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Plant residue chemical quality modulates the soil microbial response related to decomposition and soil organic carbon and nitrogen stabilization in a rainfed Mediterranean agroecosystem

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ABSTRACT

Soils play a major role in the global carbon cycle and are crucial to the management of climate change. Changes in plant cover derived from different agricultural practices induce variations in the quality of plant residue inputs and in the soil microbial community structure and activity, which may enhance the storage and protection of organic carbon (OC) and nitrogen (N) within aggregates. The aim of this study was to assess how differences in the chemical composition of plant residues in combination with tillage management practices affect the local microbial community activity and structure, and subsequent soil aggregation and OC and N dynamics in an organic, rainfed almond (*Prunus dulcis* Mill.) orchard. In the laboratory, three types of plant residue (shoots, roots, and the combination of both) coming from different species belonging to each agricultural practice (reduced tillage, reduced tillage plus green manure, reduced tillage plus organic manure, and no-tillage) were mixed with their respective soils and the CO_2 released was measured over 243 days at 60% WHC and 28 °C. Water-stable aggregates (including microaggregates within macroaggregates), enzymatic activities related to carbon (dehydrogenase and β-glucosidase) and N (urease) cycling, and the microbial biomass and community structure through phospholipid fatty acid analysis, were measured at the end of the incubation period. Our results indicate that the chemical composition of plant residues controls the microbial community response, mediating decomposition and the incorporation of OC and N in stable aggregates. Therefore, the incorporation of labile and N-rich plant residues into the soil by reduced tillage is recommended since mixing roots and shoots from green manure increased the formation of free micro-aggregates and improved OC and N stabilization in our semiarid agroecosystem.

1. Introduction

Soils play a major role in the global carbon (C) cycle and are crucial to the management of climate change (Rumpel et al., 2018). Intensive agriculture causes land degradation and contributes to global warming due to the CO_2 emissions released to the atmosphere as a result of the conversion of native ecosystems to agricultural ones and the increase in soil organic matter (SOM) mineralization because of tillage operations (Foley et al., 2005; Howden et al., 2007; Lal, 2013). However, the adoption of sustainable soil management practices - such as reducing tillage, compost application, growing cover crops, and implementing crop residue retention measures - can contribute to the mitigation of climate change while enabling agroecosystems to be more resilient to its impacts, by enhancing C sequestration in soils (Almagro et al., 2016, 2017; Stavi et al., 2016; Chenu et al., 2018). In this regard, inter-cropping with cover crops that include leguminous species (*i.e.*, green manure) and reducing tillage intensity to allow the establishment of a native plant cover in perennial woody crops have been recently reported as the most promising and economically feasible options to simultaneously address land degradation and climate change in

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water-limited regions (Sanz et al., 2017). However, despite the well-known benefits of plant covers (either native or planted), it is still unclear which management strategy for the plant residues (e.g., leaving them on the soil surface as a mulch or incorporating them into the soil by plowing) is the most effective to increase the capacity of semiarid soils to store C and nitrogen (N) and how local environmental conditions may constrain this process.

The chemical composition of plant residues controls their decomposition and the formation of SOM, the release of organic nutrients for plant growth, and therefore the C and nutrients sequestration efficiency in agricultural soils (Moorhead and Callaghan, 1994; Cotrufo et al., 2013; Chenu et al., 2018). Generally, above-ground plant residues have a lower C:N ratio and a lower content of lignin, and therefore are more labile and decompose faster (e.g., residence times of months to years), than their below-ground counterparts. Indeed, below-ground plant biomass is generally described as more recalcitrant (*i.e.*, it has a higher content of lignin and a lower N content), and has a longer mean residence time in soil because it decomposes slowly, promoting long-term organic C sequestration in soils (Puget and Drinkwater, 2001; Kemp et al., 2003). Although long-term soil organic carbon (SOC) sequestration is a powerful strategy in terms of greenhouse gases mitigation, labile fractions of SOC are essential as they enhance: i) soil physical quality (e.g., aggregate stability largely depends on labile C; Angers and Mehuys, 1989; Cosentino et al., 2006); ii) soil fertility (e.g., they provide nutrients to plants through their mineralization); and iii) the soil microbial community composition and functionality (e.g., they represent the main trophic resource for microorganisms; Fanin et al., 2014). Therefore, increases in both the labile and stable forms of organic matter in soils are desirable to enhance OC and N sequestration and stabilization through physical and biochemical pathways (Cotrufo et al., 2015; Chenu et al., 2018), which will ultimately improve soil health (Doran and Zeiss, 2000). However, to our knowledge, no studies have addressed the interactive effects of mixing above- and below-ground plant residues of different lability on C and N dynamics and stabilization in agricultural soils, to assess the potential of different management strategies for plant residues.

The aim of this study was to assess how differences in the chemical composition of plant residues in combination with tillage management practices affect the activity and structure of the local microbial community, and subsequent soil aggregation and OC and N dynamics and stabilization. To do so, we took advantage of a long-term experiment in an organic, rainfed almond orchard in southeastern Spain, where different sustainable soil management practices have been adopted for eight years. We expected that differences in the chemical composition of plant residues would have a strong influence on the local microbial community (response), affecting its activity and structure, and consequently its associated functions, such as the production of extracellular enzymes and aggregate-binding agents that mediate the microbial decomposition efficiency and the amount of C and N stored in the aggregates of different sizes (Six et al., 2006; Wilson et al., 2009; Garcia-Franco et al., 2015; Tiemann et al., 2015). Specifically, we hypothesized that: i) the chemical composition of plant residues controls the activity and structure of the soil microbial community and thus C and N stabilization; and ii) mixing above- and below-ground biomass components will enhance the formation of soil aggregates and the proportion of C and N from the plant residues that will be stabilized in the soil.

2. Material and methods

2.1. Study site and experimental design

Our study was conducted in an organic, rainfed almond (*Prunus dulcis* Mill.) orchard, planted with a 7 m × 7 m spacing and located in the northwest of the province of Murcia (Cehegín, 38° 3′ 15″ N, 1° 46′ 12″ W; 633 m a.s.l.), in southeastern Spain. The climate is semiarid Mediterranean, with warm, dry summers and cold, relatively wet winters. The annual precipitation averages 370 mm (2000–2015; SIAM) and it is concentrated in the fall and spring months, but with great inter- and intra-annual variability. The annual temperature averages 16 °C and the mean potential evapotranspiration reaches 1200 mm yr^{-1} (calculated by the Thornthwaite method), so the mean annual water deficit is around 900 mm. The soils in the study area are relatively shallow (the average soil depth is about 30 cm) with moderate southeast-facing slopes of around 7%. They are classified as Calcisols (FAO, 2006) and have a silt-loam texture with high contents of $CaCO_3$ (~45%).

During the 14 years immediately preceding our experiment, the habitual soil management in the study site was reduced tillage (RT) to control weeds (the most common practice among farmers in this area), and therefore was used as the reference (control) treatment. In October 2008, three different soil management practices were implemented: i) reduced tillage plus green manure (RTG), ii) reduced tillage plus organic manure (RTOM), and iii) no-tillage (NT). The reduced tillage consisted of chisel plowing to 15 cm depth using a cultivator, twice a year (in fall and spring), to control weeds. The green manure was produced by seeding a mixture of common vetch (*Vicia sativa* L.) and common oat (*Avena sativa* L.), in a 3:1 ratio at 150 kg ha^{-1}, in early fall to provide a cover crop during winter. The organic manure consisted of goat manure (16% OC; 2.3% N; C:N = 6.95), added at 400 and 1500 kg ha^{-1} during 2009–2011 and 2012–2016, respectively, in early fall. In the NT treatment, tillage was suppressed from the beginning of the experiment and no fertilizers or pesticides have been applied since then. Due to the changes in soil management, different plant covers were established for each tillage treatment from the beginning of the experiment: i) a mix of spontaneous herbaceous plants (*Hordeum murinum* L., *Lolium perenne* L., and *Eruca vesicaria* (L.) Cav.) in the RT and RTOM treatments; ii) a plant cover dominated by the shrub *Teucrium capitatum* L. in the NT treatment; and iii) a mix of *Vicia sativa* L. and *Avena sativa* L. in the RTG treatment (Table 1). In all treatments, the plant covers were allowed to grow from mid-fall to mid-spring, when they were cut to avoid competition for

Table 1
Description of the different farming activities performed in each management practice.

Management practice	Plant cover type††	Fall activity	Spring activity
RT		Chisel ploughing to 15 cm depth	Plant cover mowing and residue incorporation into soil by chisel ploughing to 15 cm depth
RTG		Chisel ploughing to 15 cm depth Seeding a mixture of common vetch and common oat in a 3:1 ratio at 150 kg ha^{-1}	Plant cover mowing and residue incorporation into soil by chisel ploughing to 15 cm depth
RTOM		Chisel ploughing to 15 cm depth Goat manure addition at 400 kg ha^{-1} (2009–2011) or 1500 kg ha^{-1} (2012–2016)	Plant cover mowing and residue incorporation into soil by chisel ploughing to 15 cm depth
NT		None	Plant cover mowing and mulching

RT: reduced tillage combined with the presence of *H. murinum*, *L. perenne* and *E. vesicaria;* RTG: reduced tillage combined with the presence of *A. sativa* and *V. sativa* (green manure); RTOM: combined effect of reduced tillage plus organic manure addition and the presence of *H. murinum*, *L. perenne* and *E. vesicaria*;*and* NT: no tillage combined with the presence of *T. capitatum*.
† Spontaneous vegetation growing in the different treatments starts to grow in fall, once the summer dry season is over, thereby insignificant plant residue incorporation occur during fall tillage operations in the RT, RTG and RTOM treatments.

water with the almond trees. After this, the plant residues were incorporated into the soil by chisel plowing to 15 cm using a cultivator in the RT treatments (RT, RTG, and RTOM), or were left on the soil surface as a mulch in the NT treatment, to provide nutrients and C inputs to the soil.

The experimental design consisted of 12 plots (49 m × 7 m), each enclosing seven almond trees, in a randomized-block design, with three replicate plots for each of the four soil management practices. For more details on the study site characteristics, experimental design, and soil management practices, see Martínez-Mena et al. (2013) and Almagro et al. (2017).

2.2. Soil and plant biomass sampling and analysis

Soil samples were collected from the plow layer (0–15 cm) in spring 2016, eight years after the soil management practices were implemented. Three soil samples were taken in the strips between the almond trees, 3.5 m from the tree trunk, for each management practice and block. A total of 36 samples were collected (4 management practices x 3 blocks x 3 sampling points). They were stored at 4 °C until they were processed for chemical analyses and incubations. The soil was homogenized and sieved through a 5-mm-mesh sieve to remove stones and large plant residues. The maximum water holding capacity (WHC) for each soil management practice was estimated in triplicate, following the procedure of Howard and Howard (1993). Subsamples sieved at 2 mm were used for initial soil chemical analyses (C and N), and to determine enzymatic activities related to C (dehydrogenase and β-glucosidase), N (urease), and phosphorous (alkaline phosphatase) cycling.

Total plant biomass (including roots) samples were collected in the peak growing season (in late spring) from five randomized sampling points per block, using quadrats (0.5 m × 0.5 m). The dominant species present in the different plant covers of each management practice (i.e., *A. sativa* and *V. sativa* in RTG; *H. murinum*, *L. perenne* and *E. vesicaria* in RT and RTOM; and *T. capitatum* in NT) were sampled. The plant biomass was carefully separated into shoots (leaves and stems) and roots. To ensure more homogeneous mixing with the soil during incubation, the shoots and roots were cut into small pieces (less than 1 cm). Shoot and root subsamples were ground to a fine powder with a ball mill (PM4 planetary grinder; Retsch, Haan, Germany). The initial C and N concentrations of the shoots and roots were determined using an elemental C/N analyzer (Flash 1112 EA, Thermo-Finnigan, Bremen, Germany). The above-ground plant residue chemical composition - including the cell soluble fraction (that is, soluble carbohydrates, proteins, and lipids; hereafter, cell solubles), hemicellulose, cellulose, and lignin - was analyzed using the sequential extraction technique (Van Soest et al., 1991). Subsamples (0.5 g), previously ground using a Retsch Centrifugal Grinding Mill ZM 1000 with screen aperture size of 1 mm (Retsch GmbH, Hann, Germany), were subjected to neutral fiber detergent, acid fiber detergent, and sulfuric acid digestions using an Ankom^{2000} Fiber Analyzer (Ankom Technology Corp., Macedon, NY, USA). After the sulfuric acid digestion, the samples were combusted in a muffle furnace at 550 °C for 5 h to correct for any mineral particles in the lignin fraction.

To characterize the quality of the above-ground plant residues based on chemical composition criteria, well-known indicators such as the percentage of cell solubles as well as the C:N and lignin:N ratios were used, as in previous works (Palm et al., 2001; Melillo et al., 1989).

2.3. Aerobic incubations under controlled conditions and laboratory assays

A factorial experiment was performed in the laboratory to simulate the effect of soil-plant residue mixing after tillage operations on organic carbon mineralization rates, enzymatic activities related to the C (β-glucosidase) and N (urease) cycles, soil microbial biomass and community composition, soil aggregate formation, and OC and total N stabilization.

For the construction of the mesocosms (125-cm^3 containers), the dominant species present in the different plant covers for each management practice (see Table 2) were separated into three types of plant residue (shoots, roots, and the combination of both) and mixed with their respective soils. The exception was the NT treatment, for which roots and shoots were not combined because of the lack of representativeness (*i.e.*, as no tillage operations were performed in this treatment, shoots and roots were never mixed together with soil). The soil samples (40 g dry mass) were mixed with plant residue samples (1 g dry mass) corresponding to a rate ranging between 9.5 and 10.75 g C kg^{-1} dry soil, depending on the residue type and the species present in each management practice; Table 2). A mesocosm containing only soil (as the control) for each management practice x block x replicate combination was also incubated to correct for the soil contribution to CO_2 production. The experimental design consisted of 4 management practices x 3 or 4 plant residue types (soil, soil plus shoots, soil plus roots, and soil plus shoots and roots) x 3 blocks x 3 sampling points, resulting in a total of 126 mesocosms.

2.3.1. Organic carbon mineralization and CO_2 analysis

All mesocosms were moistened to 60% WHC prior to incubation at 28 °C in air-tight, 125-cm^3 containers under aerobic, controlled conditions. The CO_2 (%) evolved from the containers was regularly measured with an infrared gas analyzer (CheckMate II, PBI Dansensor, Denmark) and the containers were opened for 1 h after each measurement to balance the atmosphere inside and outside them. Measurements were performed more frequently during the first phase of the incubation (on days 2, 3, 6, 8, 10, 13, 15, and 17), weekly during the intermediate phase (between days 17 and 156), and bi-weekly during the final phase (day

Table 2
Carbon and nitrogen contents and C:N ratios (mean ± SD) of the plant residue types present in the different management practices used for the incubation experiment.

Management practice	Species	Plant residue	C (%)	N (%)	C:N
RT	*H. murinum*, *L. perenne* and *E. vesicaria*	shoots	43.34 ± 0.09**b**	1.38 ± 0.15**b**	31.53 ± 3.44**b**
RT		roots	40.57 ± 0.28**B**	0.84 ± 0.05**A**	48.17 ± 3.21**B**
RTG	*A. sativa* and *V. sativa*	shoots	41.94 ± 0.51**a**	2.21 ± 0.08**c**	18.95 ± 0.45**a**
RTG		roots	37.63 ± 0.08**A**	1.42 ± 0.11**B**	26.51 ± 2.19**A**
RTOM	*H. murinum*, *L. perenne* and *E. vesicaria*	shoots	42.36 ± 0.19 **ab**	1.18 ± 0.09**b**	35.96 ± 2.84**b**
RTOM		roots	41.69 ± 0.29**B**	0.76 ± 0.03**A**	54.50 ± 1.71**B**
NT	*T. capitatum*	shoots	43.39 ± 0.20**b**	0.69 ± 0.04**a**	62.52 ± 4.58**c**
NT		roots	43.76 ± 0.38**C**	0.62 ± 0.03**A**	70.77 ± 4.12**C**

RT: combined effect of reduced tillage and the presence of *H. murinum*, *L. perenne* and *E. vesicaria*; RTG: combined effect of reduced tillage and the presence of *A. sativa* and *V. sativa*; RTOM: combined effect of reduced tillage plus organic manure addition and the presence of *H. murinum*, *L. perenne* and *E. vesicaria*; NT: combined effect of no tillage and the presence of *T. capitatum*. Within the same column, significant differences in the C and N contents as well as in the C:N ratios of above-ground plant residues (shoots) and below-ground plant residues (roots) among the management practices are denoted by bold lowercase and uppercase letters, respectively, according to Tukey's test ($P < 0.05$). The C:N ratios of roots were always significantly higher than those of shoots, regardless of the management practice, and no significant interaction was found for management practice x plant residue type.

156 onwards). The moisture content of samples was also checked periodically by weighting the mesocosms and evaporated water was replaced when necessary during the experiment. We used linear interpolations between sampling dates and then summed them across all dates to estimate the cumulative amount of CO_2 released (mineralized) after 243 days of incubation, to estimate the potential OC mineralization rates (mg CO_2 kg^{-1} soil; Nannipieri et al., 1990). These rates were then converted into percentages of the OC inputs added to each mesocosm (Table 2). Basal respiration was calculated as the averaged C content respired daily per gram of OC in the soil incubated with or without plant residues, and was expressed as mg CO_2–C g^{-1} OC per day.

2.3.2. Soil microbial activity and efficiency

We estimated soil microbial parameters indicative of the total microbial biomass (as the sum of the bacterial and fungal phospholipid fatty acid (PLFA) contents; as described in section 2.3.3), the general activity (respiration; as described in section 2.3.1) and the C, N, and phosphorus (P) cycling (dehydrogenase, β-glucosidase, urease, and alkaline phosphatase activities). In addition, we calculated three indices reflecting the microbial and enzymatic efficiencies. Since previous research has demonstrated a strong relationship between the microbial biomass obtained through PLFAs analysis and the microbial biomass C (Frostegård and Bååth, 1996), we used the former to modify two indices reflecting the soil microbial efficiency in the utilization of organic resources. Firstly, the ratio between the soil microbial biomass (in ng PLFAs g^{-1} soil) and SOC (g C kg^{-1} soil) was calculated as an index reflecting the efficiency of conversion of OC into microbial biomass, as equivalent to the microbial quotient (*i.e.*, the ratio between microbial biomass C and total organic C). Secondly, a proxy for the metabolic quotient (qCO_2), proposed by Wardle and Ghani (1995), was calculated as the cumulative CO_2–C production at the end of the incubation period divided by the soil microbial biomass. The lower this index, the more efficient the soil microbiota becomes at preserving C. Thirdly, we determined the enzymatic efficiency (C released per unit enzyme activity) as the ratio between the cumulative C mineralized (in mg C–CO_2 kg^{-1} soil) and the β-glucosidase activity (in mg PNP kg^{-1} soil) at the end of the incubation period (Sinsabaugh et al., 2002; Amin et al., 2014). Higher values of this index indicate increased enzymatic efficiency.

To assess the potential microbial activity related to C, N, and P cycling processes, the activities of extracellular enzymes (dehydrogenase, β-glucosidase, urease, and alkaline phosphatase) were determined in pre-incubated soils of each management practice, as well as for the different plant residue type-management practice combinations at the end of the incubation period, using standard protocols (see Appendix S1 for further details).

2.3.3. Soil microbial community composition

At the end of the incubation, subsamples (~6 g) from each mesocosm - containing only soil or soil mixed with the different plant residue types, for the different management practices - were kept frozen at −20 °C for the determination of the biomass and structure of the soil microbial communities through PLFAs analysis. Given that no significant differences in organic carbon mineralization rates and extracellular enzymatic activities were found between the RT and RTOM treatments, probably because their plant covers had the same species composition, the RTOM treatment was not included for the PLFAs analyses. Phospholipids were extracted from 6 g of soil using chloroform:methanol:citrate buffer (1:2:0.8 v/v/v), as described by Bligh and Dyer (1959). The PLFAs were then fractionated and transformed into fatty acid methyl esters (FAMEs) by alkaline methanolysis, being designated as described by Frostegård et al. (1993). The completely dried FAME fraction was dissolved in isooctane containing 0.23 mg ml^{-1} of 21:0 FAME as an internal standard. The analysis was performed using a Trace Ultra Thermo Scientific gas chromatograph fitted with a 60-m capillary column (Thermo TR-FAME 60 m × 0.25 mm ID x 0.25 μm film), with helium as the carrier gas. The following fatty acids are characteristic bacterial fatty acids and were chosen as bacterial biomarkers: i15:0, a15:0, 15:0, i16:0, i17:0, cy17:0, cy19:0, 16:1ω7c, and 16:1ω7t (Frostegard et al., 1993). The fatty acid 18:2ω6 was used as an indicator of fungal biomass (Rinnan and Bååth, 2009; Brant and Chen, 2015). The fatty acids used to represent Gram-positive bacteria were i15:0, a15:0, i16:0, and i17:0. The fatty acids used to represent Gram-negative bacteria were cy17:0, cy19:0, 16:1ω7c, and 16:1ω7t (Frostegard et al., 1993). The 10Me-branched FAMES (10Me16:0 and 10Me18:0) were taken as specific actinobacterial biomarkers within the Gram-positive bacteria (Dungait et al., 2011).

2.4. Fractionation of water-stable soil aggregates

Water-stable aggregates were separated from composite samples of soil of each management practice by plant residue type combination at the end of the incubation using a modified wet-sieving method adapted from Elliott (1986). A series of three sieves (2000, 250, and 63 μm) was used to obtain four aggregate-size classes: (i) large macro-aggregates (LM; > 2000 μm); (ii) small macro-aggregates (SM; 250–2000 μm); (iii) micro-aggregates (m; 63–250 μm); and (iv) silt plus clay-sized particles (s + c; < 63 μm). The protected micro-aggregates contained within the small macro-aggregates (hereafter, the occluded micro-aggregates (SMm; 63–250 μm)) were obtained using the micro-aggregate isolation method described by Six et al. (2000) and Denef et al. (2004). Two additional occluded organic matter fractions were obtained through this method: the intra-aggregate particulate organic matter (iPOM; > 250 μm) and the occluded mineral fraction (SMs + c; < 63 μm). Further details on the fractionation method are provided in Appendix S1.

2.5. Organic carbon and total nitrogen determination

The OC and total N concentrations were analyzed separately for each water-stable aggregate-size class, as well as for the occluded fractions within macro-aggregates, using the elemental analyzer mentioned above, after soil carbonates had been eliminated by acid digestion with 2 N HCl. Briefly, between 0.05 and 0.07 g of sample was weighted in a tin capsule and placed on a stainless-steel heating plate at 120 °C. Drops (~100 μL) of HCl 2N were added to each soil sample until bubbling stopped. The digested soil sample was them combusted and total carbon as CO_2 was determined by infrared analysis. A paired soil sample was analyzed in the same way but without previous digestion. The samples were analyzed in triplicate. The OC concentration within each water-stable aggregate-size class and in the occluded fractions was expressed on a sand-free aggregate basis (Elliott et al., 1991). The amounts of OC and total N contained in the micro-aggregates inside macro-aggregates will be considered, respectively, as indicators of SOC and total N stabilization (Denef et al., 2007).

2.6. Statistical analyses

Pre-incubated soil samples were analyzed for differences in their OC and total N contents, as well as in their extracellular enzymatic activities, using a one-way ANOVA, in which management practice was considered as the main fixed factor. Differences in organic carbon mineralization rates (i.e., CO_2 released), soil aggregate sizes and the associated OC and total N contents, extracellular enzymatic activities, total microbial biomass, and specific functional groups (PFLAs) among the soil management practices and plant residue types at the end of the incubation period were analyzed using a two-way ANOVA, in which "management practice" and "plant residue type" were considered as the main fixed factors. Tukey's multi-comparison test was used to detect differences among management practices and plant residue types. Since significant management practice x plant residue type interactions were found for many of the response variables, further analyses were conducted separately for each management practice and plant residue type.

The Pearson correlation test was used to examine the relationships among plant residue quality parameters, microbial groups and activity, and soil OC and total N stable pools across management practices. Prior to these analyses, the data were tested for ANOVA assumptions, and were log-transformed when necessary. All the statistical analyses were performed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). The results are presented as the means of nine replicates for CO_2 mineralization rates and of three replicates for soil aggregates distribution, enzymatic activities, and PFLAs analyses.

3. Results

3.1. Plant residue chemical composition and soil extracellular enzyme activities before the incubation

According to the C:N ratio, the plant cover residues of the RTG treatment had the highest chemical quality, for both shoot and root components, while those of the NT treatment had the lowest quality among the management practices (Table 2). Likewise, the lignin:N ratios of the above-ground component (shoots) of the RTG, RT, and RTOM treatments were much lower than that of the NT treatment (Table 3). Overall, the plant residues of the RTG treatment had the highest cell solubles content and the lowest lignin content, while those of the NT treatment had the lowest cell solubles content and the highest lignin content.

The effect of the sustainable soil management practices on the soil extracellular enzymatic activities (as a surrogate of microbial activity) was evident eight years after their implementation, the observed trends being consistent among the management practices (Table 4). Regarding the extracellular enzymes involved in soil C, N, and P cycling and assayed here, no differences in the dehydrogenase activity (a good indicator of soil microorganism metabolic activity and SOM dynamics) were observed among the management practices, but the activities of the other enzymes slightly differed among them. It is noteworthy that: i) the NT treatment showed the lowest β-glucosidase activity among the management practices; ii) the alkaline phosphatase activity was highest in the RTG treatment, followed by the RTOM treatment, and was lowest in the RT and NT treatments; and iii) the RTG and RTOM treatments had higher urease activities than the RT and NT treatments, although significant differences were only detected for the RTG treatment (Table 4).

3.2. Effect of plant residue chemical quality on organic carbon mineralization rates

The two-way ANOVA revealed significant differences in the OC mineralization rates among management practices and plant residue types at the end of the incubation period. The NT treatment showed the lowest OC mineralization rates among the management practices ($df = 3$; $F = 37.10$; $P < 0.001$), while the OC mineralization rates in the RTG and RTOM treatments did not differ from those in the RT treatment. The $C–CO_2$ released from shoots was always higher than that released from roots, regardless of the management practice ($df = 2$; $F = 739.37$; $P < 0.001$). Significant interactions were found between the management practices and plant residue types ($df = 6$; $F = 11.29$; $P < 0.001$). No differences in the OC mineralization rates in soils incubated without plant residues were observed among the management practices at the end of the incubation period (Table 5; Fig. 1A). However, the OC mineralization rates for shoots and roots in the NT treatment were

Table 4
Soil quality and microbial productivity indicators for different soil management practices (0–15 cm depth).

Variable/ management practice	RT	RTG	RTOM	NT	F value	P value
Enzymatic activities (pre-incubated soils)						
Dehydrogenase activity (μg g^{-1} soil h^{-1})	3.99 ± 0.26**a**	4.54 ± 0.38**a**	4.64 ± 0.60**a**	3.63 ± 0.43**a**	1.18[a]	0.332
β-glucosidase activity (μmol PNP g^{-1} soil h^{-1})	94.70 ± 0.06**a**	108.61 ± 0.06**a**	90.84 ± 0.06**a**	59.46 ± 0.08**b**	6.20[a]	0.002
Phosphatase activity (μmol PNP g^{-1} soil h^{-1})	1.39 ± 0.09**b**	1.80 ± 0.15**a**	1.55 ± 0.18**ab**	1.36 ± 0.21**b**	4.48[a]	0.047
Urease activity (μmol NH_4^+-N g^{-1} soil h^{-1})	9.93 ± 1.40**ab**	14.05 ± 2.91**a**	12.84 ± 3.0**ab**	7.34 ± 2.54**b**	4.27[a]	0.074
General indicators (at the end of the incubation)						
Organic carbon (g kg^{-1} soil)	10.58 ± 0.79**ab**	11.78 ± 0.75**a**	11.68 ± 0.72**a**	9.50 ± 0.88**b**	3.17	0.035
Total nitrogen (g kg^{-1} soil)	1.05 ± 0.07**b**	1.21 ± 0.08**a**	1.22 ± 0.09**a**	0.95 ± 0.08**b**	3.53	0.026
OC mineralization rate (mg $C–CO_2$ g^{-1} OC d^{-1})	0.16 ± 0.01**a**	0.19 ± 0.02**a**	0.16 ± 0.04**a**	0.26 ± 0.09**a**	1.26	0.351
Microbial biomass-to-OC ratio (μg PLFAs g^{-1} OC)	54.03 ± 10.76**a**	52.6 ± 8.41**a**	no data	48.27 ± 15.3**a**	0.13	0.878
Basal respiration-to-microbial biomass ratio (mg $C–CO_2$ $μmol^{-1}$ PLFAs d^{-1})	0.86 ± 0.17**b**	1.02 ± 0.14**ab**	no data	1.49 ± 0.18**a**	3.91	0.082
Enzymatic efficiency (mg $C–CO_2$ mg^{-1} PNP)	6.72 ± 1.23**a**	6.82 ± 0.97**a**	7.71 ± 0.67**a**	2.56 ± 0.47**b**	6.71	0.014

Significant differences among the management practices are indicated with bold lowercase letters, according to Tukey's test ($P < 0.05$).
Average ± SE for $n = 3$. All data are given on an oven-dried weight soil basis.
RT: combined effect of reduced tillage and the presence of *H. murinum*, *L. perenne* and *E. vesicaria*; RTG: combined effect of reduced tillage and the presence of *A. sativa* and *V. sativa*; RTOM: combined effect of reduced tillage plus organic manure addition and the presence of *H. murinum*, *L. perenne* and *E. vesicaria*; NT: combined effect of no tillage and the presence of *T. capitatum*.
[a] Data were log-transformed prior to statistical analyses.

Table 3
Above-ground biomass chemical composition of the most abundant species present in the plant covers of each management practice.

Management practice	Species	Hemicellulose (%)	Cellulose (%)	Lignin (%)	Cell solubles (%)	Lignin:N
RT, RTOM	*Lolium perenne*	31.56	38.51	3.87	26.06	4.52
	Hordeum murinum	36.07	35.70	2.51	25.73	
	Eruca vesicaria	19.83	41.86	10.91	27.40	
RTG	*Avena sativa*	29.34	27.49	2.11	41.06	1.51
	Vicia sativa	11.74	20.96	4.57	62.73	
NT	*Teucrium capitatum*	21.80	38.87	15.72	23.61	22.60

RT: combined effect of reduced tillage and the presence of *H. murinum*, *L. perenne* and *E. vesicaria*; RTOM: combined effect of reduced tillage plus organic manure addition and the presence of *H. murinum*, *L. perenne* and *E. vesicaria*; RTG: combined effect of reduced tillage and the presence of *A. sativa* and *V. sativa*; NT: combined effect of no tillage and the presence of *T. capitatum*.

Table 5
Organic carbon mineralization rates (mg C–CO_2 g^{-1} OC d^{-1}) at the end of the incubation period in soil incubated without plant residues, and in soil incubated with roots, shoots, and roots plus shoots coming from different species belonging to each management practice.

Plant residue type addition/management practice	RT	RTG	RTOM	NT	*df*	*F value*	*P* value
Soil (control)	0.16 ± 0.01**a**	0.19 ± 0.02**a**	0.16 ± 0.04**a**	0.26 ± 0.09**a**	3	0.02	ns
Soil plus shoots	27.46 ± 0.12**a**	27.61 ± 0.09**a**	26.54 ± 2.17**a**	19.03 ± 0.9**b**	3	16.99	<0.001
Soil plus roots	23.49 ± 0.38**b**	26.90 ± 1.50**a**	24.94 ± 1.88 **ab**	12.36 ± 0.6**c**	3	42.69	<0.001
Soil plus shoots plus roots	25.93 ± 1.71**b**	29.48 ± 0.97**a**	26.35 ± 0.63 **ab**		2	3.016	0.068

Within the same type of plant residue addition (none, shoots, roots, and shoots plus roots), different lowercase letters indicate significant differences among management practices, according to Tukey's test ($P < 0.05$).
RT: combined effect of reduced tillage and the presence of *H. murinum*, *L. perenne* and *E. vesicaria*; RTG: combined effect of reduced tillage and the presence of *A. sativa* and *V. sativa*; RTOM: combined effect of reduced tillage plus organic manure addition and the presence of *H. murinum*, *L. perenne* and *E. vesicaria*; NT: combined effect of no tillage and the presence of *T. capitatum*.

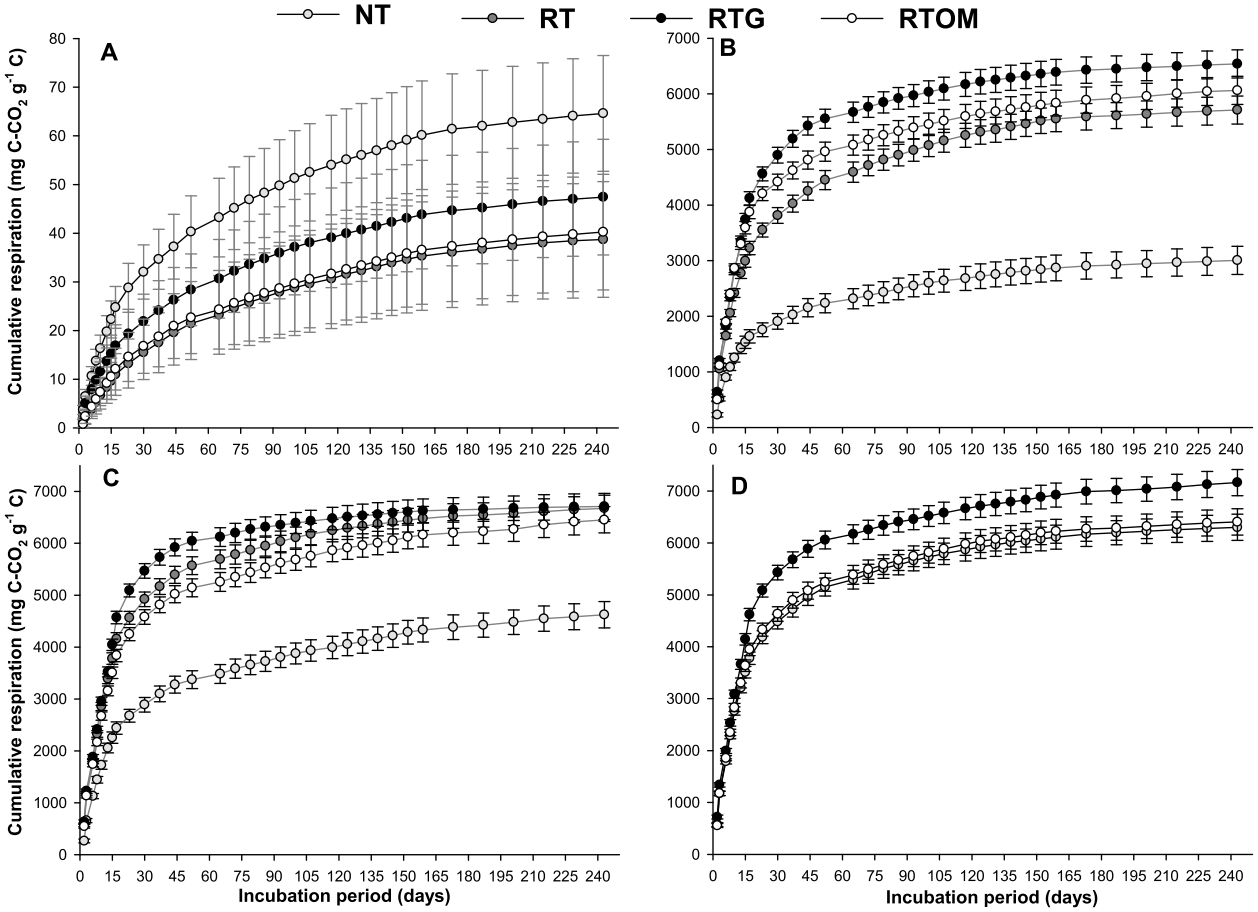

Fig. 1. Cumulative respiration curves (mg C–CO_2 g^{-1} C) during the incubation period in soil incubated without plant residues (control; panel A), and in soil incubated with roots (panel B), shoots (panel C), and roots plus shoots (panel D) coming from different species belonging to each management practice: *A. sativa* and *V. sativa* in RTG; *H. murinum*, *L. perenne* and *E. vesicaria* in RT and RTOM; and *T. capitatum* in NT.

significantly lower than those in the rest of the treatments (Table 5; Fig. 1B and C). When shoots and roots were combined, the OC mineralization rates were significantly higher for RTG, compared to the RT treatment, while RTOM showed rates similar to those of the RT treatment (Table 5; Fig. 1D).

3.3. Soil microbial response and efficiency

At the end of the incubation period, the soil microbial biomass (as the sum of the bacterial and fungal PLFA contents) was significantly higher in the RTG treatment than in RT and NT treatments (Fig. 2). However, significant differences were only detected in some functional groups: i) the bacterial PLFA content (including Gram-positive bacteria) was significantly higher in the RTG treatment than in RT and NT treatments; and ii) the saturated PLFA content was significantly higher in the RTG treatment than in NT treatment. The microbial biomass ratios (Gram-positive:Gram-negative, fungi:bacteria, and saturated:monounsaturated PLFAs) were not significantly influenced by the management practices or plant residue types.

As expected, the extracellular enzyme activities at the end of the incubation period were lower than those determined in the pre-incubated soils, and slightly different trends were also observed among the management practices depending on the plant residue added to the soil. Among the management practices, the NT treatment showed the lowest β-glucosidase ($P = 0.014$) and urease ($P = 0.018$) activities regardless of the plant residue type (Fig. 3A; 3B). Overall, there were no significant differences in the β-glucosidase and urease activities among the rest of the management practices. Only when shoots and roots were

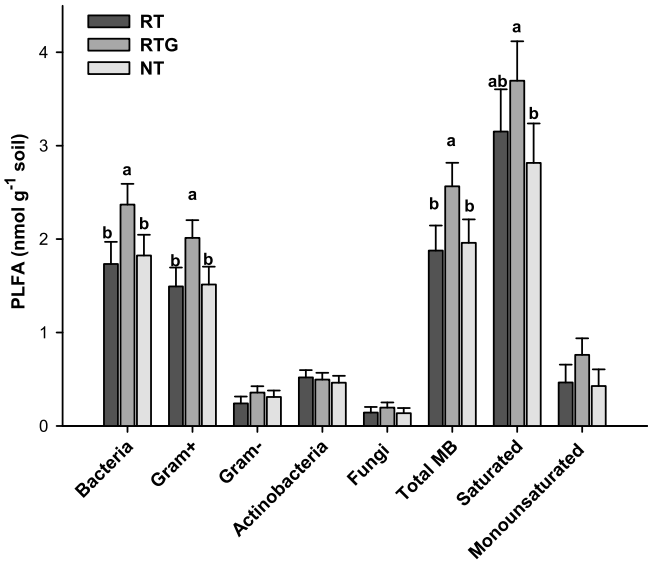

Fig. 2. Content of the different functional microbial groups under different soil management practices: bacteria (including Gram positive (Gram+) and Gram negative (Gram-), Actinobacteria, and fungi, as well as the total microbial biomass (as the sum of bacteria and fungi) and the membrane saturation status (monounsaturated and saturated). Within each functional group, different lowercase letters denote significant differences among management practices according to Tukey's test ($P < 0.05$). RT: combined effect of reduced tillage and the presence of *H. murinum*, *L. perenne* and *E. vesicaria*; RTG: combined effect of reduced tillage and the presence of *A. sativa* and *V. sativa*; NT: combined effect of no tillage and the presence of *T. capitatum*.

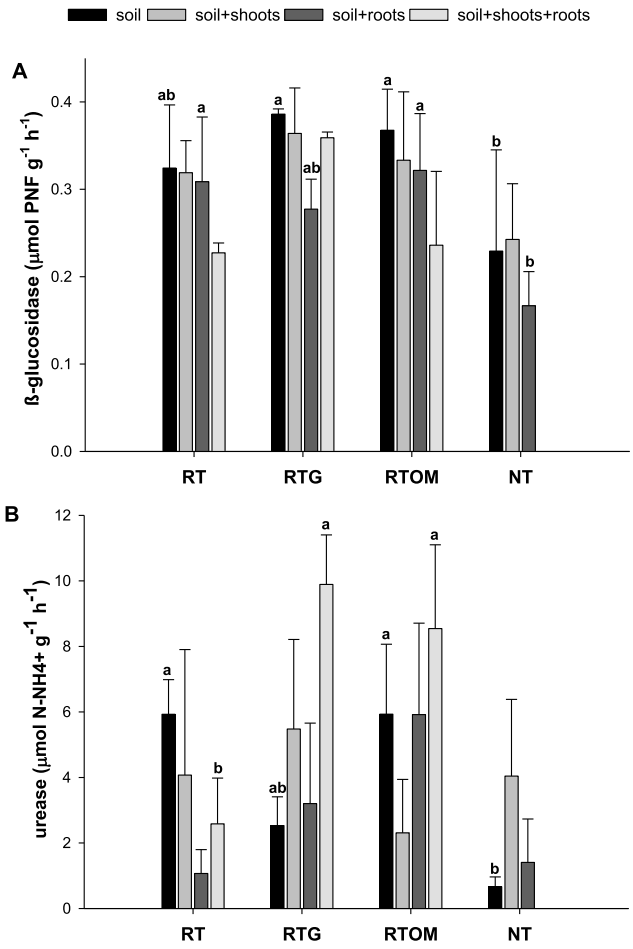

Fig. 3. Beta-glucosidase (panel A) and urease (panel B) activities for each plant residue type (shoots, roots, and the combination of shoots and roots) and management practice (RT: reduced tillage combined with the presence of *H. murinum*, *L. perenne* and *E. vesicaria;* RTG: reduced tillage combined with the presence of *A. sativa* and *V. sativa* (green manure); RTOM: reduced tillage and organic manure addition combined with the presence of *H. murinum*, *L. perenne* and *E. vesicaria; and* NT: no tillage combined with the presence of *T. capitatum*) after the incubation period. Different lowercase letters denote significant differences among plant residue types within each management practice according to Tukey's test ($P < 0.05$).

combined, did the RTG and RTOM treatments show a significantly higher urease activity than RT (Fig. 3B). There were no significant differences in the basal respiration or the soil microbial biomass:OC ratio among the management practices (Table 4). However, the highest basal respiration:soil microbial biomass ratio was observed for NT. Moreover, among the treatments, NT showed the lowest enzymatic efficiency (Table 4).

3.4. Effect of plant residue chemical quality on soil aggregation and OC and N pools

At the end of the incubation period, the total OC and N contents were higher in the RTG and RTOM treatments than in the RT treatment. However, they were lower in the NT treatment compared to the RT treatment (Table 4).

Overall, the distribution of the water-stable soil aggregates as well as the OC and total N contents of each aggregate size were not affected by the quality of the above-ground and below-ground plant residues, or by their combinations, in any management practice at the end of the incubation period (Figs. S1; S2; S3). The only exception was the RTG treatment, in which the combination of shoots and roots from *A. sativa* and *V. sativa* increased the content of the free micro-aggregates by 30% compared to the soil without plant residue addition (control) (Fig. 4A). As a result, the RTG treatment was the only practice in which the micro-aggregate-associated OC was increased on a whole soil basis: by 29%, on average, when shoots and roots were incubated separately, and by 73% when they were combined, with respect to the soil incubated without plant residues (Fig. 4B). Likewise, there was an increase in the total N associated to the free micro-aggregates when shoots (by 33%), roots (by 58%), and the combination of shoots and roots (by 65%) from *A. sativa* and *V. sativa* were incubated, compared to the soil incubated without plant residue (Fig. 4C). Moreover, the total N associated to the small macro-aggregates increased when roots alone (by 65%) or in combination with shoots (by 34%) were incubated in the RTG treatment. Noteworthy, a significant enrichment in the total N content (by 58%, on average) of the small macro-aggregates and of the free micro-aggregates did occur when roots from green manure were incubated, compared to the soil incubated without plant residues ($P = 0.049$ and 0.021, respectively). In relation to the occluded fractions and their associated OC and total N contents, there were no significant differences among the plant residue types within each tillage treatment (data not shown).

3.5. Relationships among the plant residue quality parameters, soil microbial response, and OC and N pools

Pearson correlations showed that the plant residue chemical composition controlled the total OC and N contents in the soil, across the distinct management practices (Table 6). While the N and cell solubles contents of the plant residues were strongly and positively correlated with the total OC and N contents in the soil, the opposite was observed for the lignin content. Basal respiration was positively correlated with the N content of the plant residues and strongly and negatively correlated with the lignin content. Basal respiration was also positively correlated with the soil N content, as well as with the OC and total N

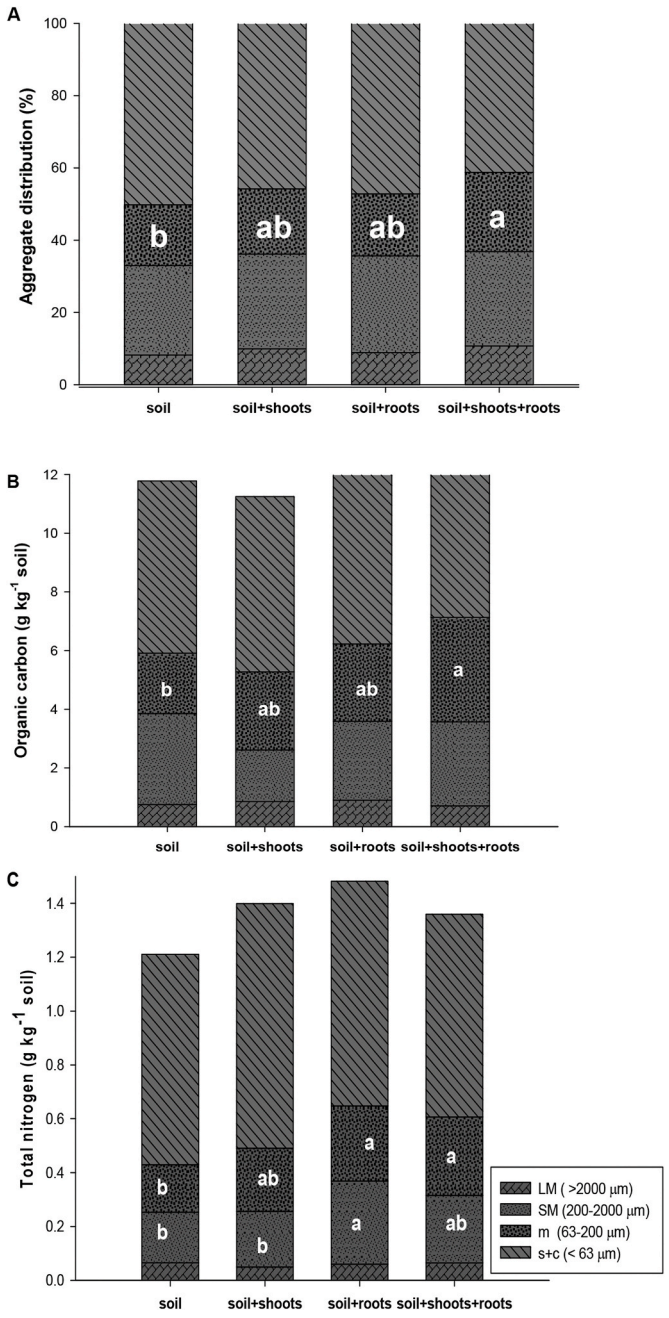

Fig. 4. Distribution of water-stable soil aggregates (panel A), and the associated contents of organic carbon (OC; panel B) and total N (panel C), in the reduced tillage combined with *A. sativa* and *V. sativa* (green manure; RTG), according to the type of plant residue addition (soil, shoots, roots, and shoots plus roots). For each aggregate size, different lowercase letters indicate significant differences among crop residue types according to Tukey's test ($P < 0.05$).

contents of the free micro-aggregates. It is noteworthy that the OC and N contents of the free and occluded micro-aggregates were positively correlated with the β-glucosidase activity. The enzymatic efficiency was negatively correlated with the N content in the bulk soil and in the free micro-aggregates.

4. Discussion

Regarding our first hypothesis, we found that the chemical composition of the plant residues returned to the soil influenced the activity of the local microbial communities and their associated functions. However, changes in the microbial community structure were only observed under the RTG treatment.

The labile and N-rich plant residues from common vetch and oat used as green manure in the RTG treatment stimulated the soil microbial biomass, specifically Gram-positive bacteria, enhancing microbial decomposition and the incorporation and preservation of OC and N within soil aggregates, in line with results observed by other authors (Schutter and Dick, 2002; Six et al., 2006; Schimel and Schaeffer, 2012; Geyer et al., 2016; Lashermes et al., 2016). The strong relationship between the N content of the plant residues and basal respiration ($r = 0.51$; $P < 0.01$; Table 6) confirms that increased availability of N due to green manure addition supported higher levels of microbial activity. Indeed, a significant increase in urease activity was only observed in the RTG treatment. Our results are in agreement with those of García-Orenes et al. (2016), who also found an increase in Gram-positive bacteria when *Vicia villosa* used as green manure was incorporated into the soil, together with pruning residues, in a semiarid Mediterranean vineyard where inorganic fertilization had been applied formerly. Our results seem to contradict previous studies showing that Gram-positive bacteria utilize more complex sources of C while Gram-negative bacteria preferentially use more easily degradable C compounds (Kramer and Gleixner, 2008; Torres et al., 2015; Fanin et al., 2019). However, several studies have reported increases in Gram-positive bacteria with increasing availability of soluble N compounds in soils as a result of green manure addition (Mbuthia et al., 2015; Frasier et al., 2016), supporting the idea that Gram-positive bacteria are more sensitive and respond faster than other soil microorganisms, such as Gram-negative bacteria and fungi, to the addition of easily degradable N sources (Moore et al., 2005). Nevertheless, other authors found the opposite, *i.e.*, that vetch decreased the proportion of Gram-positive and favored that of Gram-negative when adding cover crops in a tomato cropping system (Buyer et al., 2010). Although we cannot conclude from our data that Gram-positive are more copiotrophic than Gram-negative bacteria, it appears that Gram-positive bacteria are well adapted to the harsh living conditions in our study site (*i.e.*, low OC and N contents, water scarcity). This explains the growth of Gram-positive bacteria with increasing availability of easily degradable N compounds when *Vicia sativa* used as green manure was incorporated into the soil. Noteworthy, the low bacteria PLFA content observed in our soil, and particularly that of Gam-negative bacteria, is in line with previous studies reporting higher contents of Gram-positive than of Gram-negative in semiarid environments (Hueso et al., 2012; Li et al., 2020). Unlike Gram-negative bacteria (with a single-layer cell wall and an outer membrane), Gram-positive bacteria have a strong, thick cell wall and the ability to form endospores, which make them inherently resistant to stress conditions and thus more responsive to improved soil environmental conditions such as increased water and/or nutrient availability (Schimel et al., 2007; Chodak et al., 2015).

It is clear from our results that microbial decomposition, and so the incorporation of OC and N in stable aggregates, was enhanced by the addition of easily degradable and N-rich plant residues. However, we cannot state that the efficiency of microbial decomposition was improved by the addition of common vetch and oat as green manure, in comparison to the RT treatment, dominated by spontaneous herbaceous plants (i.e., *H. murinum*, *L. perenne* and *E. vesicaria*). The observed microbial response to the RTG treatment (growth of Gram-positive bacteria that do not significantly increase the microbial decomposition efficiency) is in agreement with the home-field advantage hypothesis (Hunt et al., 1988; Gholz et al., 2000), which suggests that local microbial communities are more efficient at decomposing the plant residues that are produced in their own environment than exogenous ones.

The recalcitrant and N-poor plant residues of *T. capitatum* in the NT treatment, on the other hand, did not affect the structure of the microbial community, but significantly reduced the soil microbial efficiency (e.g., greater production of CO_2 per unit of microbial biomass) and the enzymatic activity and efficiency (Table 4), hampering the soil OC and N

Table 6
Pearson correlation coefficients between plant residue quality parameters, microbial functional group ratios and activity, and soil OC and N stabilization indicators.

	Plant residue chemical quality parameters							Bulk soil quality			SOC and N stable pools			
	N (%) n = 24	C:N n = 24	Hemicellulose (%) n = 12	Cellulose (%) n = 12	Lignin (%) n = 12	Cell soluble (%) n = 12	Lignin: N n = 12	TOC (g kg^{-1}) n = 45	TN (g kg^{-1}) n = 45	C:N n = 45	m-OC (g kg^{-1}) n = 45	SMm-OC (g kg^{-1}) n = 45	m-N (g kg^{-1}) n = 45	SMm-N (g kg^{-1}) n = 45
N (%)	1	**-.938**[a]	.159	**-.606**[b]	**-.971**[a]	**.705**[b]	**-.962**[a]	**.548**[a]	**.750**[a]	**-.558**[a]	.243	.401	.222	.385
C (%)	-.268	**.429**[b]	.261	**.764**[a]	**.773**[a]	**-.803**[a]	**.794**[a]	**-.550**[a]	**-.587**[a]	.198	-.227	**-.414**[b]	-.141	-.234
C:N	**-.938**[a]	1	.104	**.802**[a]	**.978**[a]	**-.875**[a]	**.988**[a]	**-.538**[a]	**-.735**[a]	**.546**[a]	-.258	**-.505**[b]	-.182	**-.485**[b]
Hemicellulose (%)	.159	.104	1	**.672**[b]	-.076	-.564	-.003	-.047	-.172	**.835**[a]	.122	.106	-.189	-.004
Cellulose (%)	**-.606**[b]	**.802**[a]	**.672**[b]	1	**.688**[b]	**-.991**[a]	**.739**[a]	**-.645**[b]	**-.690**[b]	.547	-.299	-.491	-.325	-.459
Lignin (%)	**-.971**[a]	**.978**[a]	-.076	**.688**[b]	1	**-.780**[a]	**.997**[a]	**-.823**[a]	**-.760**[a]	-.082	-.523	**-.766**[a]	-.252	**-.689**[b]
Cell soluble (%)	**.705**[b]	**-.875**[a]	-.564	**-.991**[a]	**-.780**[a]	1	**-.824**[a]	**.711**[a]	**.737**[a]	-.456	.356	.567	.327	.518
Lignin:N	**-.962**[a]	**.988**[a]	-.003	**.739**[a]	**.997**[a]	**-.824**[a]	1	**-.829**[a]	**-.775**[a]	-.021	-.515	**-.760**[a]	-.266	**-.684**[b]
Gram+:Gram- (n = 26)	.330	-.307	.665	-.159	-.569	.247	-.539	.031	.178	-.218	**.415**[b]	.155	.144	-.053
Fungi:bacteria (n = 26)	-.175	.138	**-.909**[b]	-.364	.133	.274	.089	.309	.188	.108	.268	.313	**.616**[b]	.103
Saturated:monosaturated (n = 26)	-.127	.083	**-.893**[b]	-.228	.249	.135	.211	.050	.206	-.236	.184	.012	.305	-.248
OC mineralization rate (mg $C–CO_2$ g^{-1} SOC d^{-1}) (n = 45)	**.516**[a]	**-.562**[a]	.327	-.455	**-.933**[a]	.568	**-.912**[a]	.018	**.454**[a]	**-.615**[a]	**.336**[b]	.138	**.393**[a]	.183
β-Glucosidase activity (μmol PNP g^{-1} h^{-1}) (n = 45)	.304	-.325	.382	-.142	-.565	.229	-.539	**.478**[a]	.239	.235	**.580**[a]	**.470**[a]	**.383**[a]	**.394**[a]
Urease activity (μmol $N–NH_4^+$ g^{-1} h^{-1}) (n = 45)	.181	-.105	.271	.020	-.238	.027	-.219	.287	.260	-.022	**.386**[a]	.181	.264	.083
Basal respiration:microbial biomass (mg $C–CO_2$ μmol^{-1} PLFAs d^{-1}) (n = 26)	.232	-.232	-.120	-.349	-.350	.359	-.355	.224	**.505**[a]	**-.464**[a]	**.396**[b]	.243	**.394**[b]	.250
Enzymatic efficiency (mg $C–CO_2$ mg^{-1} PNP) (n = 45)	-.070	.084	.142	.233	.174	-.234	.185	.077	**-.392**[a]	**.642**[a]	-.211	-.041	**-.305**[b]	-.100

The number of samples (n) is provided for each parameter.
[a] Correlation is significant at the 0.01 level (2-tailed).
[b] Correlation is significant at the 0.05 level (2-tailed).

dynamics and decreasing C storage and nutrient availability. Our results contradict previous research reporting that C inputs rich in lignin can increase enzyme efficiency and change the structure of the microbial community in soils (Blagodatskaya et al., 2007; Elfstrand et al., 2007; Amin et al., 2014; Torres et al., 2016). These contrasting microbial community responses are most likely the result of the complex interactions between soil structure, microbial activity and composition, and the chemical quality of plant residues, that should be considered when managing soils. Although it has been widely reported that no tillage favors soil microorganisms in dry climates because of improved soil water infiltration and greater soil moisture conservation (Hobbs et al., 2008; Serraj and Siddique, 2012), the opposite was observed in this semiarid agroecosystem. The reduction in microbial activity observed in the NT treatment, despite the improved soil water content (Martínez-Mena et al., 2013), was probably due to a combination of factors that caused soil compaction, worsening the conditions for microorganisms: 1) the inherent nature of our soil, with relatively low OC and N availability; 2) the warm and dry climatic conditions; 3) the management practice, since no fertilizers were applied; and 4) the crop type (a woody permanent crop with wide row spacing). Likewise, the new plant residues - rich in lignin - reduced both the microbial activity and the decomposition efficiency, all together likely explaining the observed decrease in soil nutrient availability for the main crop in this treatment (Martínez-Mena et al., 2021). Indeed, an abrupt decrease in the main crop yields under this treatment was observed from the beginning of the experiment (Martínez-Mena et al., 2013). This indicates that the local and baseline environmental conditions are very important to ensure the success of the implementation of sustainable soil management practices.

Our results partially confirm our second hypothesis that mixing labile (shoots) and recalcitrant (roots) organic matter components from plant covers is a more appropriate management practice under semiarid rainfed conditions for improving soil structure and thus increasing soil C and N sequestration. It is of note that the synergistic effect of mixing above- and below-ground plant residues on the formation of free micro-aggregates and the protection and stabilization of OC and total N within them was only observed in the case of green manure (Fig. 4A–C). While labile and N-rich shoots from common vetch and oat used as green manure probably stimulated microbial activity, improving soil OC and N cycling, roots promoted the formation of free micro-aggregates and increased OC and N accumulation in more stable SOM pools that are protected from microbial attack (Helfrich et al., 2008; Cotrufo et al., 2013; Gunina and Kuzyakov, 2014; Tiemann and Grandy, 2015). This statement is supported by the fact that the urease activity was highest when shoots and roots from green manure were combined (Fig. 3B), while respiration did not significantly increase (Table S1), indicating that the high-quality plant residues from green manure enhanced microbial activity without compromising OC and N stabilization in soils. It is well known that changes in the substrate availability control the structure and function of soil microbial communities (Fanin et al., 2019). The enhancement of both labile and stable forms of high-quality organic matter in the RTG treatment stimulated microbial decomposition, improving OC and N stabilization in free micro-aggregates while enhancing the release of nutrients from plant residues into the soil, ultimately increasing nutrient availability for the main crop. However, improvements in the soil organic matter content and fertility under semiarid conditions are generally slow and therefore the beneficial effects of applying green manure on crop yields are not always immediate (Martínez-Mena et al., 2013). Indeed, in our experimental site, an increase in the main crop yields under the green manure treatment only began seven years after the implementation (Martínez-Mena et al., 2021).

Finally, we cannot ignore the fact that carbonates contributed to the amount of CO_2 released during our incubation experiment, and that such contribution would have been dependent on the treatment itself. Previous research has proven that the dissolution of carbonates does contribute to the evolved CO_2 from carbonate soils and that its relative contribution depends on many environmental and management factors such as soil pH and water availability, fertilization regime, and the chemical composition of the decomposing plant residues (Sanderman, 2012). This is a limitation of our study that future research could overcome by including analyses of soil carbonates before and at the end of incubation experiments.

In conclusion, the results from this study demonstrate that the chemical composition of the plant residues returned to the soil influences the microbial response as well as extracellular enzymatic activities (β-glucosidase, urease, and phosphatase) involved in biogeochemical processes. These effects alter the soil structure and OC and N dynamics, ultimately changing the functioning (*i.e.*, C sequestration, fertility, and crop productivity) of the whole agroecosystem. On the one hand, the labile and N-rich plant residues from common vetch and oat used as green manure increased the microbial biomass and slightly changed the soil microbial community structure, enhancing the dynamics and storage of OC and N in this semiarid Mediterranean woody cropping system. On the other hand, the more recalcitrant and N-poor plant residues of *T. capitatum* in the NT treatment reduced soil microbial activity and efficiency, hampering the soil C and nutrient dynamics. Our results also indicate that the mixing of roots and shoots from green manure had a positive, synergistic effect on the formation of free micro-aggregates while increasing the contents of OC and N associated with this stable SOM pool. Therefore, when adopting the use of green manure in semiarid agroecosystems, it would be desirable to incorporate above- and below-ground plant residues into the soil by reduced tillage, to allow them to decompose together. Nevertheless, further experiments under field conditions - to assess the effect of different species used as green manure on soil C sequestration and fertility under semiarid conditions - would allow the selection of the most effective and locally adapted agricultural management practices for climate change mitigation and adaptation in semiarid rainfed agroecosystems.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2021.108198.

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