 and 200 kg ha^{-1}, respectively. Nitrogen (N) was supplied at a rate of 70 kg ha^{-1} in 2002 and 2003; in 2004, the N application rate increased to 120 kg ha^{-1} yr^{-1}, and then to 130 kg ha^{-1} yr^{-1} from 2006 to the end of the commercial life of the orchard (2014). Nitrogen was applied as ammonium nitrate (N = 35 %), while P and K were applied as a binary fertilizer (P = 10%; K = 20%); no other nutrients were provided with mineral fertilization;

3. fertilization with compost (Compost) at 10 Mg dry weight (DW) ha^{-1} yr^{-1} corresponding to 240 kg N ha^{-1} yr^{-1}. Compost was applied on the 2-m wide tree row and tilled into the soil to a depth of 0.25 m. The compost used in this experiment was classified as municipal solid waste deriving from the combination of domestic organic wastes (50%) with pruning material from urban ornamental trees and garden management (50%) after a 3-month stabilization and had on average: N 21.1 g kg^{-1} DW, organic C 234 g kg^{-1} DW, and a C/N ratio of 10 (for complete analysis see Baldi et al., 2018).

Mineral fertilizer and compost were split in May (60%) and September (40%). All the treatments were compared in a complete randomized block design with four replicates; each plot was of 6 trees and measurements were taken from the 4 central plants (Figure S1).

2.2. Soil collection and analysis

At the end of the commercial life of the orchard (December 2014) a total of 48 soil samples (3 treatments x 4 depth x 4 replications) were collected with an auger at 0–0.15, 0.16–0.25, 0.26–0.45, and 0.46–0.65 m of depth in the tree row. These layers do not correspond to soil horizons. Each sample consisted of 4 sub-samples collected between two trees of the row. Soil samples were stored at 4°C and before analysis, they were sieved through a 2 mm sieve and cleaned from roots and visible plant residue.

Soil aggregates fractionation was carried out according to the method of Elliott (1986), modified by Gioacchini et al. (2016). Briefly, an aliquot of 5 g of air-dried soil samples was used to separate the aggregate fractions by wet sieving on two sieves in sequence (250 μm and 53 μm mesh, respectively). Samples were submerged in deionized water at room temperature with a slacking time of 10 min, then sieves were moved up and down for 20 min to separate water-stable aggregates. The aggregate fractions were divided as follows: particles with diameter > 250 μm were considered macroaggregates (MACRO); those with a diameter between 250 μm and 53 μm were the microaggregates (MICRO); those smaller than 53 μm were silt and clay (SC). Once dried to constant weight, the fractions were weighted and analyzed for TOC, total nitrogen (TN), ^{13}C, and ^{15}N abundance by an elemental analyzer (Flash 2000, Thermo Fisher Scientific) coupled with an isotopic ratio mass spectrometer (Delta V Advantage Thermo Fisher Scientific), after a pre-treatment with few drops of 6 M HCl to eliminate the carbonates. The isotopic composition of organic C and N of the unfractionated soil was also measured with the same procedure.

The isotopic composition of C and N were expressed as delta values, δ^{13}C ‰ and δ^{15}N ‰ and calculated as follow:

$$\delta^{13}C(‰) = \left[\left(\frac{R_{sample}}{R_{std}} \right) - 1 \right] \times 1000 \qquad (1)$$

where, R_{sample} is the isotope ratio ^{13}C/^{12}C of the sample and R_{std} is the ^{13}C/^{12}C ratio of the international standard (Vienna PeeDee Belemnite).

$$\delta^{15}N(‰) = \left[\left(\frac{R_{sample}}{R_{std}} \right) - 1 \right] \times 1000 \qquad (2)$$

where, R_{sample} is the isotope ratio ^{15}N/^{14}N of the sample and R_{std} is the ^{15}N/^{14}N ratio of the international standard (N$_2$ atmospheric air).

2.3. Data handling and statistical analysis

The parameters analyzed for every soil layer were the weight of the fractions, the organic C (C_F) and N (N_F) content of the fractions, the relative distribution of soil total organic C (C_T) and N (N_T) in the three fractions, and the δ^{13}C and δ^{15}N ‰ of both unfractionated soil and fractions.

$$C_F(gC_{FRACTION}kg_{soil}^{-1}) = M_F \times C_{fraction} \qquad (3)$$

$$N_F(gN_{FRACTION}kg_{soil}^{-1}) = M_F \times N_{fraction} \qquad (4)$$

where, M_F is the fraction weight, $C_{fraction}$ is the C concentration in the fraction and $N_{fraction}$ is the N concentration in the fraction.

$$C_T(\%) = \frac{C_F}{TOC} \times 100 \qquad (5)$$

$$N_T(\%) = \frac{N_F}{TN} \times 100 \qquad (6)$$

where, C_F and N_F are the C and N content of the fractions, TOC is the total organic C of the unfractionated soil (data from Baldi et al., 2018) and TN is the total N in unfractionated soil (data from Toselli et al., 2019).

Data were analysed using the software SAS (SAS Institute Inc., Cary, North Carolina, USA), as in a factorial experimental design with treatment (3 levels: control, mineral, compost) and soil depth (4 levels: 0–0.15 m, 0.16–0.25 m, 0.26–0.45 m and 0.46–0.65 m) as factors. When analysis of variance showed a statistically significant (P 0.05) effect of treatment, Student Newman-Keuls (SNK) test separated the means. On the other hand, when interaction between factors was significant, 2 times standard error of means (2SEM) was used as the minimum difference between two means statistically different for P ≤ 0.05.

In addition, Pearson correlation analysis was performed to evaluate the linear relationship between C_T and N_T content for each aggregate fraction.

3. Results

3.1. Distribution of soil aggregate fractions

Fertilization treatment and depth did not significantly interact for the weight of the physical fractions, thus only the main effects are reported in Fig. 1. The long-term application of compost induced a significant increase of 60% in the MACRO compared to mineral fertilization and control; the opposite was observed for the SC that diminished by 22% compared to mineral and control (Fig. 1A). The microaggregates were not influenced by the fertilization strategy.

The amount of the MACRO decreased with depth showing the highest values (250 g kg^{-1} soil) at 0–0.15 m, intermediate values ranging between 200 and 170 g kg^{-1} of soil between 0.16 and 0.45 m, and the lowest at 0.46–0.65 m (125 g kg^{-1} of soil) (Fig. 1B). The value of MICRO was around 400 g kg^{-1} of soil and did not significantly vary with depth; while the SC fraction increased with soil depth (Fig. 1B). In detail, the shallowest (0–0.15 m) and the deepest (0.46–0.65 m) soil layers had respectively the lowest and the highest values (250 g kg^{-1} of soil the shallowest and 430 g kg^{-1} of soil the deepest one) while between 0.16 m and 0.45 m of depth, intermediate values were measured.

3.2. Organic C, N content and C_F/N_F ratio of the aggregate fractions

The application of compost increased the C_F and N_F content of the MACRO compared to the other two treatments at all depths. In particular, C_F and N_F content in the compost treatment were five and six-fold higher than in the control and mineral in the two top layers and three times higher in the two deepest layers. In all the fertilization treatments,
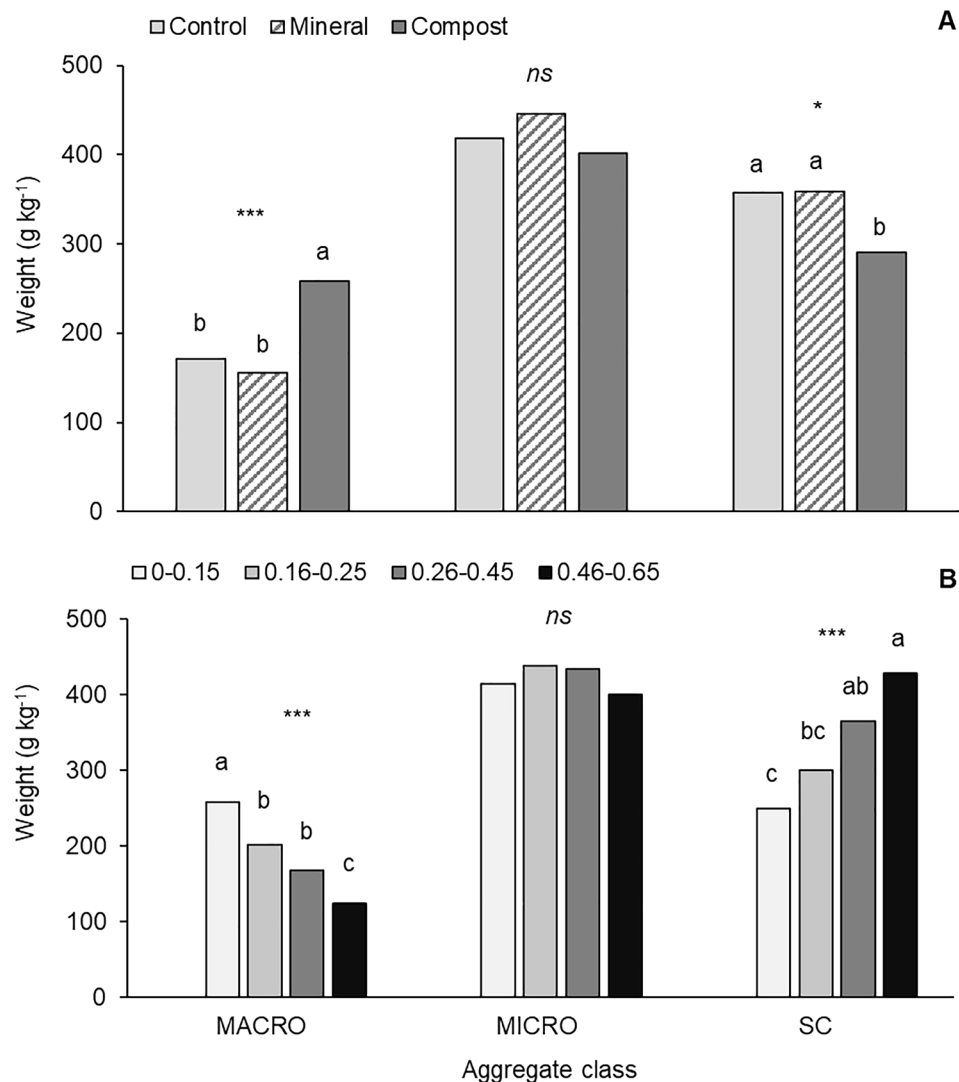
Fig. 1. Effect of fertilization treatment (A) and soil depth (B) on weight distribution (g kg^{-1}) of the soil aggregates (MACRO, MICRO, and SC) (Treatment x Depth = n.s.). Different letters represent significant differences (P≤0.05).

however, C_F and N_F content of the MACRO was higher at 0–0.25 m than in other soil layers with values progressively decreasing with depth (Fig. 2A-2B). In compost plots the values ranged between 22.5 g kg^{-1} for C_F and 2.8 g kg^{-1} for N_F in the first layer and 4.8 g kg^{-1} for C_F and 0.4 g kg^{-1} for N_F in the deepest one (Fig. 2A-2B). In control and mineral C_F and N_F content of the MACRO was similar and ranged between 4 g kg^{-1} for C_F and 0.4 g kg^{-1} for N_F in the first layer and 1 g kg^{-1} and 0.15 g kg^{-1} in the deepest one. A similar trend was also observed for the MICRO (Fig. 2C-2D), with compost supply determining a significant increase of C_F and N_F content compared to mineral and control in the two top layers. Values equal to 12.5 g kg^{-1} and 1.6 g kg^{-1} respectively for C_F and N_F were measured in the first layer in the compost treatment, while values of 5 g kg^{-1} and 0.5 g kg^{-1} of C_F and N_F respectively in mineral and control without significant differences between these two treatments. From the top to the deepest soil layer, the C_F and N_F significantly decreased in compost and mineral, with values different at each sampling depth. In control the two intermediate layers showed similar values (Fig. 2C-2D). The C_F and N_F content of the SC showed different trends than those observed for MACRO and MICRO (Fig. 2E-2F) with values around 0.5 g kg^{-1} for C_F and 0.3 g kg^{-1} for N_F and no significant differences due to the treatments, soil depth or their interaction.

In the MACRO, the C_F/N_F ratio (given by the ratio between C and N of the fraction) of control was not influenced by depth (Fig. 3A); in

mineral, the C_F/N_F at 0–0.25 m was higher (about 9) than that of the deepest layers (about 8), while in compost, the highest value was measured at 0.46–0.65 (about 9), and no differences were observed in the other soil layers (Fig. 3A). In the shallowest soil layer, the C_F/N_F ratio of the MICRO showed values around 7.4 and was not influenced by the fertilization treatment (Fig. 3B). In SC the C_F/N_F ratio decreased compared to the other two aggregate fractions. In control plots the value did not change with depth; on the contrary in the mineral treatment, a significant decrease with depth from 7.4 (in the first layer) to 4.8 (in the deepest one) was measured (Fig. 3C). In the compost treatment the highest C_F/N_F ratio was found in the first layer (7.6), then lower values, similar to those of the control, were measured between 0.16 and 0.65 m.

3.3. Relative distribution of soil total organic C and N in the three fractions

Fertilization treatment and depth did not significantly interact for the relative distribution of soil total organic C (C_T) and N (N_T) in the three fractions; therefore, in Table 1 only the main effects are reported. These parameters can be referred to also as the contribution of C and N content in the three fractions to the total soil organic C (TOC) and N (TN) and were influenced by the treatment and depth. The MACRO, in the compost treatment, contributed to TOC for 45.9% and were significantly
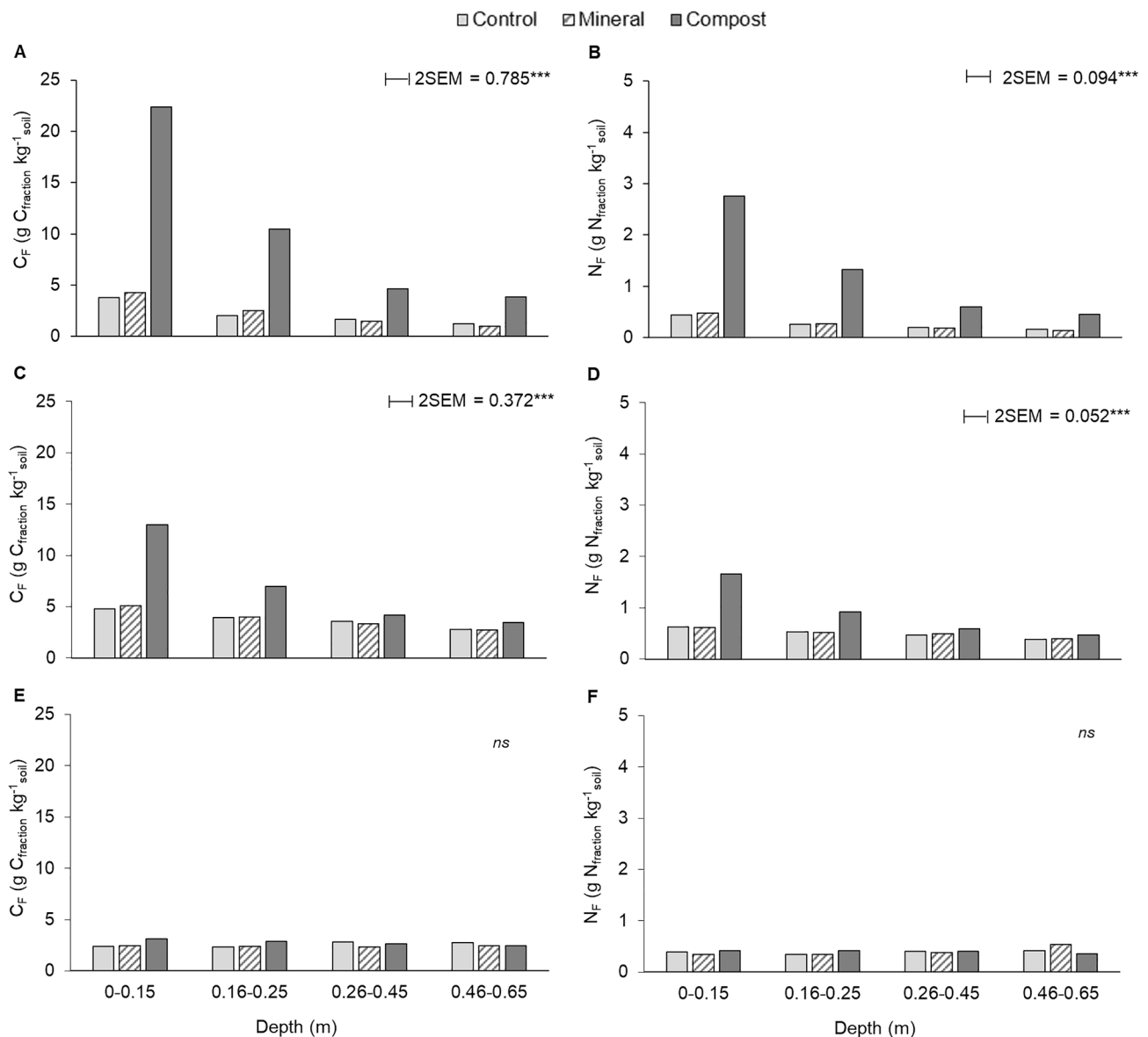
Fig. 2. Carbon (C_F) and nitrogen (N_F) content of the MACRO (A-B); MICRO (C-D) and SC (E-F) soil aggregates as affected by the treatment and depth. 2SEM = 2 times standard error of means used as the minimum difference between two means statistically different for $P \leq 0.05$.

higher than mineral and control (Table 1). The highest C_T in the MICRO was found in control (44%), whereas lower values were measured in mineral and compost (37.4 and 35.3% respectively). In the SC the highest values were found in control (31.8%), followed by mineral and compost (Table 1). As for C_T, also N_T showed higher values in compost (43.4%) than control (21.7%) and mineral (19.3%) for the MACRO, the opposite was observed for MICRO and SC (Table 1). The MACRO showed the highest C_T and N_T values in the shallowest layer (40.1% and 37.9% respectively), then a significant decrease was observed with no differences in the deepest layers. The pattern for SC was the opposite, and the highest values (33.8% for C_T and 39.9 for N_T) were measured in the deepest layer (0.46–0.65 m), then a significant decrease was measured in the top layers (16.2% for C_T and 18.7 for N_T). No significant difference was observed for the MICRO fraction with values of about 39 % for C_T and 40 % for N_T (Table 1).

C_T and N_T were positively correlated for all the fractions (Fig. 4). In the MACRO (Fig. 4A), the C_T and N_T of compost treatment were spread in the highest part of the curve, while control and mineral were overlapped in the bottom curve section. In the MICRO, no clear separation among the treatments is observed (Fig. 4B), while in the SC fraction the

values of compost were in the lowest part of the curve and those of mineral and control in the highest (Fig. 4C).

3.4. C and N isotopic composition of unfractionated soil and aggregates

Compost fertilization induced a ^{13}C enrichment of the TOC in the unfractionated soil that showed less negative $\delta^{13}C$ values (-26.6 $\delta^{13}C$) compared to mineral and control (-27.1 $\delta^{13}C$) (Table 2). Overall, the isotopic signature was lower (^{13}C-depleted) at 0–0.15 m (-27.4 $\delta^{13}C$) than at all the other sampling depths (-26.8 $\delta^{13}C$); values at 0.16–0.25 and 0.26–0.45 were not significantly different and were higher than those measured at 0.46–0.65 m (Table 2). The control was characterized by $\delta^{15}N$ values (6.22) higher than those measured in mineral and compost (4.22 and 5.23 respectively). $\delta^{15}N$ values decreased from the top to the deepest soil layer with the lowest value (4.44 $\delta^{15}N$) at 0.46–0.65 m (Table 2).

In control and mineral treatments, the aggregate fractions showed a very similar pattern of $\delta^{13}C$ in all the layers. In general the MACRO were characterized by the lowest $\delta^{13}C$ values, generally similar to those of the unfractionated soil, while MICRO and SC showed a progressive
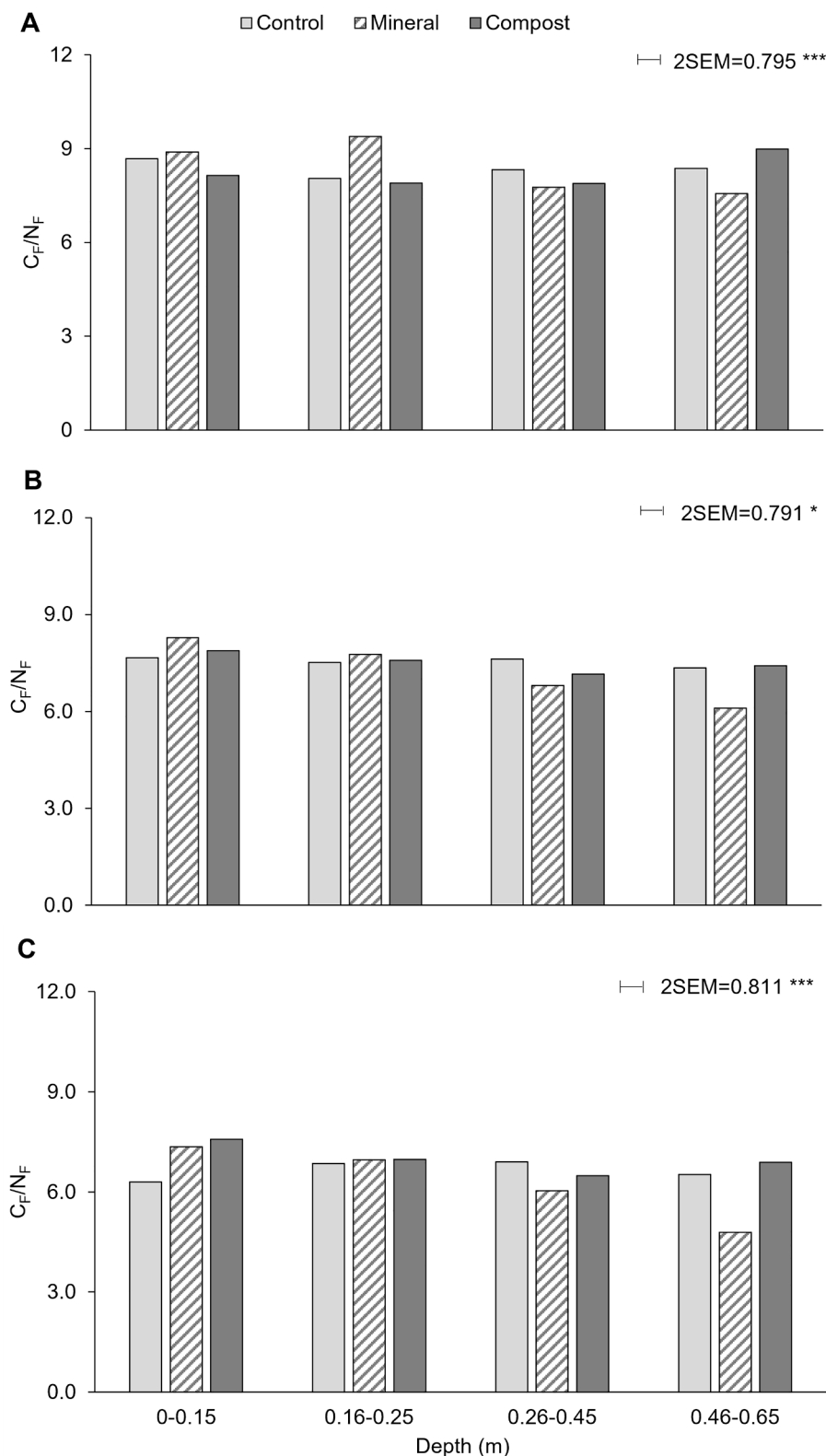
Fig. 3. Effect of fertilization treatment and soil depth on C_F/N_F ratio of MACRO (A), MICRO (B) and SC (C) soil aggregates. 2SEM = 2 times standard error of means used as the minimum difference between two means statistically different for $P \leq 0.05$.

enrichment in ^{13}C. In control, this shift from MACRO to SC ($\Delta\delta$) was similar in all the layers ranging between 0.91 in the top layer and 1.07 in the deepest one, while in the mineral the highest $\Delta\delta$ (1.25) was measured in the top layer, then it decreased to 0.96 in the second one and to 0.78 between 0.26 and 0.65 m. In the soil treated with compost

the three fractions were characterized by similar δ^{13}C values, in the first layer comparable to those of the unfractionated soil (-26.7 δ^{13}C). From of 0.16 to 0.65 m depth we observed a progressive increase of the δ^{13}C values from MACRO to SC ($\Delta\delta$), from 0.66 in the second layer, to 1.24 and 1.42 in the third and fourth layer, respectively (Fig. 5). Moreover, In

Table 1

Relative distribution of unfractionated soil carbon and nitrogen in the different soil aggregates (C_T and N_T, respectively).

TREATMENT	C_T (%)			N_T (%)		
	MACRO	MICRO	SC	MACRO	MICRO	SC
Control	24.2 b	44.0 a	31.8 a	21.7 b	43.3 a	35.0 a
Mineral	20.7 b	37.4 b	25.3 b	19.3 b	41.8 a	33.7 a
Compost	45.9 a	35.3 b	18.8 c	43.4 a	35.9 b	20.7 b
Significance	***	***	***	***	*	***
DEPTH (m)						
0–0.15	40.1 a	37.5	16.2 c	37.9 a	37.8	18.7 d
0.16–0.25	32.8 b	40.8	22.6 b	30.2 b	40.8	24.8 c
0.26–0.45	25.0 c	39.1	28.7 a	23.4 c	41.1	33.2 b
0.46–0.65	23.3 c	38.2	33.8 a	21.0 c	41.5	39.9 a
Significance	***	n.s.	***	***	n.s.	***
Treatment x Depth	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s., *, *** effect not significant, significant at P ≤ 0.05 and P ≤ 0.001, respectively. Different letters represent significant differences (P ≤ 0.05) according to the Student Newman-Keuls (SNK) test.

the two deepest layers (0.26–0.65 m), the $\Delta\delta$ between the unfractionated soil and the MACRO progressively increased due to the ^{13}C enrichment of the unfractionated soil TOC while the C isotopic signature in the MACRO remained similar in all soil layers. The MICRO was always characterized by the same $\delta^{13}C$ values of the unfractionated soil TOC, whereas the SC, with depth, progressively shifted towards less negative values than the unfractionated soil TOC (Fig. 5).

In control soil, all the fractions in the two top layers showed values ranging from 2.66 and 4.89 $\delta^{15}N$ and were ^{15}N-depleted compared to the unfractionated soil (6.2); at 0.26–0.65 the fractions, were characterized by $\delta^{15}N$ values not different or slightly higher than those of the unfractionated soil. The mineral treatment showed a ^{15}N depletion of MACRO and MICRO compared to the unfractionated soil in the top layer, the SC was similar to the unfractionated soil. In the two intermediate layers a progressive convergence of the $\delta^{15}N$ values of the fractions to the $\delta^{15}N$ value of the unfractionated soil was observed, while in the deepest soil layer, all the fractions were ^{15}N-enriched (5.5) compared to the unfractionated soil's (3) (Fig. 6). In the mineral treatment, a clear and progressive ^{15}N enrichment from the large to the finest aggregate fractions was observed only in the first two layers.

Compost supply induced more homogeneous $\delta^{15}N$ values among the fractions and in all the layers that were closer to those measured in the unfractionated soil (Fig. 6).

4. Discussion

4.1. Distribution of soil aggregate fractions

The repeated C inputs with compost fertilization led, after 14 years, to the increase of 60% of macroaggregates and the 20% reduction of the silt and clay fraction, in line with the results of previous research (Mikha and Rice, 2004; Ghosh et al., 2016; Mangalassery et al., 2019). In detail, Mangalassery and co-authors (2019) observed, compared to control, an increase of macroaggregates by 18–52% after 5 years of application of manure, biofertilizer, and organic amendments. In addition, Mikha and Rice (2004) evidenced a 38% increase of this fraction after 10 years in a no-tillage system with continuous cultivation of corn and a 57% increase in the same system as a consequence of manure supply. In the same experiment, the supply of manure induced a reduction of the silt and clay fraction by 18% (Mikha and Rice, 2004). Moreover, a linear relationship between the C input and the increase in aggregation was reported by Kong and co-authors (2005).

In all treatments, the shallowest soil layer (0–0.15 m) was characterized by the highest amount of macroaggregates mainly due to the higher amount of organic C, supplied from compost and orchard litter, incorporated in the first 0.25 m depth. In the control and mineral fertilized soil, the amount of the macroaggregates was lower than in compost since it was only related to the decomposing litter that yearly accumulates in the orchard. These organic matter sources directly act as a nucleus for the formation of macroaggregates and as energy substrates for the growth and activity of the microbial biomass that contributes to macroaggregate formation. With the increasing depth, the C input from compost and plant residues decreased, leading to a general 50% reduction of the macroaggregates observed in the deepest layer.

According to the model proposed by Six et al. (2000), the C and N accumulated can be stabilized in the finer fractions (i.e., microaggregates and silt and clay fractions) occluded inside the macroaggregates (King et al., 2019). In microaggregates, organic compounds are physically protected by enzyme accessibility and oxygen diffusion and can accumulate longer, while in the silt and clay fraction, they are stabilized through chemical adsorption on the mineral surface (Six et al., 1998; 2000). The repeated input of compost and the scarce soil disturbance favored this mechanism and allowed to enhance C and N content in the macroaggregates between three and six times compared to the other two treatments that did not receive extra C input. Other authors also found significant increase in the C and N content of macroaggregates as a consequence of the repeated input of organic material such as crop residues (Zhao et al., 2019) or organic amendments (Zhang et al., 2021) confirming that extra C input first accumulates in this aggregate fraction.

Compost addition also induced higher level of microbial biomass and soluble C as measured previously by Baldi and co-authors (2018). The enhancement of these parameters favors the formation of the aggregates (Bissonnette et al., 2001, Czachor et al., 2015) and the build-up of microbial C and N necromass. Indeed, microbial necromass, thanks to its resistance to degradation and ability to stabilize rapidly (Kallenbach et al., 2016; Kopittke et al., 2018), could effectively contribute to soil C and N accumulation and stabilization. Moreover, the continuous organic amendment can induce a shift in the microbial community towards the fungal population leading to a higher amount of microbial necromass in soils (Ye et al., 2019). Although to a less extent, also in the deeper layers the supply of compost enhanced by three times the amount of C and N accumulated in the macroaggregates. The particulate organic matter derived from the compost itself may have been transferred and redistributed from the top to the deeper layers by the action of mesofauna, promoting the C accumulation also in soil deeper layers, that are important for creating C stock (Tautges et al., 2019). Therefore, in the compost treatment, almost half of the soil TOC and TN were contained in the macroaggregates while, in the control and mineral treatments, the fraction of C_T and N_T in this fraction was significantly lower only ranging between 20 and 24%.

The microaggregates fraction was not affected by the treatment nor by soil depth confirming previous results (Ashagrie et al., 2007). The effects of soil management techniques on the microaggregates fraction and the C and N retained within them were lower than those on macroaggregates since the binding agents of the microaggregates were stronger (Christensen, 2001). The microaggregates were also able to accumulate higher amount of C and N in the compost treatment than in control and mineral, but only in the two surface layers. This fraction is generally less affected by C addition than the macroaggregates fraction (Sarker et al., 2022; Choudhury et al., 2014; Benbi and Senapati, 2010). Therefore, the percentage of TOC (C_T) and TN (N_T) contained in the microaggregates was around 35%, and was lower than that in the macroaggregates. On the contrary, in the other two treatments where the amount of macroaggregates was lower, the microaggregates fraction contained the highest percentage of TOC (C_T) and TN (N_T) and played a major role in stabilizing C and N. From macroaggregates to microaggregates we also observed a decreasing trend in C_F/N_F ratio indicating
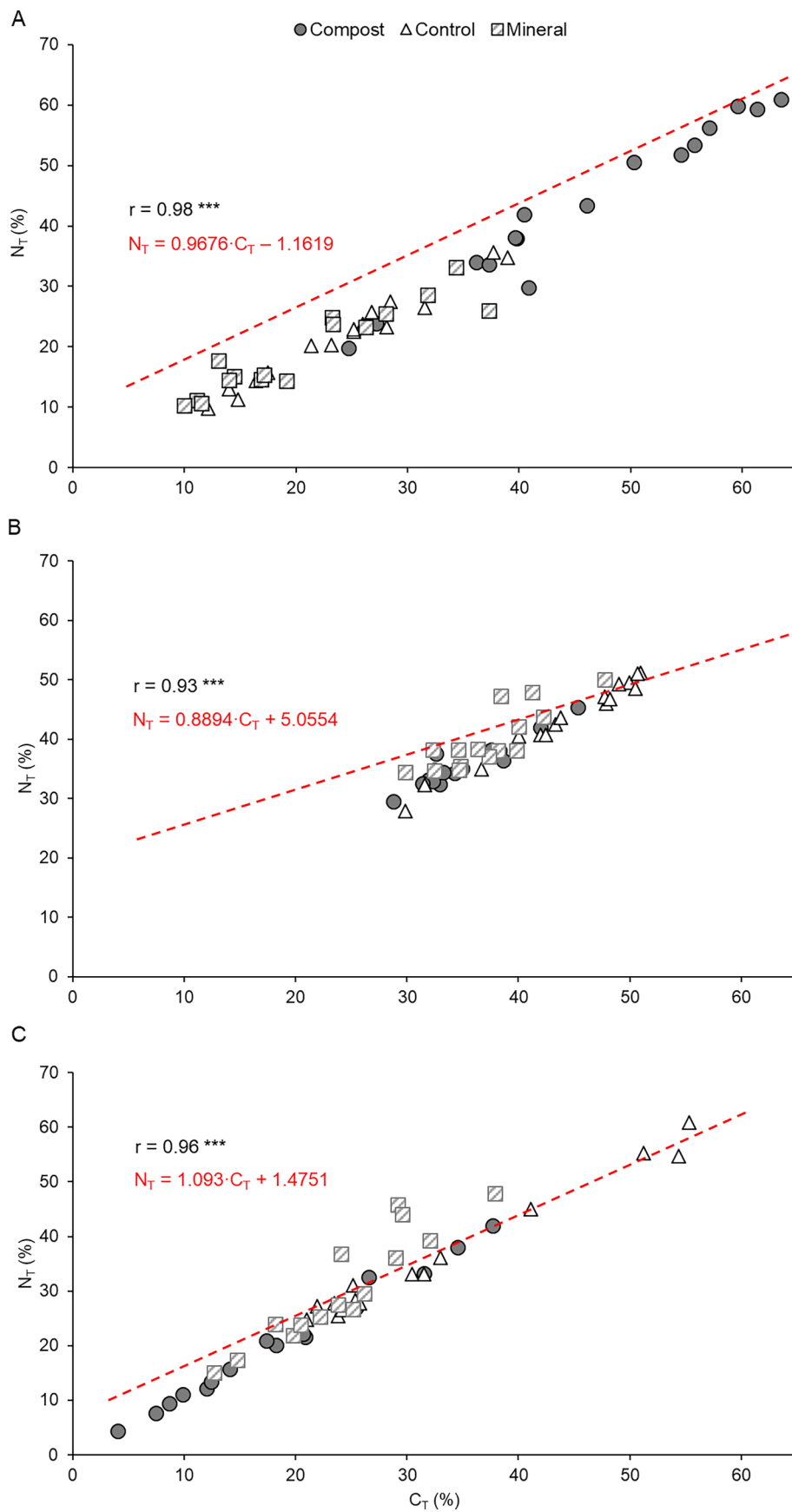
Fig. 4. Relationship between carbon (C_T) and nitrogen (N_T) in MACRO (A), MICRO (B) and SC (C) aggregates. r = Pearson correlation coefficient.

Table 2

Effect of fertilization treatment and depth on unfractionated soil $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰). Different letters represent significant differences (P < 0.05) according to the Student Newman-Keuls (SNK) test.

TREATMENT	$\delta^{13}C$	$\delta^{15}N$
Control	−27.2 b	6.22 a
Mineral	−27.1 b	4.22 c
Compost	−26.6 a	5.23 b
Significance	***	***
DEPTH (m)		
0–0.15	−27.4 c	5.62 a
0.16–0.25	−26.9 b	5.60 a
0.26–0.45	−26.9 b	5.19 a
0.45–0.65	−26.7 a	4.44 b
Significance	***	***
Treatment x Depth	*n.s.*	**

n.s., **, *** effect not significant, significant at P ≤ 0.01 and P ≤ 0.001, respectively. Different letters represent significant differences (P ≤ 0.05) according to the Student Newman-Keuls (SNK) test.

that in the microaggregates fraction the organic substrates were processed by the microbial biomass (Elliott, 1986). Lower C/N ratio in microaggregates than in macroaggregates was found also by Zhao and co-authors (2019) after eight year of crop residues return.

The silt and clay fraction was significantly lower in the compost treatment compared to the control and mineral, and it increased with depth, with a trend opposite to that of the macroaggregates. The reduction observed in the present experiment was around 14–17% very similar to that observed by Mikha and Rice (2004) that evidence a reduction of 18% as a consequence of manure supply.

In the finest fraction, the accumulated organic compounds were mainly of microbial origin (Plaza et al., 2013) and the further decreasing trend of SC C_F/N_F ratio from the other two fractions is indicative of this origin. According to the model proposed by Cotrufo and co-authors (2013), the C and N in the silt and clay fraction mainly derived from labile components of plant residues which were processed by microbial biomass and released as microbial by-products. These were then stabilized by chemical interaction with the soil organo-mineral complexes. The repeated addition of compost, led to the highest concentrations of dissolved organic C and microbial activity in the orchard (Baldi et al., 2018), therefore guaranteeing the most favorable condition for the organic compounds' stabilization through chemical interaction on the silt and clay fraction. The C and N associated to this fraction were stabilized through persistent binding agents (Christensen, 2001), and thus were the most stable C and N in soil (Hassink and Whitmore, 1997;
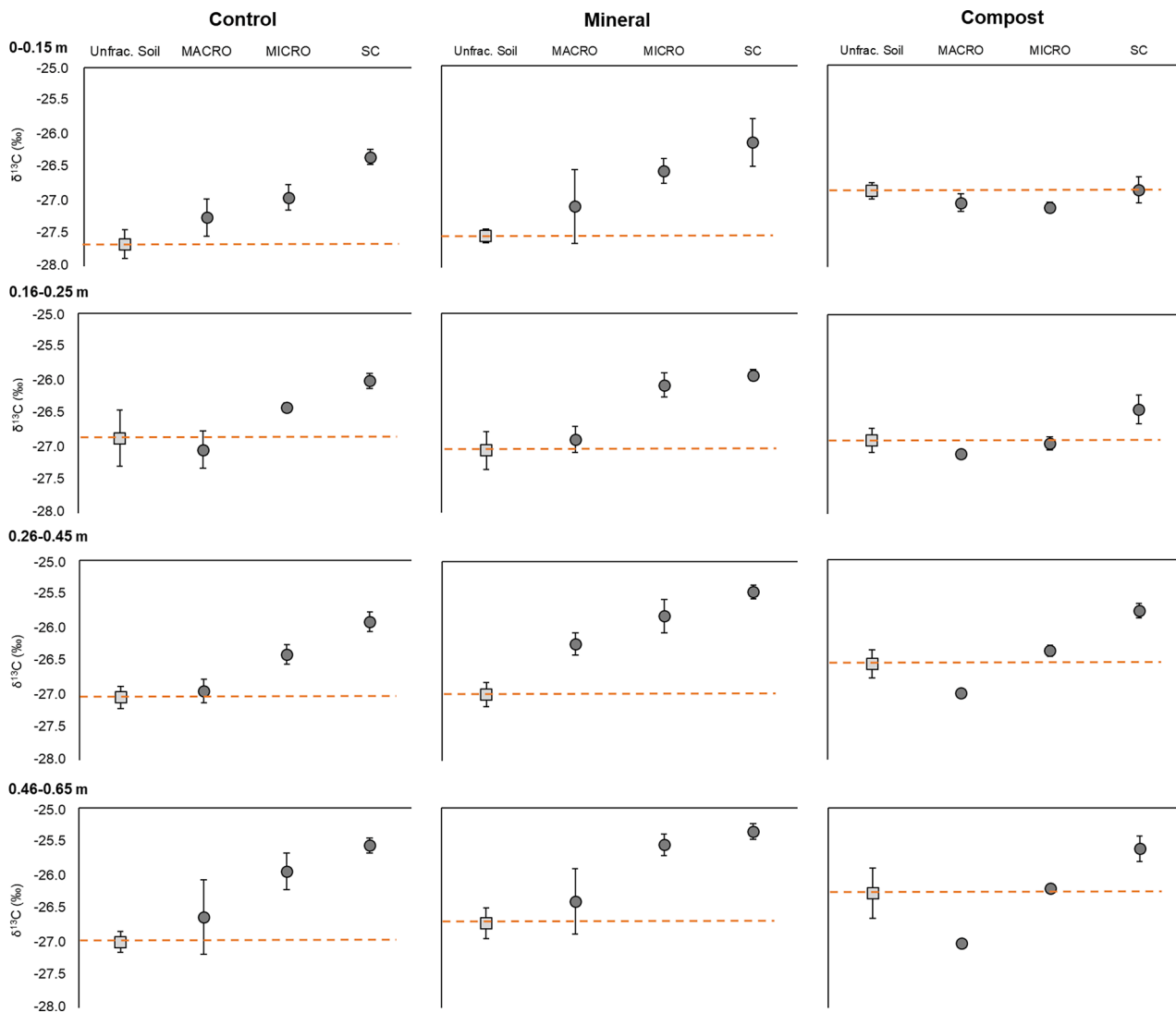

Fig. 5. $\delta^{13}C$ of unfractionated soil (Unfrac. Soil) and soil aggregates (MACRO, MICRO, and SC) in the different treatments and at different depth. All the values are means of four replicates and bars represent standard deviation. The empty square and the dashed line indicate the level of $\delta^{13}C$ of unfractionated soil. The full circle represents the values of $\delta^{13}C$ in the soil aggregates.
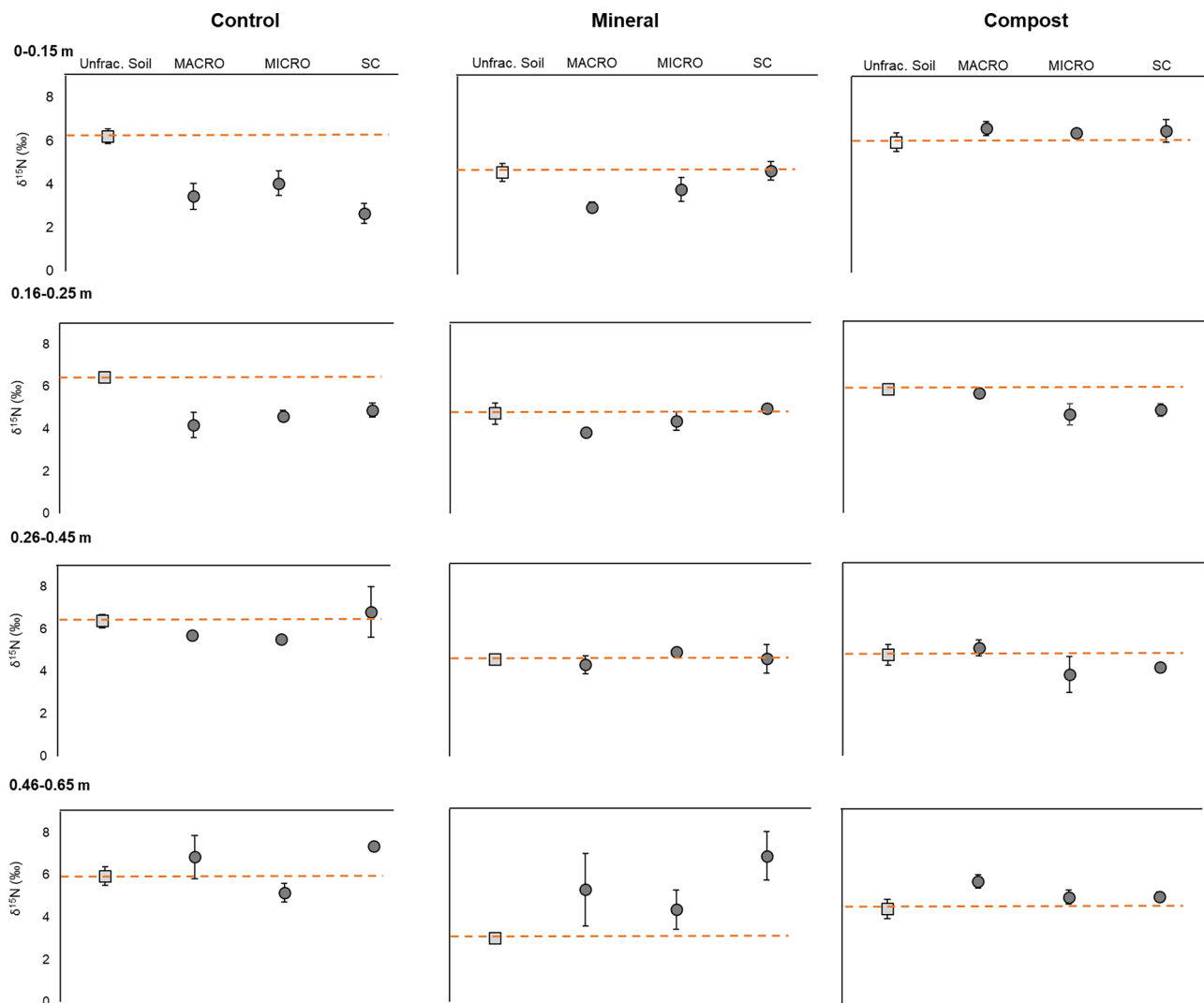
Fig. 6. δ^15N of unfractionated soil (Unfrac. Soil) and soil aggregates (MACRO, MICRO, and SC) in the different treatments and at different depth. All the values are means of four replicates and bars represent standard deviation. The empty square and the dashed line indicate the level of δ^15N of unfractionated soil. The full circle represents the values of δ^15N in the soil aggregates.

Stewart et al., 2008; Matus, 2021).

4.2. δ^13C of soil and aggregate fractions

The C isotope composition of soil TOC reflects that of the plant with a shift towards a ^{13}C enrichment (Nadelhoffer and Fry, 1988; Melillo et al., 1989; Šantrůčková et al., 2000) that usually increases with depth (Bird and Pousai, 1997). This change in δ^13C may depend on the selective use by the microbial biomass of molecules with different isotope compositions and on the isotope fractionation that accompanies the microbial metabolism that preferentially uses, for biomass growth, molecules enriched in ^{13}C (Nadelhoffer and Fry, 1988, Šantrůčková et al., 2000). The δ^13C of soil TOC in the control and mineral treatment was similar and resulted from the sole input of C from trees that had an isotopic signature of −28 δ^13C‰. On the contrary, the repeated compost input led to an enrichment in ^{13}C of soil TOC compared to the other two treatments. This difference could be in the isotopic signature of the C added with the compost itself that was on average equal to −26 δ^13C‰ and/or in the transformation processes mediated by the microbial biomass and the associated isotopic fractionation that the repeated addition of compost induced. In addition, the repeated application of compost for 14 years may have selected a microbial community, at least partly different from that of the control and mineral treatment, thus affecting the isotopic composition of soil TOC (Zhang et al., 2015; Miao et al., 2021). However, from the top to the deepest layer, an enrichment in ^{13}C due to the presence of more processed organic matter in the deepest layers was observed in all the treatments in agreement with other authors (De Clercq et al., 2015; Wang et al., 2015).

The three aggregate fractions also showed a ^{13}C enrichment from the macroaggregates to the finest fraction that was evident in all the soil layers in both control and mineral treatments, whereas with compost this enrichment was not detected in the first layer and progressively increased from the second layer to the deepest one.

This shift may depend on the δ^13C value of the molecules that are preferentially stabilized and on the number of microbial utilization cycles through which lighter CO_2 is released and heavier C is stabilized (Werth and Kuzyakov, 2010). Consequently, the δ^13C value could be a helpful tool for highlighting the C sources, transformation, and flows in and between the aggregates and is indicative of the stabilization degree of C in the different fractions (Baisden and Amundson, 2002; Gunina and Kuzyakov, 2014).

This progressive stabilization of C from macroaggregates to silt and clay evidenced by the ^{13}C enrichment is also accompanied by a narrower C_F/N_F ratio of the finer fractions, in particular silt and clay, compared to the macroaggregates (Gunina and Kuzyakov, 2014; De Clercq et al., 2015). Decomposed fresh residues in the soil may act as a core for

macroaggregate formation; in the macroaggregates, the organic substrates are used by microorganisms as a source of energy for their growth and activity. The microbial residues formed, as the result of this process, are richer in N because microorganisms generally retain this element which is often the limiting factor for their growth, while part of the metabolized C is lost as CO_2. The microbial residues, richer in N and heavier C compared to the original substrates, are then physically and chemically protected by the mineral soil particles in the microaggregates and silt and clay fraction occluded inside the macroaggregates.

The breakdown of macroaggregates then led to the release of these finer fractions and their associated C, which is mainly of microbial origin and resistant to degradation (Verchot et al., 2011; Plaza et al., 2013). In a recent paper, Klink and co-authors (2022) by measuring ^{13}C of microbial residues demonstrated the greater contribution of fungal residues compared to plant residues to the more stabilized organic matter associated to the mineral fraction in a deciduous forest.

With compost, the positive shift of δ^{13}C from coarse to fine fractions was not measured in the first layer and only partly in the second one, while progressively increased in the two deepest layers. Every year during the 14 years of orchard life, compost was buried, and the soil was tilled between 0 and 0.25 m, thus these two layers received the highest amount of C from compost. The similar isotopic composition of C associated with the three fractions in the layers that directly received compost indicates that macro-, micro-aggregates, and silt and clay hold the same C source that still has to undergo enough utilization cycles by microorganisms to measure a significant ^{13}C enrichment in the finer fractions in spite of the highest amount of microbial biomass among the treatments (Baldi et al., 2018).

However, in 14 years of compost addition, not only the microbial community amount but also its composition might have changed. In 20 years of compost application, Zhang et al. (2015) found, together with an increase in aggregation, either an increase of total microbial biomass and a shift towards a microbial community with more facultative and/or obligated anaerobes and Gram-positive bacteria. This has favored the accumulation of C since anaerobes decompose organic C less efficiently than aerobes and Gram-positive bacteria release more resistant organic compounds. This shift in the microbial community could be happened also in our experimental site; however, a deep and specific study of the microbial community and its activity would reinforce this hypothesis also in consideration of the results on the microbial biomass reported in Baldi and co-authors (2018).

Between 0 and 0.60 m depth, the C associated with macroaggregates in the compost treatment had the same isotopic composition indicating the same origin of C accumulated in the coarse fraction. This results from a massive redistribution year after year of the C of compost still little transformed by soil microbial biomass. The extent of this redistribution was in such a way to keep unchanged the δ^{13}C value of C associated with macroaggregates from surface to deep layers. This observation further demonstrates that the macroaggregates are the first storage structures of fresh C that still have to undergo microbial transformations. Therefore, with depth, the δ^{13}C values of macroaggregates progressively disentangled from the δ^{13}C values of soil that became enriched in ^{13}C and from the δ^{13}C values of the microaggregates and even more of silt and clay where the microbially processed C is stabilized.

4.3. δ^{15}N of soil and aggregate fractions

The isotopic composition of soil TN exhibited a different pattern compared with that of TOC, and we did not observe the expected increase in δ^{15}N with depth, not even in the control samples where the values remained constant. In general, the mineral treatment showed the lowest δ^{15}N value among the treatments. Indeed, inorganic N fertilizers are ^{15}N-depleted and characterized by values around 0 ‰ δ^{15}N, and the repeated application of mineral fertilizer caused a general decrease in soil δ^{15}N value compared to the other two treatments. In the deepest layer, this was consistent as a possible result of the leaching of fertilizer-derived N to depth.

In the top layer of control, we observed the widest difference between the δ^{15}N of the soil TN and the values of the three fractions that were ^{15}N-depleted compared to the unfractionated soil samples. This would indicate that plant residues returning to the soil as litter, characterized by a low δ^{15}N value (-2.74 ‰), strongly contributed to the N content of the aggregates, while the soluble N components, which were not considered in the fractionation procedure, mainly derived from the mineralization of SOM that was richer in the heavier N isotope (6.22 ‰). However, the difference in N isotopic composition between the aggregate fractions and the unfractionated soil TN diminished with depth disappearing in the deepest layer, where the three fractions approached the values of the unfractionated soil. In control plots, the N input for both microorganisms and plant uptake derives from the decomposition of plant residues returning to the soil. The mineralization process is not or slightly responsible for isotopic fractionation; on the contrary, nitrification causes a consistent N fractionation that leaves in soil ^{15}N-enriched ammonium preferentially immobilized by the microbial biomass and ^{15}N-depleted nitrate that is readily taken up by plants thanks to its easy movement into the soil (Jones and Dalal, 2017).

The scarce N availability in the control treatment, might have induced more competition between plants and microorganisms, and a higher rate of SOM mineralization together with a lower level of aggregation or with a faster turnover of the aggregates, and also a higher nitrification rate accompanied by a high isotopic fractionation. Going to depth, however, the progressive ^{15}N enrichment of the N fractions means that the N was recycled many times by the microbial biomass and progressively stabilized (De Clercq et al., 2015).

In the mineral treatment, the greater N availability supported plant uptake and microbial growth, thus probably reducing microorganisms' mining activity for N. Therefore, the difference between the N isotopic composition of soil and that of the fractions was lesser than the control. Indeed, this system was strongly affected by the repeated supply of ^{15}N depleted mineral N that lowered the δ^{15}N value of the soil. However, going into depth, there was a general trend of the fractions becoming enriched in ^{15}N, opposite to that of the unfractionated soil TN. Moreover, in silt and clay fraction, that tendency was accompanied by a significant decrease in the C_F/N_F ratio, suggesting that the greater N availability with mineral fertilization favored microbial immobilization and stabilization in the finest fraction of N of microbial origin (De Clercq et al., 2015; Buckeridge et al., 2022).

In the compost treatment, the isotopic composition of the fractions was much closer to that of unfractionated soil TN in the whole soil profile. In the first layer, the three soil fractions had the same N isotope composition, thus holding the same N source. As already observed for C, also δ^{15}N value indicates that compost was occluded in the aggregates, and its N was associated with the three fractions. Then going to depth, as already seen for δ^{13}C, macroaggregates maintained a constant isotopic signature, further suggesting that the compost redistributed year after year in the soil profile was occluded in the macroaggregates. Unexpectedly the two finest fractions turned out to be less ^{15}N enriched than macroaggregates (except the first layer), regardless their associated N is supposed to be more microbially processed and stabilized. It is possible, however, that some of the N associated with the finest fraction, would derive from the selective mineralization of compost components with a different isotopic composition (Yun and Ro, 2009) or from the mineralization of the leaf litter that is another N source ^{15}N-depleted and much more available to microorganisms.

5. Conclusions

Our findings indicate that, in this context, continuous compost supply and typical orchard agronomic management can generate a positive feedback loop for soil macroaggregate formation and the associated C and N storage. Indeed, the fraction of macroaggregate was increased by 66 % compared to control and mineral treatment. The C and N content

was 5 times higher in surface and 3 times higher than in the control and mineral in deeper layers. Therefore, about half of the soil TOC and TN was accumulated in macroaggregates along the 0–0.60 m depth of this trial. The C and N content in microaggregates was 2 times higher than in the control and mineral in the first two layers. Both delta values indicate the same source of C and N associated to the macroaggregate fraction from surface to depth, suggesting a consistent compost redistribution and accumulation of compost-derived C and N also in deeper soil layers. In surface layer similarities in delta values among the coarse and finer aggregate fractions suggest that compost was just occluded and not yet transformed and recycled enough to measure a change in isotopic composition due to microbial transformations. However, the enrichment in delta values from the coarse to the fine fraction increased with depth, confirming the presence of more stabilized C and N in the finest fraction and depth.

These findings are extremely relevant from an environmental point of view since demonstrate that, in a context of reduced soil disturbance, long-term application of compost is able to build up reservoir of C and N by favoring the aggregation and the progressive stabilization playing a fundamental role in maintaining soil fertility in the long-term and in the mitigation of climate change.

CRediT authorship contribution statement

P. Gioacchini: Data curation, Formal analysis, Writing – original draft. **E. Baldi:** Data curation, Investigation, Writing – original draft. **D. Montecchio:** Data curation, Investigation, Writing – original draft. **M. Mazzon:** Data curation, Formal analysis, Writing – original draft. **M. Quartieri:** Formal analysis, Investigation. **M. Toselli:** Supervision, Writing – review & editing. **C. Marzadori:** Supervision.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.catena.2024.107968.

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