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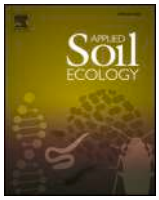

Waste-derived organic soil amendments for a sustainable vineyard management: Linking microbiome responses to soil biochemistry

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ABSTRACT

Products obtained from various organic wastes recycling processes can represent sustainable alternatives to traditional fertilizers in vineyard management. In this study, three waste-derived organic-based amendments were applied consistently for the first three years from plantation in an experimental vineyard in Northern Italy. The amendments included municipal organic waste compost, sewage sludge compost, and 'plasters' derived from the alkaline hydrolysis and pH neutralization of sludges. Aim was exploring the effects of these amendments on soil biochemistry, including physicochemical properties and main enzymatic activities, together with soil microbiome variations. Microbial community composition was analyzed by bacterial 16S and fungal ITS regions sequencing and network analysis, also studying its correlations to changes in soil C and N pools. The matrices did not act as microbial inocula. Instead, they stimulated specific autochthonous microbial populations and had a deep and varied impact on the cumulative fungal and bacterial network of interactions. Compost-based amendments representing a source of recalcitrant carbon, significantly promoted soil biodiversity and increased microbial biomass. Sludge plasters, a more easily degradable C and N source, promoted the proliferation of copiotrophic bacteria and unexpectedly resulted in a deep rearrangement of the fungal population, leading to increased laccase activity. Although the organic matrices were applied considering their different susceptibility to release available N over time, by applying different mineralization coefficients, significant differences were found in the accumulation of soluble forms of N and C in soil, with the consequent differences in microbial compositional and functional responses. These findings suggest the need for in-depth studies to formulate specific guidelines for the use of compost-based matrices, and especially sludge plasters, in the field. Compost-based matrices behaved like soil amendments, while sludge plasters behaved like mineral fertilizers. Together they could be used to maintain soil fertility and meet plant nutritional needs.

1. Introduction

Agricultural soils often show a decline in organic matter (OM) content, which negatively impacts their quality and fertility (Plaza et al., 2012). Adding organic amendments restores soil OM content and improves its quality (Lal, 2004; Rastogi et al., 2023; Soria et al., 2021). According to the European Compost Network (ECN), 71 million tons of organic waste are produced per year in Europe (Gilbert and Siebert, 2022), including agricultural and forestry wastes, sludges from water treatment plants, food residues, and the organic fraction of municipal solid waste (Sánchez-Monedero et al., 2019). The recycling processes of these organic wastes provide possible alternatives to mineral fertilizers in agriculture (Nafez et al., 2015).

Composting is one of the best-known processes to treat organic waste, combining its stabilization and the recovery of macro- and micronutrients (Ciavatta et al., 2022; Montejo et al., 2015). Municipal Organic Waste Compost (MOWC) derives from the combined aerobic and anaerobic digestion of municipal and food-industry solid wastes (Baldi

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et al., 2022). MOWC application in agricultural soil is subjected to specific biochemical evaluation due to potential chemical or biological contaminants that have to be removed during the process to improve compost quality and suitability for its use. In Italy, compost quality is defined by the Legislative Decree no. 75/2010 (Cesaro et al., 2015; Ciavatta et al., 2022).

Sewage sludges, from municipal wastewater treatment plants, are another example of materials rich in organic carbon (C), macronutrients, essential and nonessential trace elements, as well as organic contaminants or heavy metals. They must be properly treated to achieve chemical and biological stabilization, to avoid environmental contamination, before being used to improve soil fertility (FAO, 2004; Jamali et al., 2008; Fernández et al., 2009). Sewage sludge could be combined with municipal and industrial organic wastes, and subsequently composted, originating the sewage sludge compost (SSC) (Nafez et al., 2015; Baldi et al., 2022). Another possible treatment of sewage sludge is liming stabilization (FAO, 2004), a process in which high pH values (>10) are reached, allowing to reduce the solubility of trace and toxic metals (Jamali et al., 2008). This treatment is not widespread and the product has excessively high pH values for soil application. Nevertheless, in Italy it has been improved by treating the sewage sludge in two steps: a first alkaline or acidic hydrolysis, followed by pH neutralization with the addition of sulfuric acid or calcium oxide, respectively. The obtained product can be named Sludge Plaster (SP) and is disciplined by the Italian fertilizer law (Legislative Decree 75/2010).

The application of organic amendments to agricultural soils aims not only at restoring soil OM content and soil quality, but also at sustaining fruit trees growth and yields. Several studies have focused on the effects of compost on grapevine growth, yield and soil biochemical properties (Nendel and Reuter, 2007; Morlat and Symoneaux, 2008; Gaiotti et al., 2017; Sánchez-Monedero et al., 2019; Baldi et al., 2022; Lucchetta et al., 2023). In this scenario, evaluating soil quality and microbial ecosystem features is essential to understand the effects of organic amendments application and to optimize their use to promote food and environmental health.

Soil quality is assessed exploring chemical (pH, organic C, total nitrogen (N), and available phosphorous (P)) and biochemical (microbial activity and biomass) indicators (Bünemann et al., 2018). Among these, soil enzymatic activities are sensitive indicators of changes in soil quality (Sinsabaugh et al., 2008; Nannipieri et al., 2018), as microbial enzymes drive key soil processes such as OM decomposition, nutrient release, and nutrient cycles (Wallenstein et al., 2012; Kwiatkowski et al., 2020; Mazzon et al., 2021). Soil microbiome – comprising an incredibly biodiverse assembly of bacteria, archaea and fungi and their interactions among each other and with the environment (Berg et al., 2020) - is responsible for a large portion of the soil nutrient cycling, acts as reservoir for plant-colonizing bacteria and prevents the colonization of microorganisms possibly harmful for plants and animals (Peay et al., 2016; Fierer, 2017; Thompson et al., 2017). In vineyards, the soil microbiome is recognized not only as a key component of the soil quality, but also as crucial for the health, yield and quality of the grapevine, as well as a biodiversity reservoir from which the fruit microbiome is drawn, ultimately playing a role in the sensory properties of the wine (Belda et al., 2017).

The present study investigates the effect of three different organic-based amendments (MOWC, SSC, and SP) on soil biochemical and microbiological properties in an experimental vineyard located in Northern Italy. The study focuses on the potential beneficial effects of each amendment by considering the complex relationship between soil microorganisms, their enzymatic activity and the quality of the soil in which the grapevine are settled. We extensively analyzed samples taken in differently treated blocks of the vineyard, in which amendments were consistently applied for three years starting from grapevine plantation, evaluating chemical and biochemical parameters, as well as both the bacterial and fungal communities. Specifically, the main hypotheses of the work were that (i) the organic matrices differ in both chemical characterization and behavior into the soil; (ii) the supply of organic amendments to the soil changes its microbiological composition, in its prokaryotic and eukaryotic components, with a consequent impact on the chemical and biochemical features relevant to the quality of the soil in which the grapevines are set to grow.

2. Materials and methods

2.1. Experimental site description

An experimental vineyard, cv. Sangiovese (*Vitis vinifera* L.) grafted on 110 Richter (*V. berladieri* × *V. rupestris*) rootstock, was planted in February 2019 at the facility of the University of Bologna (Italy, 44°33′N; 11°24′E) on a clay-loam Cambisol soil (WRB-IUSS, 2015) characterized by a neutral pH (6.52), a low content of total N (TN, 0.80 g kg^{-1}) and organic matter (OM, 1.09 %), and a C.E.C. of 12.5 (Baldi et al., 2022). The climate of the area is temperate with mean annual precipitation of 631 mm, average air temperature of 14.1 °C, and average soil temperature (0.3 m of depth) of 15.7 °C.

Since plantation, the following treatments (each replicated 4 times, with 5 plants constituting a replicate) were compared according to a randomized block design:

(i) No-treated control (CK);
(ii) Mineral Fertilization (MF) with a N, P and potassium (K) fertilizer (26 - 17.4 - 13 kg ha^{-1}) in spring and urea in summer considering a N application of 120 kg N ha^{-1} yr^{-1};
(iii) Municipal Organic Waste Compost (MOWC) applied considering the N rate of 120 and 240 kg N ha^{-1} yr^{-1} (MOWC1 and MOWC2, respectively);
(iv) Sewage Sludge Compost (SSC), N rate of 120 and 240 kg N ha^{-1} yr^{-1} (SSC1 and SSC2);
(v) Sludge Plasters (SP), N rate of 120 and 240 kg N ha^{-1} yr^{-1} (SP1 and SP2).

As a common agronomic practice in the agricultural area where the experiment was conducted (Emilia Romagna Region, Italy), the amounts of N added by organic amendments were calculated considering the potentially available N by applying a specific mineralization coefficient for each organic matrix, specifically 0.3 for MOWC, 0.4 for SSC and 0.6 for SP.

In spring, before organic amendments application, a 0.25-m-depth furrow was opened under the vines in a 1.5-m-wide strip immediately closed with a disk harrow after the fertilization. The vineyard was watered with a drip irrigation system and the soil was tilled superficially (0.25 m) on the row, while alleys were covered with a mixture of *Festuca rubra*, *Festuca ovina*, *Lolium perenne* and *Trifolium repens* mowed 3 times a year (Baldi et al., 2022).

2.2. Chemical characteristics of the organic amendments and C and N introduced in the soil

A dried subsample of MOWC, SSC, and SP was chemically characterized. The total element content was determined by wet mineralization in an Ethos TC microwave lab station (Milestone, Bergamo, Italy); element concentration was determined by inductively coupled plasma optical emission spectrometer (ICP-OES; Ametek Spectro, Arcos, Kleve, Germany). Organic contaminants analyses were performed by CSA S.p. A. group (Rimini, Italy) external laboratory, ISO 11725 certified. Aromatic organic acids (EPA 5021A 2014 + EPA 8260D 2018), C10-C40 hydrocarbons (UNI EN 14039:2005), polycyclic aromatic hydrocarbon (PAH) and chlorinated phenols (EPA 3550C 2007 + EPA 8270E 2018) content were determined following the respective methods as reported in parentheses for each organic contaminant. Their pH and Electrical Conductivity (EC, expressed as dS m^{-1}) were determined on matrices suspension in water, while total organic carbon (TOC) and TN were

determined on air-dried samples with an elemental analyzer (Flash 2000; Thermo Fisher Scientific, USA). The three organic matrices had a constant TN content (ranging from 2.1 to 2.4 %), but the two composted matrices (MOWC and SSC) had significantly higher values of TOC (on average +31 %) compared to the SP matrix (Table 1). This clearly determined that the three organic matrices were also characterized by different values of C/N ratio with MOWC showing the highest one (13.5).

Table 1
Physico-chemical characteristics of the organic amendments used. The reported data correspond to the mean ± standard deviation of the values measured during the three years of treatment. Italian legislation for compost and soil correctives (D.Lgs. 75/2010) and the fixed limits are reported too.

Characteristics [unit]	MOWC	SP	SSC	D.Lgs. 75/2010
Humidity [%]	24.8 ± 2.9	64.3 ± 2.5	48.3 ± 18.5	<50
pH (in water)	8.7 ± 0.1	7.8 ± 0.7	7.6 ± 0.7	6 ÷ 8.8
Electrical Conductivity (EC)	2.9 ± 0.4	2.6 ± 0.1	2.0 ± 0.2	
Total Organic C [% dw]	28.3 ± 0.1	16.9 ± 2.1	25.1 ± 0.0	>20
Total N [% dw]	2.1 ± 0.0	2.2 ± 0.0	2.4 ± 0.5	
C/N ratio	13.5 ± 0.0	7.7 ± 0.8	9.5 ± 0.2	<25
Total element content				
Phosphorus [P_2O_5, %]	0.83 ± 0.34	2.10 ± 0.85	0.86 ± 0.30	
Potassium [K_2O, %]	1.44 ± 0.50	0.26 ± 0.07	0.78 ± 0.14	
Sulfur [SO_3, %]	0.41 ± 0.10	8.08 ± 2.94	0.87 ± 0.08	
Calcium [CaO, %]	8.22 ± 2.41	16.9 ± 8.1	6.35 ± 2.30	
Magnesium [MgO, %]	0.68 ± 0.15	0.50 ± 0.19	0.70 ± 0.26	
Copper [Cu, mg kg_{dw}^{-1}]	51.8 ± 20.1	118 ± 56	76 ± 39	<230
Iron [Fe, mg kg_{dw}^{-1}]	6150 ± 919	10,625 ± 3147	7825 ± 3429	
Manganese [Mn, mg kg_{dw}^{-1}]	237 ± 121	243 ± 234	345 ± 3	
Molibdenum [Mo, mg kg_{dw}^{-1}]	1.23 ± 0.66	2.23 ± 0.60	1.98 ± 1.24	
Zinc [Zn, mg kg_{dw}^{-1}]	91.5 ± 36.1	198 ± 86	116 ± 58	<500
Potential toxic element				
Cobalt [Co, mg kg_{dw}^{-1}]	2.55 ± 0.64	2.96 ± 2.58	2.76 ± 1.36	
Cadmium [Cd, mg kg_{dw}^{-1}]	0.22 ± 0.12	0.32 ± 0.19	0.26 ± 0.13	
Chromium [Cr, mg kg_{dw}^{-1}]	39.0 ± 35.4	55.3 ± 52.7	42.0 ± 19.8	
Chromium VI [Cr(VI), mg kg_{dw}^{-1}]	LoQ	LoQ	LoQ	0.5
Mercury [Hg, mg kg_{dw}^{-1}]	LoQ	LoQ	LoQ	<1.5
Nickel [Ni, mg kg_{dw}^{-1}]	17.2 ± 13.7	15.2 ± 4.5	22.1 ± 11.5	<100
Lead [Pb, mg kg_{dw}^{-1}]	20.2 ± 17.0	10.8 ± 9.8	31.2 ± 3.8	<140
Main xenobiotics				
Aromatic organic acids [mg kg_{dw}^{-1}]	LoQ	0.022 ± 0.005	0.007 ± 0.002	
PAHs [mg kg_{dw}^{-1}]	0.230 ± 0.058	0.04 ± 0.01	0.05 ± 0.01	
Chlorinated phenols [mg kg_{dw}^{-1}]	<0.01	<0.01	<0.01	
C10-C40 hydrocarbons [mg kg_{dw}^{-1}]	490 ± 110	1670 ± 380	540 ± 120	

MOWC = Municipal Organic Waste Compost, SSC = Sewage Sludge Compost, SP = Sludge Plasters.

Simultaneous Thermogravimetric and Differential Thermal Analysis (TG-DTA, SETARAM 92B - France) was used to measure the temperatures where decomposition, reduction or oxidation occur. Five grams of amendment sample were weighed in alumina crucible and heated from 25 to 800 °C in a flux of air (8 L h^{-1}) with a heating rate of 10 °C min^{-1}. Two exothermic peaks (Exo I and Exo II) at which the bonds in organic compounds break with the release of CO_2 are highlighted (Fig. 1). Exo I peak corresponds to oxidation of labile compounds (i.e., proteins, amino acids, carbohydrates) while in the Exo II peak the oxidation of recalcitrant compounds occurs (i.e., aromatic compounds) (Francioso et al., 2005; Díaz et al., 2021). ExoII/ExoI ratio is a widely used indicator of the recalcitrance degree of organic compounds (higher ExoII/ExoI ratio indicate matrices with higher recalcitrance degree) (El Ouaqoudi et al., 2015; Marouani et al., 2019). The T max of the Exo I peak (in the range of 325–337 °C) showed lower values in the MOWC sample compared to SSC and SP, with a weight loss that was similar in the two composts (SSC and MOWC) and lower (−22 %) in SP. At those temperatures, the mass loss is mainly due to the breakdown of labile compounds, such as polysaccharides and proteins (Gioacchini et al., 2015). As for Exo II peak, MOWC and SP exhibited similar T max values (483 and 452 °C, respectively), but a significant difference in the percentage of mass loss (21 % and 6 %, respectively). At these temperatures, it is possible to observe the loss of thermostable compounds, such as aromatic rings and lignin. The results indicate that these compounds were present in higher quantities in MOWC than in SP. ExoII peak of the SSC matrix was

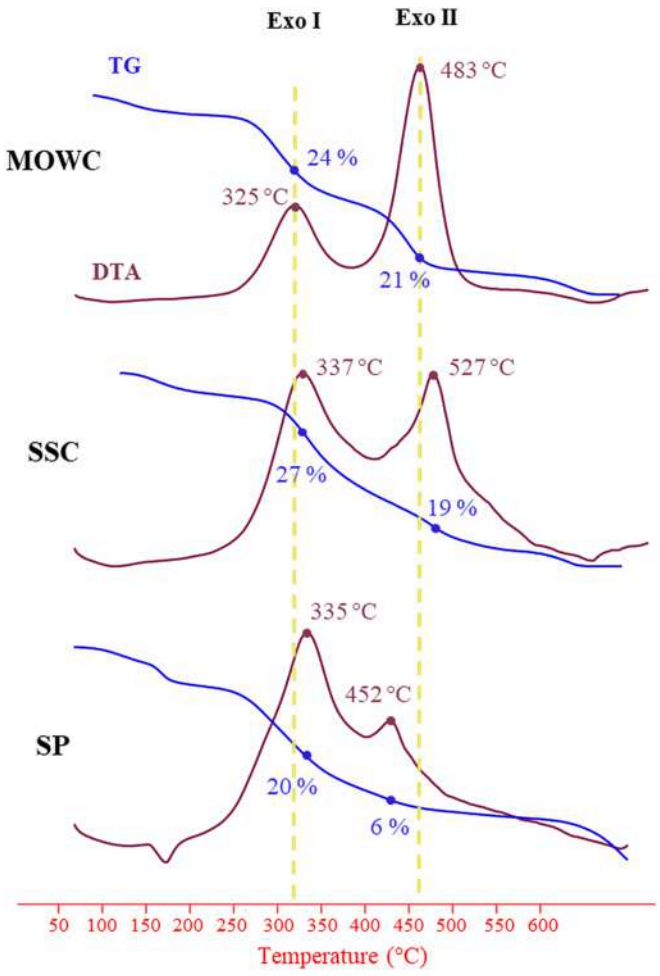

Fig. 1. Thermogravimetric and Differential Thermal Analysis (TG-DTA) of the organic amendments. For each peak (Exo I and Exo II) the corresponding T max (°C), in dark red, and mass lost (%), in blue, are reported. MOWC = Municipal Organic Waste Compost, SSC = Sewage Sludge Compost, SP = Sludge Plasters.

observed at 527 °C, with a mass loss similar to that of MOWC (19 %). At these temperatures, oxidation of thermally resistant organic compounds occurs, such as esters, fatty acids, and aromatics (Gioacchini et al., 2015). Exo II/Exo I ratio showed a clear decreasing trend following the order MOWC (0.85) > SSC (0.69) > SP (0.31).

The C/N ratio and the Exo II/Exo I ratio clearly show that the organic matrices tested have different degrees of recalcitrance (Table 1 and Fig. 1) (El Ouaqoudi et al., 2015; Marouani et al., 2019), corresponding to a different predisposition to mineralization, which follows the hierarchy MOWC > SSC > SP. Indeed, the amounts of N added by organic amendments were calculated considering the potentially available N by applying a specific mineralization coefficient (Table 2) for each organic matrix (Baldi et al., 2022). Since the application was based on the potentially available N supply, the amount of C added annually and from plantation was determined (Table 2). As a result, a reduction in the organic C supplied from MOWC to SSC and SP can be observed.

2.3. Soil sampling

At the third year of experimentation (autumn 2021), after grape harvesting, 64 composite soil samples were taken with an auger from each plot at 0–0.2 and 0.3–0.5 m of depth. A subsample was stored at −80 °C for microbial community analysis. Samples for biochemical analysis were sieved to 4 mm and cleaned with forceps from roots and plant residues, then homogenized and divided in two aliquots, one stored in plastic bags at 4 °C and one air-dried.

2.4. Soil biochemical analyses

Microbial biomass C (MBC) and N (MBN), dissolved organic C (DOC) and total dissolved N (TDN) were determined using the fumigation-extraction method (Vance et al., 1987). The K sulfate extracts were analyzed using an elemental analyzer for liquid samples (TOC - TN Hypertoc; Shimadzu Corp., Kyoto, Japan) and expressed as mg per kg of dry soil (mg kg_{ds}^{-1}). The ratio between DOC and TDN content (DOC:TDN ratio) was also calculated. Soil samples were analyzed with an elemental analyzer (Flash 2000; Thermo Fisher Scientific, USA) to determine the TOC and TN content.

The activity of 5 enzymes, widely considered good indicators of soil quality and functionality (Gil-Sotres et al., 2005; Rao and Gianfreda, 2014), were measured:

- Dehydrogenase (Dehy), intracellular and oxidative, and β-glucosidase (β-glu), extracellular and hydrolytic, involved in the C cycle;
- alkaline Phosphatase (Phos), extracellular and hydrolytic, linked to P availability;
- Tyrosinase (Tyr) and Laccase (Lac), extracellular and oxidative, involved in phenols and lignin degradation processes and OM degradation/formation.

Dehy activity was determined on 1 g of moist soil incubated with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) at 37 °C for 2 h. The 5-(4-iodophenyl)-1-(4-nitrophenyl)-3-phenylformazan (INTF) release was measured at 464 nm and the activity was expressed as $\mu g_{INTF}\ g_{ds}^{-1}\ h^{-1}$ (Von Mersi and Sehinner, 1991). The activities of β-glu and Phos were measured on 1 g of moist soil incubated at 37 °C for 1 h with *p*-nitrophenyl-β-glucoside (pNG) and *p*-nitrophenyl-phosphate (pNP) as substrates, respectively (Eivazi and Tabatabai, 1977, 1988). The reaction product was p-nitrophenol (pN) measured at 400 nm; the activities of Phos and β-glu were expressed as $\mu g_{pN}\ g_{ds}^{-1}\ h^{-1}$. Lac activity was determined with the ABTS substrate as described by Floch et al. (2007); the reaction product was measured at λ = 420 nm and the activity was expressed as μmol ABTS+ $g_{ds}^{-1}\ min^{-1}$. Tyr activity was determined by measuring the dopachrome released after substrate (L-DOPA) oxidation reaction (Sinsabaugh et al., 1999); the reaction product was measured at λ = 475 nm and the activity was expressed as μmol dopachrome $g_{ds}^{-1}\ h^{-1}$.

Specific enzymatic activities, i.e. the ratios between the potential enzymatic activities and the MBC, were used as soil quality indicators. An increase of these ratios suggests an increase of the enzymatic activity not corresponding to a greater microbial biomass thus suggesting a potential nutrient stressing condition for the microbial community (Kandeler and Eder, 1993; Gil-Sotres et al., 2005; Trasar-Cepeda et al., 2008).

2.5. 16S rRNA and ITS gene profiling

Total microbial DNA was extracted from 250 mg of soil sample or amendment using DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) as previously described (D'Amico et al., 2018). DNA samples were quantified using Qubit 3.0 fluorimeter (Invitrogen, Waltham, MA, USA) and stored at −20 °C until processing. The V3-V4 hypervariable region of the 16S rRNA gene and fungal ITS2 were PCR amplified as previously described (D'Amico et al., 2018; White et al., 1990). PCR products were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA). Indexed libraries were prepared by limited-cycle PCR with Nextera technology and cleaned-up with the same magnetic beads protocol. Libraries were normalized to 4 nM and pooled, prior to denaturation with 0.2 N NaOH. Sequencing was performed by Well-micro service (Bologna, Italy) on Illumina MiSeq platform using a 2 × 250 bp paired-end protocol, following the manufacturer's instructions (Illumina, San Diego, CA, USA). Raw sequences were merged using the VSEARCH algorithm (v2.15.2) (Rognes et al., 2016) and analyzed using QIIME2 (version 2022.2) pipeline (Bolyen et al., 2019). The DADA2 (Divisive Amplicon Denoising Algorithm 2) (Callahan et al., 2016) plugin was used to remove noise, chimeras, and to generate Amplicon Sequence Variants (ASVs). Rarefaction was applied using the recorded lowest number of reads per sample (4483 reads for 16S rRNA sequencing, 5656 reads for ITS sequencing). Taxonomy attribution of 16S rRNA genes was obtained using the feature classifier VSEARCH with SILVA SSU database release 138 as reference (Yilmaz et al., 2014). UNITE database version 9.0 was used for the taxonomy assignation of fungal ITS (Abarenkov et al., 2021).

Table 2
Mineralization coefficient (μ_m), dose of nitrogen, real average yearly apport of matrix and related total nitrogen provided yearly, average amount of C added to the soil yearly and sum of the C added after 3 years of treatment for each matrix used in the study.

	μ_m	Dose of N (t $ha^{-1}\ yr^{-1}$)	Matrix apported (t $ha^{-1}\ yr^{-1}$) average of 3 years	Total N provided (t $ha^{-1}\ yr^{-1}$)	C/N ratio (average of 3 yrs)	C added (t $ha^{-1}\ yr^{-1}$) average of 3 years	C added over 3 years (t ha^{-1})
MOWC	0.3	0.12	18.8	0.4	13.5	5.3	15.8
		0.24	37.5	0.8		10.6	31.7
SSC	0.4	0.12	12.8	0.3	9.5	3.1	9.4
		0.24	25.6	0.6		6.3	18.8
SP	0.6	0.12	8.9	0.2	7.7	1.6	4.8
		0.24	17.8	0.4		3.7	9.6

MOWC = Municipal Organic Waste Compost, SSC = Sewage Sludge Compost, SP = Sludge Plasters.

2.6. Statistical analysis and networks construction

Statistical analyses were performed using the R statistical environment (www.r-project.org), v. 4.2.2, and the libraries "rstatix", "agricolae", "vegan", "made4", "randomForest", and "rfPermute".

Alpha diversity and richness indices were calculated using different metrics, Faith's Phylogenetic Diversity (for bacteria only), Shannon-Wiener, Chao1 for both bacterial and fungal communities. Bacterial beta-diversity was measured by calculating the weighted and unweighted UniFrac distance matrices, while for fungal beta-diversity, chemical and biochemical data Bray-Curtis dissimilarity was used. Significance of chemical, biochemical and microbiological data separation in the Principal Coordinates Analyses (PCoA) was tested using a permutation test with pseudo-F ratios (function "adonis" in the vegan package) for multiple comparison, while "pairwise.adonis" function was used to perform pairwise comparisons.

Soil chemical and biochemical parameters were analyzed by checking the normality of distribution and homogeneity of variance and by applying one-way ANOVA with replicates ($P < 0.05$) followed by Tukey HSD post-hoc test ($P < 0.05$). Wilcoxon rank-sum test and Kruskal-Wallis test for multiple comparisons, followed by Tukey post-hoc test, were used to evaluate significant differences among values (alpha-diversity index, relative abundance of selected taxa) measured in two or more groups of samples, respectively.

The sharing of microbial taxa between amendment matrices and amended soil samples was explored at ASV level by computing Venn diagrams and identifying shared sequences, whereas family level was chosen for network analysis.

Co-abundance of combined fungal and bacterial communities (Durán et al., 2018; Araya et al., 2020; Li et al., 2024) at the family level was explored using network inference based on strong negative and positive Kendall correlation ($\tau < -0.4$ and $\tau > 0.4$) and visualized using hierarchical Ward clustering with a Spearman correlation distance metrics. Microbial (bacterial and fungal) families with relative abundance higher than 1 % in at least 10 % of the samples were selected for the analysis. The significant associations observed were controlled for multiple testing by the Benjamini-Hochberg method, with a false discovery rate (FDR) ≤ 0.05. Networks were created using Cytoscape version 3.9.1 (https://cytoscape.org/). Edges were represented as grey or red line for positive and negative significant correlation, respectively. Nodes sizes were proportional to families mean relative abundance or over-abundance, calculated as the ratio between the mean relative abundance in a single amendment group and in all samples (meanAmendment/meanAllsamples). NetworkAnalyzer tool was used to calculate network topology parameters. Putative hub nodes were identified as defined by a combination of highest values of closeness centrality, betweenness centrality and degree on Cytoscape, as proposed by Agler et al. (2016). Networks were also used to visualize Kendall correlation between microbial families and soil properties (TOC, DOC, TN, TDN, C/N ratio) identified as stronger "predictors" using Random Forest regression approach (variance explained >30 %). Positive and negative correlation having FDR ≤ 0.05 were displayed as blue and red node, respectively, with sizes proportional to Kendall-τ correlation coefficient, both negative and positive.

2.7. Accession number

Nucleotide sequences generated and analyzed during the current study are available in the National Center for Biotechnology Information (NCBI) repository with the BioProject ID: PRJNA1096138.

3. Results

3.1. Chemical and biochemical soil indicators

The impact of sampling depth on soil biochemical parameters was explored, to guide the subsequent statistical analyses. Overlapping between 0–0.2 and 0.3–0.5 m was observed on PCoA analysis based on Bray-Curtis distances (Supplementary Fig. S1) and adonis test ($P > 0.05$). Consequently, subsequent analyses of the impact of the different treatments were carried out without distinguishing between samples taken at different depths.

The soil did not show significant changes on its pH due to the treatments (average pH value with its standard deviation was 7.41 ± 0.24, data not shown). The extractable fraction of C (DOC) showed the highest value in correspondence of the SP2 treatment (+34 %) with respect to the controls (MF and CK); also, MOWC2 and SSC2 addition increased the DOC with respect to the controls (+19 %) but not at the same extent (Fig. 2A). The rest of the treatments are not clearly distinguishable from the CK and MF. The extractable fraction of N (TDN) showed a 69 % and 81 % increase respectively in SP2 and SSC2 treatments compared to CK, indicating a significant N release in these soil samples (+66 % on average compared to MF and MOWC) (Fig. 2B). The ratio between soil DOC and TDN (DOC:TDN) was calculated. A decrease in the DOC:TDN ratio was observed between the different treatments according to the hierarchy MOWC > SSC > SP (14.2, 10.4, and 8.0 respectively). The two controls CK and MF have higher values, 18.1 and 15.0 respectively. Consequently, there is a progressive increase in the relative availability of N compared to C, passing from MOWC to SSC and SP, confirming the observed hierarchy of recalcitrance. The microbial biomass C and N (MBC and MBN) showed the highest value in MOWC2 (Fig. 2C–D). SP2 supply induced a decrease of the MBC content compared to CK and MF (−32 and 46 %, respectively), whereas the MBN showed the lowest values in CK (6.00 mg of MBN kg_{ds}^{-1}). Finally, the TOC and TN significantly increased with the MOWC2 treatment by 50 % and 94 %, respectively, compared to the controls (CK and MF). Additionally, both SSC and SP increased TOC and TN by an average of 36 % compared to the controls (Fig. 2E–F). However, the higher TOC and TN levels observed with the MOWC2 treatment were predictable due to the greater amount of matrix added to the soil (Table 2). It should be noted, however, that these results are consistent with the trends in MBC and MBN contents, but not in DOC and TDN contents.

Within the potential enzymatic activities (Supplementary Table S1), β-glu and Lac did not highlight significant differences, while Dehy, Phos and Tyr resulted enhanced by different organic matrices; specifically, Dehy showed the highest value in correspondence of SP2 (78.6 μg_{INTF} g_{ds}^{-1} h^{-1}) with respect to all the other treatments, Phos showed increased activity with MOWC2 (+64 % with respect to CK and MF controls), and Tyr increased by 24 % with the SSC at both the doses applied compared to the controls. Concerning the specific enzymatic activities (i.e., the ratio between enzymatic activity and the MBC), the β-glu did not show significant differences among treatments (Fig. 3A). The specific Phos and Dehy activities (Fig. 3B–E, respectively) exhibit the same trend with the highest value in correspondence of the SP2 treatment (3.40 and 1.35, respectively) followed by the SSC2. The two specific extracellular oxidative activities (Lac and Tyr) showed highest values in CK, followed by SP2 and SSC (both doses) treatments (Fig. 3C–D, respectively). In general, all the specific enzymatic activities showed the lowest values in correspondence of the MOWC supply. The greater stimulating effect on specific phosphatase activity was observed with SP2 and partly with SSC2; all the other treatments were positioned at levels similar to CK. For laccase and tyrosinase activities, all treatments have values significantly lower than CK, except SP2, which also tends to be higher than all other organic and mineral treatments. Also in this case, the main differences between the treatments could be attributed to the degree of their recalcitrance.

3.2. Characterization of the microbial community

Both bacterial and eukaryotic portions of the microbial communities inhabiting the vineyard soil were characterized using amplicon sequencing approach, together with the microbial community carried by

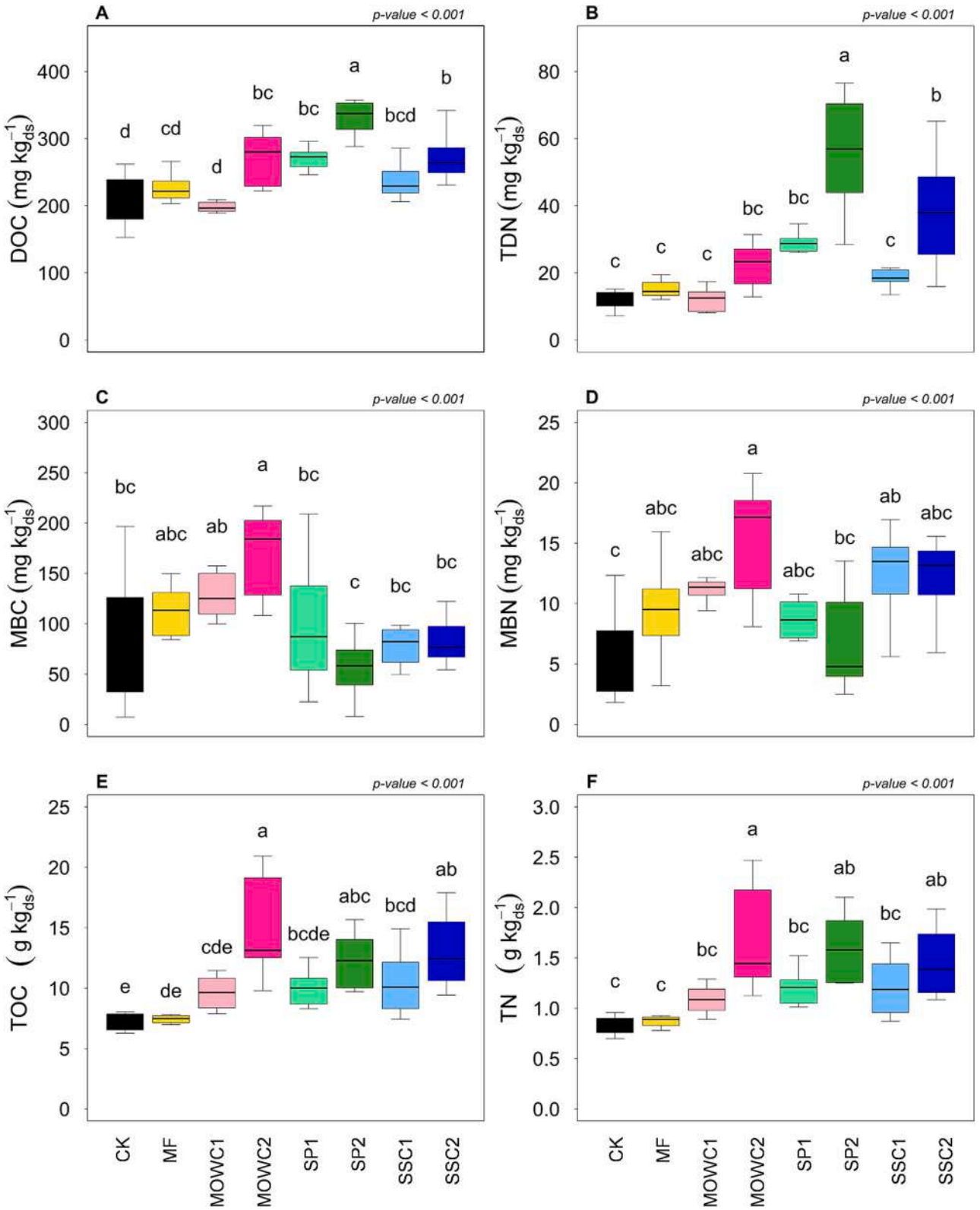

Fig. 2. C and N pools distribution in the different groups of samples. Means of (A) soil dissolved organic C (DOC), (B) total dissolved N (TDN), (C) microbial biomass C and (D) N (MBC and MBN), (E) total organic C (TOC), and (F) total N (TN). Different lower-case letters indicate significant differences (p-value < 0.05). CK = Control, MF = Mineral Fertilization, MOWC = Municipal Organic Waste Compost, SSC = Sewage Sludge Compost, SP = Sludge Plasters.

the bulk amendments MOWC and SSC. From samples of the SP matrix it was not possible to extract detectable microbial DNA. Sequencing of the V3/V4 region from bacterial 16S rRNA gene and of the fungal ITS2 provided a total of 407,782 and 954,165 high-quality reads, with a mean of 6178 and 14,478, respectively.

Firstly, the impact of sampling depth on the microbial communities was explored. A consistent overlapping of the phylogenetic composition of samples taken between 0 and 20 cm and between 30 and 50 cm was

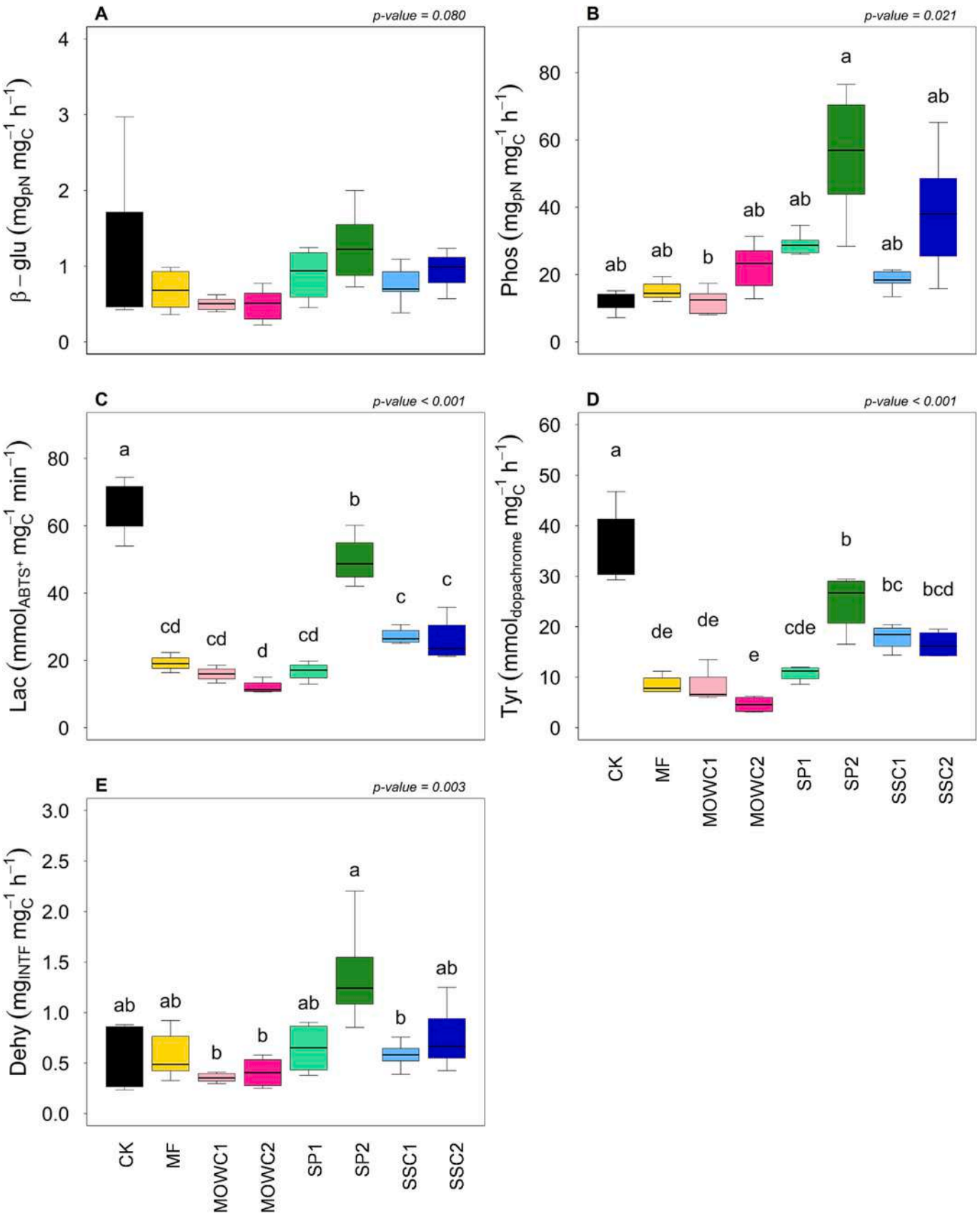

Fig. 3. Specific enzymatic activities in the different groups of samples. Means of soil specific activities of (A) β-glucosidase (β-glu), (B) Phosphomonoesterase (Phos), (C) Laccase (Lac), (D) Tyrosinase (Tyr), and (E) Dehydrogenase (Dehy). Different lower-case letters indicate significant differences (p-value < 0.05).

observed for both bacterial and fungal communities on PCoA analysis based on UniFrac and Bray-Curtis distances, respectively (Fig. 4, Adonis test, p value > 0.05 for all PCoA analyses). Random forest machine learning approach confirmed that microbial community composition was not a predictor for sampling depth ("out-of-bag" estimate of error rate 54.3 and 41.1 %, for 16S rRNA gene and ITS sequence-based analyses, respectively). Finally, samples taken at different depth did not show significant differences in terms of biodiversity (Supplementary Tables S2 and S3). Consequently, subsequent analyses of the impact of different amending strategies on soil microbial communities were carried out without distinguishing between samples taken at different depths.

MOWC (both dosages) and SSC2 amendments were associated to a significantly higher bacterial diversity with respect to CK soil. A similar trend was observed for fungal biodiversity, even if statistical significance was not reached (Supplementary Fig. S2). A peculiar trend was associated to soil samples treated using the lower dosage of SSC (SSC1), which were characterized by the highest values of fungal biodiversity, without inducing a significant increase in bacterial diversity with respect to CK. No significant variations in either bacterial or fungal biodiversity were detected in MF and SP amended soils, with respect to CK.

Conversely, multivariate analysis highlighted that SP-based amendments induced the most appreciable compositional changes in soil microbiome, especially in fungal community. SP soil samples clustered separately from all other samples on a PCoA based on Bray-Curtis distances among the ITS sequencing profiles of soil samples, whereas SSC, MOWC and MF samples overlapped with controls (Adonis test, p value = 0.001) (Fig. 4C). Pairwise PERMANOVA analysis confirmed a significant separation between SP samples (both dosages) and all the other treatments (adjusted p value < 0.03). Such separation was still significant (Adonis test, p value = 0.001), even if less striking on the plots, when the bacterial community was considered (Fig. 4A–B). Briefly, MF treatment did not induce relevant changes in soil bacterial community, as CK and MF samples overlap in PCoA based on both weighted and unweighted UniFrac distances among 16S rRNA gene sequencing profiles (no significant separation based on pairwise PERMANOVA test in both PCoA), whereas SP samples clustered on the other side of the plots (unweighted UniFrac-based PCoA, SP samples vs. CK or MF samples, adjusted p value < 0.03). MOWC and SSC samples spread in between the two clusters, with partial overlapping with SP samples but clear separation with respect to MF and CK. Pairwise PERMANOVA highlighted significance of the separation between MOWC2 samples (high dosage) and CK and MF (p value = 0.028).

At a compositional level, most represented bacterial phyla in all samples were Acidobacteriota, Actinobacteriota, and Proteobacteria,

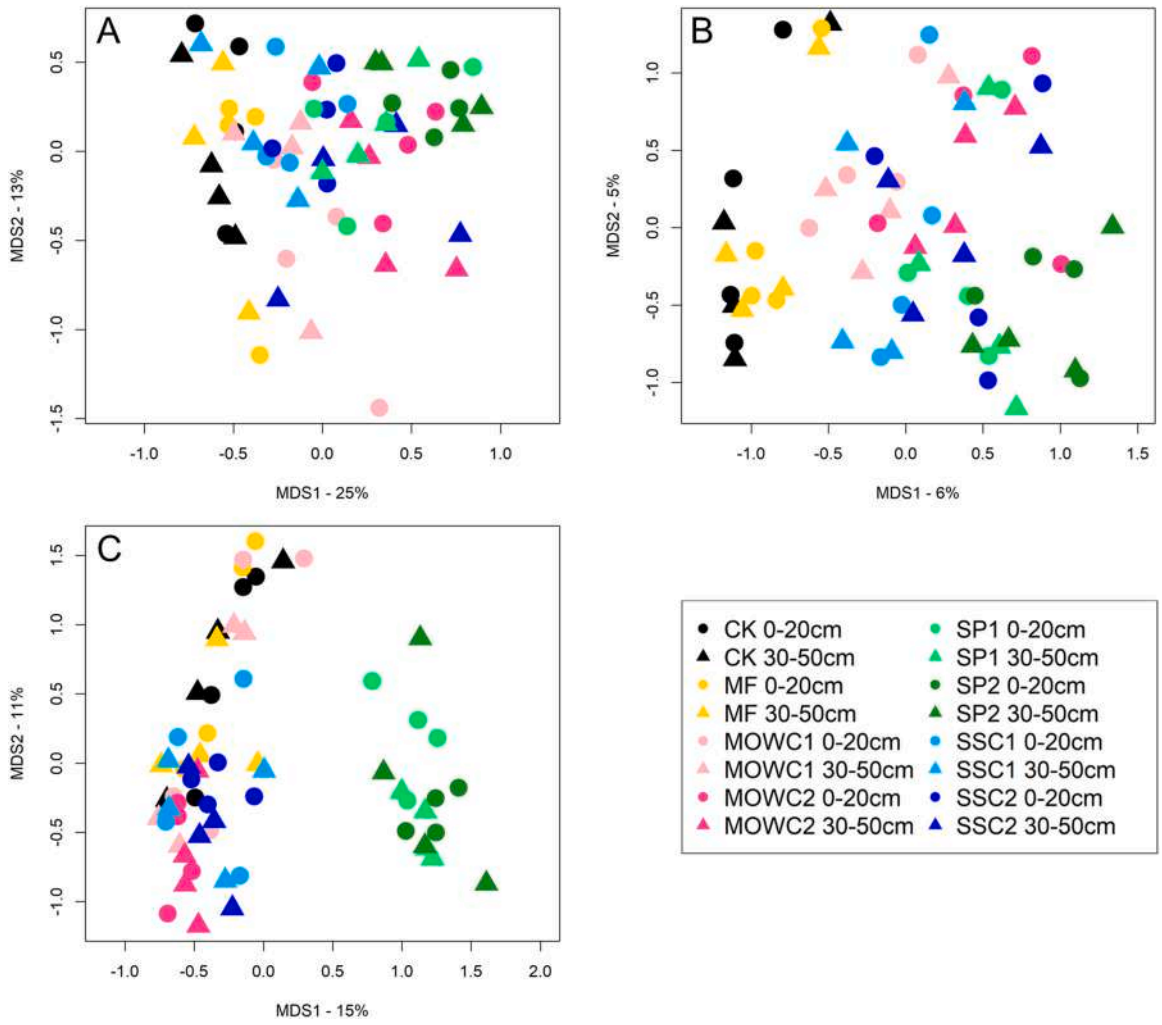

Fig. 4. Impact of different amendments on soil bacterial (A–B) and fungal (C) communities. PCoA based on weighted (A) and unweighted (B) UniFrac distances among 16S rRNA-based phylogenetic profiles, and based on Bray-Curtis dissimilarity matrix among fungal community (ITS sequences-based phylogenetic profiles) (C) of soil samples taken at 0–20 cm (circles) and 30–50 cm depth (triangles). Color legend for all plots is reported in a bottom-right panel. First and second coordination axes (MDS1 and MDS2) are plotted for each analysis. Percentages of variance in the dataset accounted for by MDS1 and MDS2 are reported.

with Bacteroidota becoming more abundant in soil amended using MOWC, SSC and SP to the detriment of Acidobacteriota and Actinobacteriota (Supplementary Table S4). This is mirrored by the composition of MOWC and SSC amendments samples that were enriched in Bacteroidota members, with *Sphingobacteriaceae* (22.2 % in MOWC) and *Microscillaceae* (15.0 % in SSC) as most represented families. For what concerns the eukaryotic component, the most represented phylum in all samples was Ascomycota, with Basidiomycota and Mortierellomycota

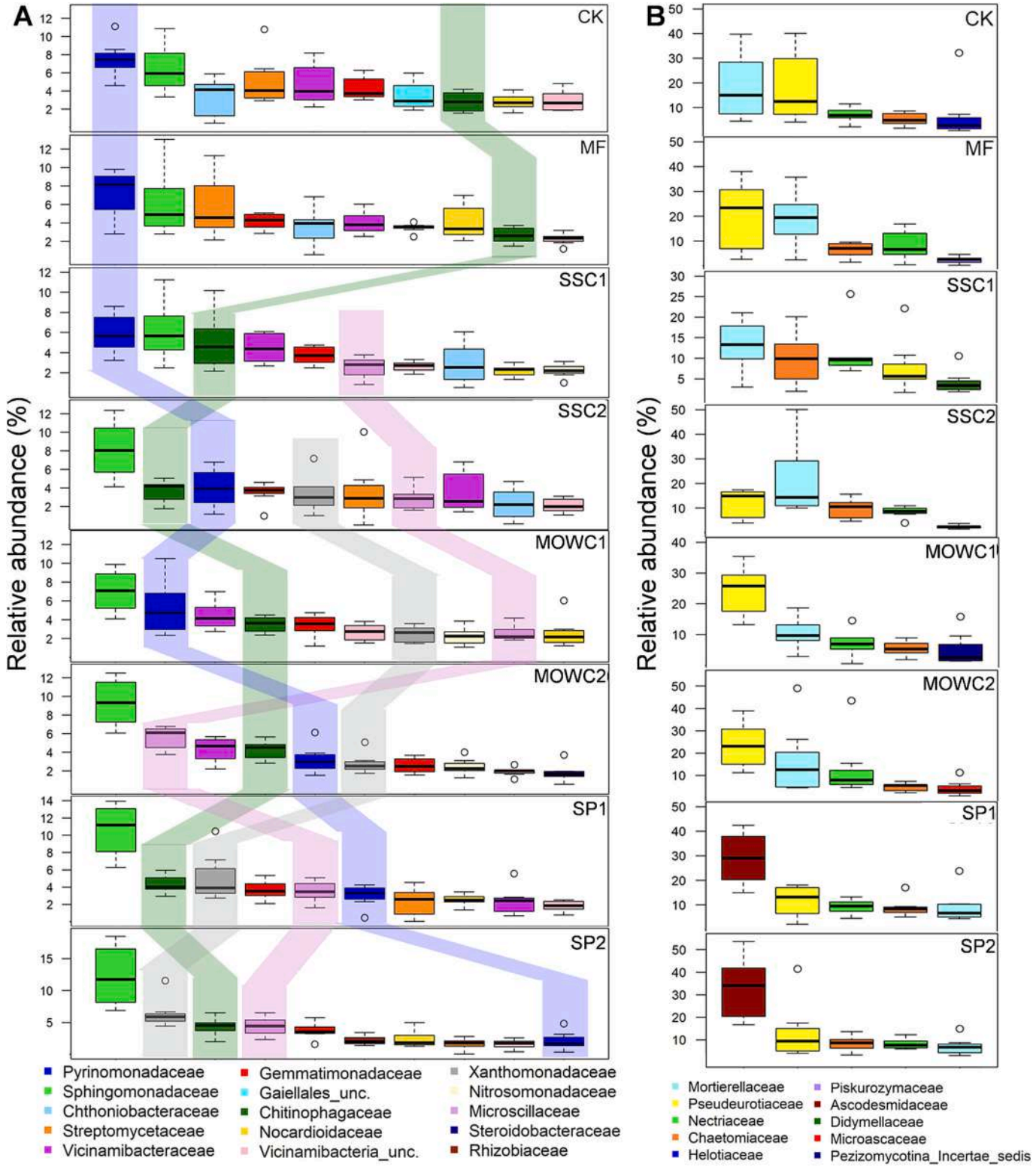

Fig. 5. Impact of different amendments on most abundant soil microbial families. Boxplot showing the top 10 bacterial families (A) and top 5 fungal families (B) in terms of average relative abundance in control soil (CK) and soils treated using the different amendments (indicated at the top-right corner of each plot). Bacterial families are plotted using a color code (see legends at the bottom). Relevant changes in "rank" among the top10 bacterial families are highlighted using a shadow ribbon for clarity.

also consistently contributing. An analysis of ASV shared among the different groups of samples highlighted that the different amendments deeply rearranged the soil microbiome structure without acting as true inoculum (data not shown). Indeed, MOWC matrix and MOWC-amended soil shared only 3 fungal ASV out of 2955 (each one accounting for a relative abundance <0.5 % in the matrix and <0.06 % in the amended soil) and none of the bacterial ASV (total number 28,352). Analogously, SSC matrix and SSC-amended soil shared no bacterial ASV and 7 fungal ASV. Among the latter, 2 ASV belonging to the Sordariomycetes class were abundant in the matrix (48 % and 6 % relative abundance, respectively) but were retrieved only in few SSC soil samples (1 and 4 out of 16, with relative abundances <0.05 % and <1 %, respectively). The other 5 shared fungal ASV accounted for <0.4 % of the matrix fungal community and were retrieved only in 1 of the SSC soil samples with <0.06 % relative abundances.

Focusing the attention on the "top 10" bacterial families (ten most abundant families ordered by mean relative abundance) and on the "top 5" fungal families (Fig. 5), the most abundant bacterial family in CK and MF soils, *Pyrinomonadaceae*, in the present dataset composed entirely of uncultivated RB41 taxa (in blue in Fig. 5A), decreased in soil treated using organic amendments, especially in MOWC and SP-amended samples where the average abundance reached values <4 %. As a consequence, other families gained rank, such as *Sphingomonadaceae* (light green), *Chitinophagaceae* (dark green), *Xanthomonadaceae* (grey) and *Microscillaceae* (pink) (Fig. 5A). As for fungi, the family *Ascodesmidaceae* became dominant in SP amended soil, to the detriment of *Pseudeurotiaceae* and *Mortierellaceae* (Fig. 5B).

To better understand the extent of the modification induced by the different amendments at a community level (both prokaryotic and eukaryotic), an overall network was generated. Briefly, the co-abundance associations between bacterial and fungal families were assessed. Co-abundance groups (CAG) assignment relied on a heatplot showing co-abundance of each taxon, clustered by the Spearman correlation coefficient and Ward-linkage hierarchical clustering (Supplementary Fig. S3). Five different CAGs were detected. The generated network was composed of 75 nodes (Fig. 6). CAG1, CAG3, and CAG4 were the most interconnected, whereas the smaller CAG2 and CAG5 showed less connections, either within the CAG or with members of

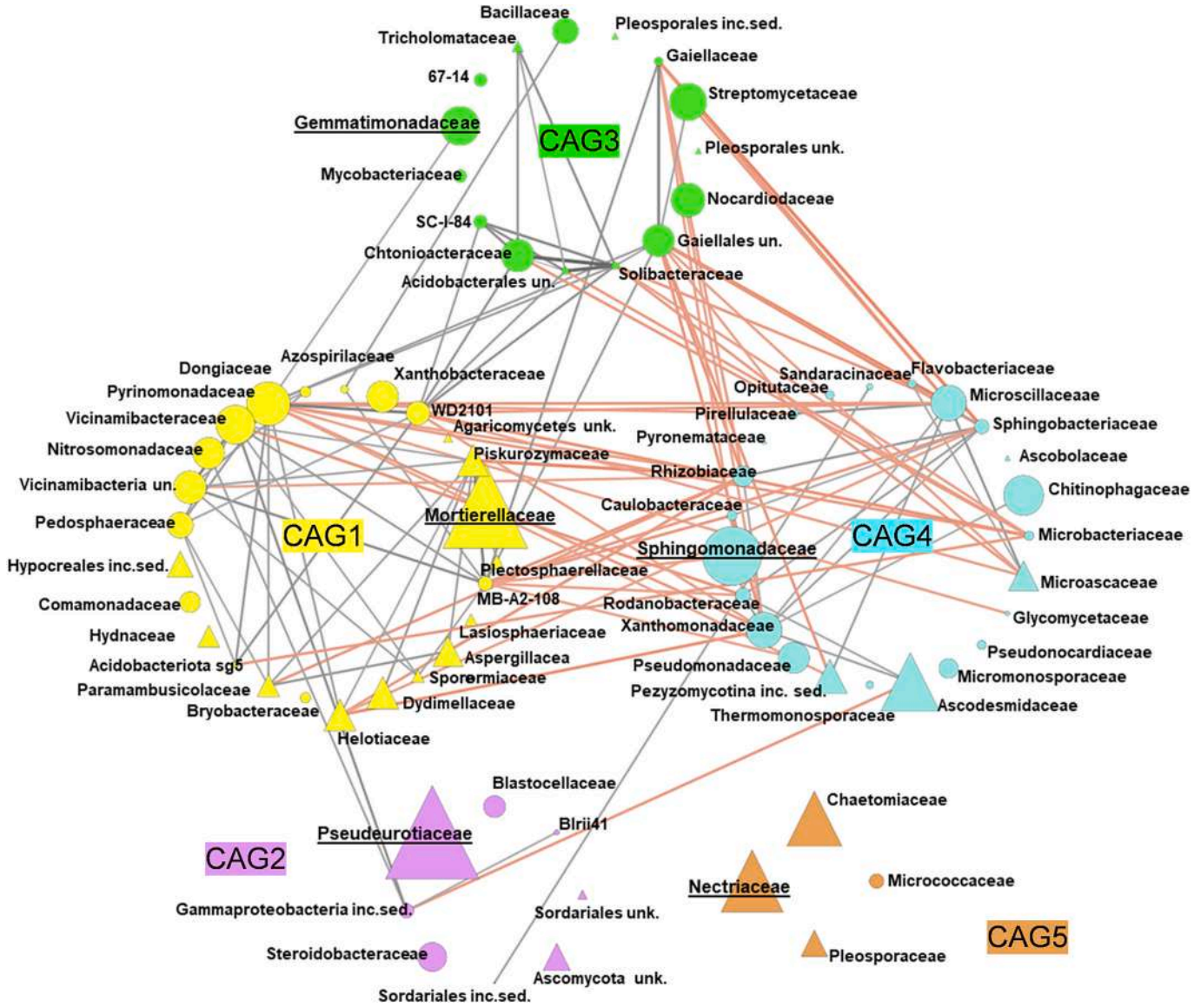

Fig. 6. Prokaryotic and eukaryotic soil community network. Wiggum plot representing the overall relative abundance of bacterial and fungal families in the five identified co-abundance groups (CAGs). CAGs are color coded as follows: CAG1, yellow; CAG2, purple; CAG3, green; CAG4, cyan; CAG5, orange. Nodes' size is proportional to the overall mean relative abundance (%) of bacterial (circles) and fungal (triangles) families. Connections between nodes represent strong positive ($\tau > 0.4$, grey) and negative ($\tau < -0.4$, red) significant Kendall correlations (FDR ≤ 0.05). Abbreviations: un, uncultured; unk, unknown; inc. sed., incertae sedis.

other CAGs. Identified potential hub nodes in the network (Supplementary Table S3) were spread across the 3 richest CAGs: *Gaiellaceae* and Gaiellales unclassified in CAG3, *Pyrinomonadaceae* in CAG1, *Xanthomonadaceae* and *Rhizobiaceae* in CAG4. CAG1 and CAG3 members showed positive inter-CAG correlations among members, whereas members of CAG4 were found negatively correlated with members of both CAG1 and CAG3.

The variations of the network in soil treated using different amendments have been explored by observing the cumulative abundance of bacterial and fungal members of each CAG (Fig. 7A and B) and computing patterns of over-abundance in nodes (Fig. 7C–I). For nodes with strikingly high overabundance values (>2) the relative abundance distribution in the differently amended soils is provided in Supplementary Fig. S4. CK and MF soil (showed as unique network in Fig. 7C for clarity and in accordance with the similarity emerged from previous analyses) showed overabundant nodes spread across CAG1 and CAG3, with the fungal families *Tricholomataceae*, *Helotiaceae* and unknown Agaricomycetes showing the highest overabundances (2.8, 2.1, and 2.2, respectively). Conversely, all organic-based amendments mainly impacted nodes belonging to CAG4, the cumulative abundance of which noticeably increased with respect to CK soil especially in SP soil, in terms of both bacterial (Fig. 7A) and fungal (Fig. 7B) contributions. MOWC and SSC impact on the overabundance pattern was most appreciable when highest dosages were used. Both induced an increase in abundance in the fungi group Sordariales *incertae sedis* (CAG2) (overabundance 2.4, 2.7 and 2.0 in MOWC2, SSC1 and SSC2 soil, respectively), whereas only MOWC caused an increase in the bacterial group "Birii41" (of the Polyangiales order) (overabundance 1.9 and 2.7, in MOWC1 and MOWC2 soil, respectively). The MOWC2 and SSC2 dosages also induced an increase in abundance of several families belonging to CAG4, including *Flavobacteraceae* and *Sandaracinaceae*, and the fungal families *Ascobolaceae*, *Microascaceae* and *Pezyzomycotina* (specifically responding to MOWC2) (Fig. 7D, G, F and I). The most striking impact on members of CAG4 was reported for SP amendment (Fig. 7E and H). Indeed, overabundance >2 was reported for *Thermomonosporaceae*, *Sphingobacteriaceae*, *Rhodanobacteraceae*, *Xanthomonadaceae*, *Ascodesmidaceae*, and *Pyronemataceae* (Supplementary Fig. S4).

3.3. Correlation between microbial and biochemical data

In order to explore how the variations in the microbial network, induced by the different amendments, are associated to differences in soil parameters of agronomical interest, pairwise Kendall correlations were computed between prokaryotic and eukaryotic soil community profiles at the family level and soil parameters that showed higher "predictivity" for microbial composition using Random forest regression approach (i.e. DOC, TOC, TN, TDN, C/N) (Fig. 8). All considered soil parameters (Fig. 8A–D), with the exception of the C/N ratio (Fig. 8E), showed positive correlations with members of CAG4, and negative correlations with members of CAG1, CAG2 and CAG3. Within CAG4, peculiarly high Kendall correlation coefficient values were obtained for *Sphingobacteraceae*, *Microscillaceae* (with DOC, TDN, TOC, TN), *Pirellulaceae* (TDN, TOC, TN), *Rhodanobacteraceae* and *Xanthomonadaceae* (DOC, TDN, TN), and *Ascodesmidaceae* (DOC, TDN). Conversely, within CAG1 and CAG3, the putative hub nodes *Gaiellaceae*, Gaiellales uncultured, and *Pyrinomonadaceae* showed significant negative correlations with all soil parameters but C/N. For the latter, only one positive (Gammaproteobacteria *Incertae Sedis* unknown family, Kendall $\tau = 0.4$, fdr corrected p value = 0.01) and one negative (*Ascodesmidaceae*, $\tau = -0.4$, p value = 0.001) correlations were reported (Fig. 8E). When trying to correlate microbial family abundances with the specific enzymatic activities, none of the obtained correlation values passed the chosen threshold ($\tau < -0.4$ and $\tau > 0.4$). It is interesting to point out that significant correlation trends (FDR corrected p value < 0.05, $\tau < -0.3$ or $\tau > 0.3$) were obtained between Dehydrogenase activity and the abundance of Gammaproteobacteria Incertae Sedis ($\tau = -0.34$) and *Ascodesmidaceae* ($\tau = +0.36$), between Phosphatase activity and *Bacillaceae* ($\tau = -0.33$), and between Laccase activity and Pezizomycotina Incertae Sedis ($\tau = -0.38$) (data not shown).

4. Discussion

The effects on soil biochemistry and microbiology of different amendments were studied in an experimental vineyard in its third year of planting. Concerning the potential to introduce allochthonous bacterial and fungal species into agricultural soil, the present study suggests that the changes in soil microbiome were unlikely caused by microbial inoculation. This is supported by the lack of shared bacterial sequences between the amendment matrices and the corresponding treated soil samples, as well as by the detection of only sparse fungal sequences retrieved both in the matrix and in a minority of amended soil samples, with a very low relative abundance.

Regarding the effects on soil biochemistry, the most significant results were observed at the highest dose of matrices supplied, while at the lowest dose, even though the supply of OM was not negligible, no significant variations were observed, for example, in TOC, MBC and specific enzymatic activities compared to the control. This can be linked to the initial condition of the soil, particularly poor in TOC and prone to the rapid mineralization of OM. This, combined with the processing (disk harrowing) of burying the matrices, was such that 3 years of experiments were not sufficient to highlight significant effects at the lower dose of matrices.

Since the doses applied were calculated to provide the same potentially bioavailable N contribution, the matrices added different amounts of organic C to the soil. Additionally, the provided C source varied in the content of low and high-thermal stable compounds. According to the presented Exo I and Exo II peaks analyses, and their ratio, SP matrix was the one with the lowest quantity of recalcitrant components, whereas MOWC provided a higher fraction of recalcitrant compounds, followed by SSC (El Ouaqoudi et al., 2015; Marouani et al., 2019). This suggests that SP is the least stable matrix and is expected to be more easily degraded by soil microbes. Indeed, the C/N ratio of SP was significantly lower than that of SSC and MOWC, indicating a higher tendency towards mineralization and consequent higher release of N in the short term, confirmed by the lower DOC:TDN ratio obtained with SP compared to both the addition of the other two organic amendments and to the controls. This aspect has been considered in the calculation of the amount of mineral N that organic matrices added to the soil can make available to plants each year. The amount of organic matrices added to the soil is calculated considering a mineralization coefficient that estimates the predisposition of the matrix to mineralization. This method of operation is intended to prevent excessive and too rapid release of mineral N from those matrices, which could be detrimental both to the quality of the grapes and to the distribution of leachable nitric N in the environment. However, despite the use of mineralization coefficients, the DOC:TDN ratio accurately reflects the C/N of the organic matrices (SP < SSC < MOWC). Results show the main consequence of these relationships, with a greater accumulation of TDN in SP treatments, at both doses, compared to the relative doses of MOWC and SSC. This suggests that there may be an imbalance in the relative soil content of soluble C and N, which could also affect microbial activity (Moorhead and Sinsabaugh, 2006). On the other hand, the supply of chemically stable organic compounds (i.e., MOWC) favored the increase of the microbial biomass both compared to SP, SSC and both CK and MF. A similar trend was also observed by Bastida et al. (2008) in a 2-year field study where fresh and composted organic matrices were applied to a sandy clay loam soil in southeastern Spain (semi-arid Mediterranean climate). Specifically, Bastida et al. (2008) found MBC values that were two to three times higher with compost application compared to the control just three months after amendment application.

After three years of application, the abovementioned differences in terms of quantity and quality of C added to the soil not only impacted on

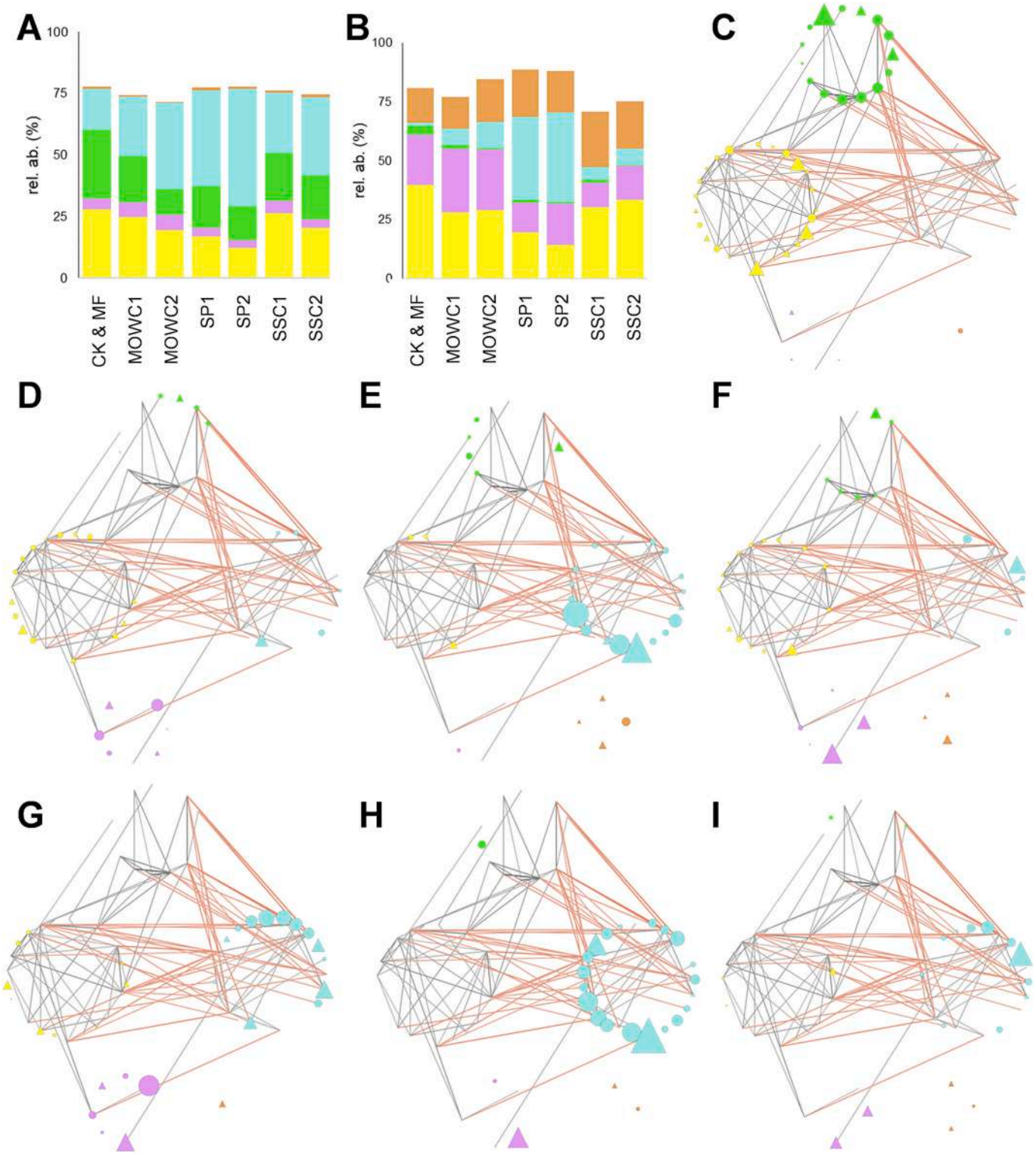

Fig. 7. Prokaryotic and eukaryotic community network variations in differently amended soils. Average cumulative abundance of the bacterial (A) and fungal (B) families in each CAG and Wiggum plots representing the over-abundance of bacterial and fungal families in the five CAGs (C-I) for the different amendment conditions: CK and MF (analyzed together based on results of previous analyses) (C), MOWC1 (D) and MOWC2 (G), SP1 (E) and SP2 (H), SSC1 (F) and SSC2 (I). Color code for CAGs is the same as in Fig. 6. Nodes corresponding to bacterial and fungal families with an overabundance ≤ 1 are not represented. The node size corresponding to families with overabundance > 1 is proportional to the overabundance value. Network structure (i.e., nodes position and connections) is the same provided in Fig. 6.

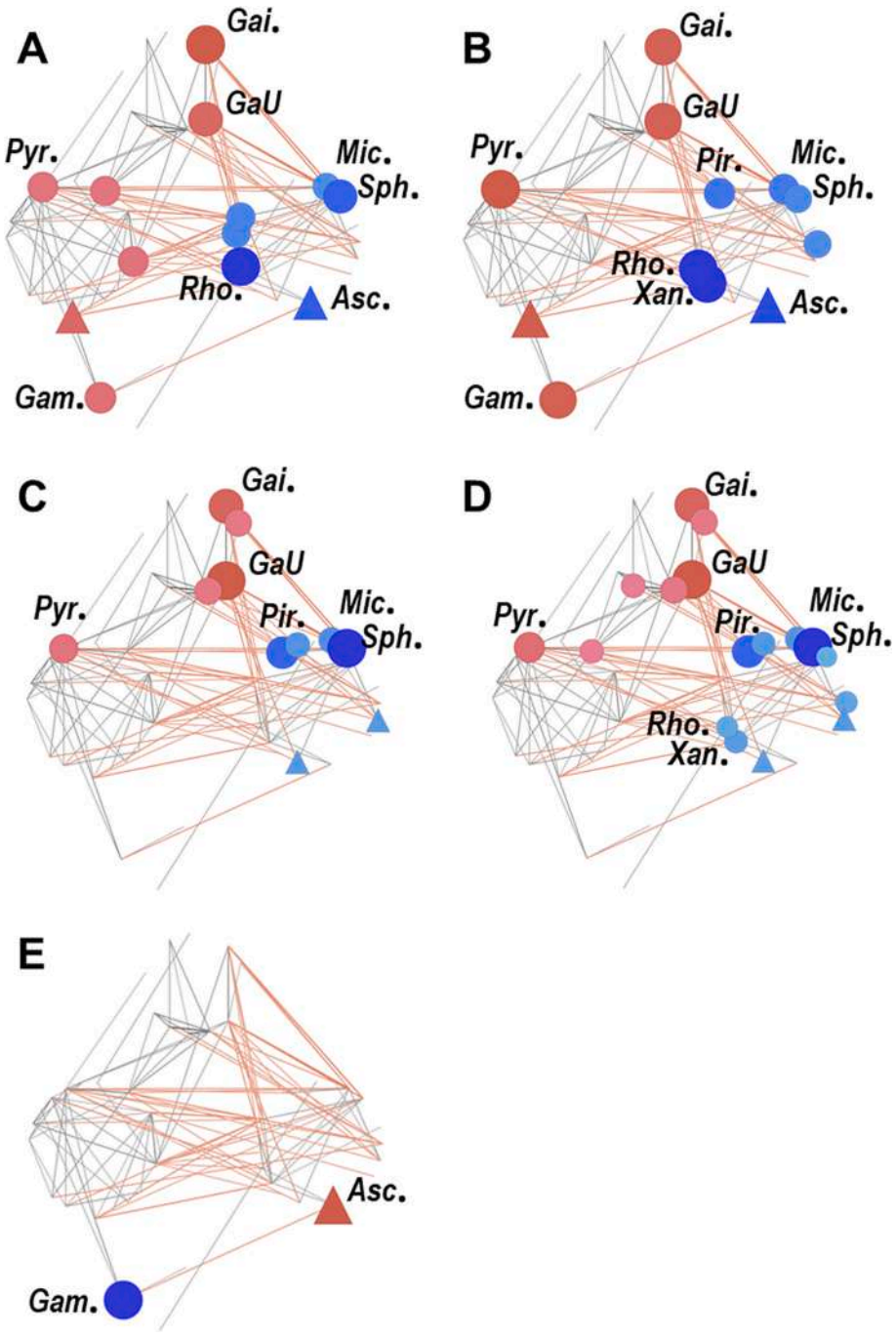

Fig. 8. Correlation between soil prokaryotic and eukaryotic community networks and soil parameters: dissolved organic C (DOC) (A), total dissolved N (TDN) (B), total organic C (TOC) (C), total N (TN) (D), and C/N ratio (E). Network structure (i.e., nodes position and connections) is the same provided in Fig. 6. Each node represents a bacterial (circle) or fungal (triangle) family. Positive and negative Kendall τ correlation coefficients with FDR-corrected p value ≤ 0.05 are represented as blue and red nodes, respectively. Nodes' size and color intensity is proportional to the absolute value of Kendall τ correlation coefficients with soil parameters. Abbreviations: Gai., *Gaiellaceae*; GaU, Gaiellales uncultured; Pir., *Pirellulaceae*; Mic., *Microscillaceae*; Sph., *Sphingobacteraceae*; Asc., *Ascodesmidaceae*; Xan., *Xanthomonadaceae*; Rho., *Rhodanobacteraceae*; Pyr., *Pyrinomonadaceae*; Gam., Gammaproteobacteria *Incertae sedis*.

the soil C and N pools, but also had effects on the microbial biomass content, activity and composition, both at the eukaryotic and prokaryotic level. The structure of the soil microbial communities reflected what reported in previous studies for vineyard soils, with Proteobacteria, Actinobacteria and Acidobacteria as the most represented bacterial phyla (Burns et al., 2016; Coller et al., 2019; Zhou et al., 2021; Gobbi et al., 2022), and Ascomycota, Basidiomycota and Mortierellomycota the most abundant fungal phyla (Liu et al., 2020), but was affected at compositional level by all tested organic matrices, whereas the traditional MF showed almost no effects with respect to CK soil.

Such impact was noticeable at the bacterial phylum level, with Proteobacteria and Bacteroidota contributes averagely increasing in all amended soils, to the detriment of Acidobacteriota and Actinobacteriota. This trend might reflect an increase in heterotrophic respiration rate in the vineyard soil (Zhang et al., 2022b), which is coherent with the increased amount and availability of C sources (TOC, DOC) in amended soils with respect to CK and MF. Indeed, Proteobacteria and Bacteroidetes tend to thrive in soil with high resources, thus being

classified as copiotrophs or *r*-strategists (Leff et al., 2015; Wang et al., 2021) in opposition to Acidobacteriota, which are recognized as prevalently oligotroph (K-strategists) (Ho et al., 2017; Wang et al., 2018). This compositional shift is most evident in SP samples, consistently with the highest amount of dissolved C and N fractions (DOC and TDN) and the lowest recalcitrance of the C fraction reported for this amendment.

At a lower phylogenetic level, a deep rearrangement in the relative contribution of the most abundant bacterial families was induced by the different organic amendments. This was particularly evident in the case of SP soil in which the distribution of the most abundant families was dominated by *Sphingomonadaceae*, followed by *Xanthomonadaceae* (both Proteobacteria) and *Chitinophagaceae* (Bacteroidota), while *Pyrinomonadaceae* (Acidobacterota), i.e. the most abundant family in CK soil, lose rank. *Pyrinomonadaceae* are prone to colonize environments with nutrient limitations (Ivanova et al., 2020) instead of soil with promptly available C and N source, such as the one treated with SP. SP also caused a deep rearrangement in the dominant eukaryotic families with the appearance of *Ascodesmidaceae* as the most abundant taxa, replacing *Mortierellaceae* (Mortierellomycota), a common fungal taxon in vineyard soil (Gupta et al., 2019; Cureau et al., 2021). Not much is known about the role of *Ascodesmidaceae* (Pezizomycetes class) in soil, a group of filamentous ascomycetes that also includes endophytic fungi (Lütkenhaus et al., 2019; Healy et al., 2022). Interestingly, the compositional rearrangement induced by SP treatment did not quantitatively affect neither the biodiversity of the ecosystem (at bacterial and fungal level) nor the amount of C and N immobilized into microbial biomass (MBC and MBN). However, the addition of SP resulted in a significant increase in specific dehydrogenase and laccase activities compared to the other treatments. Laccases are commonly produced by both Basidiomycota and Ascomycota (Baldrian, 2006; Zhang et al., 2022a). Correlating compositional changes to soil microbiome and functional effects, as measured by enzymatic assays, is not easy task because of the extremely high microbiome functional redundancy (Chen et al., 2022). Nonetheless, the increase in laccase activity could be a consequence of the reduction in DOC:TDN ratio and the increase of *Ascodesmidaceae* induced by SP application. Laccases can oxidize a wide range of organic aromatic compounds (Rivera-Hoyos et al., 2013) and their activity in soil is significantly affected by N content and variations in the C/N ratio (Moorhead and Sinsabaugh, 2006; Sinsabaugh, 2010; Jin et al., 2022). The application of SP, with its lowest content of recalcitrant compounds and determining the lowest soil DOC:TDN ratio, may have primed the decomposition of soil recalcitrant C compounds (e.g., OM or humic substances). This could have led to the activation of laccases and oxidative metabolism (dehydrogenase activity, positively correlated with the abundance of *Ascodesmidaceae*) to recover the limiting element, which in this case was C (Janusz et al., 2013; Zhang et al., 2022a). However, also in CK, laccases showed high values comparable to those measured with SP, although the DOC:TDN ratio was the highest measured, indicating a surplus of C with respect to N in the control. Thus, it seems that "extreme" DOC:TDN values (both high and low) could stimulate laccases and therefore two different deficiencies (C in the case of SP and N in the case of CK) could produce a similar effect. Furthermore, the highest SP dose resulted in a higher release of N compared to C, suggesting that phosphorus may also be a limiting element. The reported increase in phosphatase activity might indicate an unbalance in the C:N:P ratio due to the high release of N from the SP organic matrices (Acosta-Martínez and Tabatabai, 2011) with a consequent stimulation of the microbial biomass to produce/activate enzymes for enhancing the oxidative degradation processes (dehydrogenase activity) and P recover.

Conversely, MOWC and SSC caused an increased microbial biodiversity, as well as an increased immobilization of N and C (MOWC especially) in microbial biomass, with a dose dependent effect, with respect to SP and CK. Coherently with the compost-based nature of MOWC and SSC, changes in soil microbial networks included an overabundance of Sordariales (Sordariales *Incertae sedis* and Sordariales unknown family), a fungal group known for growing on decaying plant biomasses and typical of the early stages of residues decomposition (Huhndorf et al., 2004; Ma et al., 2013). Bacterial community in MOWC soil is characterized by a dose-responding loss in *Pyrinomonadaceae*, as it happens for SP amended soil, but in the case of MOWC treatment, another Acidobacteria family, *Vicinamibacteraceae*, remains among the most abundant ones. Both *Pyrinomonadaceae* and *Vicinamibacteriaceae* (Acidobacteria subgroups 4 and 6, respectively) are oligotrophic groups, preferring complex proteinaceous compounds as nutrients (Huber and Overmann, 2018; Pascual et al., 2018). However, *Vicinamibacteraceae* is less acidophilic than other Acidobacteria (Huber and Overmann, 2018) increasing its adaptability to soil treated with recalcitrant C source. Another bacterial family showing interesting overabundance in MOWC soil is the "Blrii41" clade (Myxococcales), a predatory group with high hydrolytic potential, usually dominant in compost (Wang et al., 2020; Dai et al., 2021) and associated to organic farming agricultural soil, in which diversity increases (Petters et al., 2021), as reported for MOWC.

Among those microbial families who underwent the most relevant changes of "rank", two were also identified as putative hub nodes in the network analysis, namely *Pyrinomonadaceae* and *Xanthomonadaceae*. Even if it has been suggested to use caution in inferring the relevance of keystone or hub species from microbial co-occurrence network (Berg et al., 2020), in the present experimental settings the abundance of such families emerged as highly indicative of soils amended using organic matrices, and possibly playing a relevant role in ecosystems dynamics. Indeed, the abundance of *Pyrinomonadaceae* family was negatively correlated with all measured soil parameters with the exception of C/N ratio. Recently, *Pyrinomonadaceae* was highlighted as one of the most indicative bacterial families to predict soil health parameters (Wilhelm et al., 2022). The genus RB41, main component of the *Pyrinomonadaceae* family, was indicated as responsible, together with *Bradyrhizobium* and *Streptomyces*, for half of the soil C flow through respiration (Stone et al., 2021), coherently with the inverse association found in this study between the abundance of this group and organic C in soil.

As for the other putative hub nodes, the trend showed by *Xanthomonadaceae* and *Gaiellaceae* confirms previous studies in which the first increases and the latter decreases in association with organic (Li et al., 2017) or cellulosic waste-based (Meschewski et al., 2019) amendments, the first also being correlated to the amount of soil organic C and N (Li et al., 2017). *Sphingomonadaceae* and *Xanthomonadaceae*, which increased together following all organic amendments, have been proposed as potentially inhibiting the growth of plants pathogens (Deng et al., 2022; Liu et al., 2021). Both families have also been reported among key taxa for lignocellulose catabolism in soil (Díaz-García et al., 2020), making C source more available to the ecosystem.

In contrast to the vast number of researches focusing on bacteria, studies concerning soil fungal communities and their relations to ecosystem services are very limited. Yet, the soil "mycobiome" plays a crucial role in converting biomass and cycling C sources, as well as in interacting with both bacteria and plants (Frąc et al., 2018, 2022). This finds confirmation in the present dataset, as the fungal community did not cluster as a separate module in the network analysis. Fungal families were interspersed in the microbial network, interacting, in terms of positive and negative correlations, both among each other and with the bacterial members of the ecosystem. Moreover, even if fungal species were not identified as putative keystone taxa, the fungal community seems to be the one more deeply affected in terms of both biodiversity (especially in SSC soil) and composition (SP). Such relevant effects need to be evaluated carefully in a perspective of productivity and final product quality, as it is likely to have effects on wine aroma (*terroir*) (Liu et al., 2020).

5. Conclusions

The use of waste-derived organic matrices to enhance soil quality can be a sustainable alternative to traditional fertilization in grapevine

cultivation. This study provides knowledge on the effects of three processed waste-based matrices on soil biochemistry and microbiology, as summarized as follows:

- despite having their own microbial load, the organic matrices did not act as a microbial inoculum, stimulating instead the autochthonous microbes;
- the different chemical composition of the matrices, particularly in terms of TOC and recalcitrant C sources, can explain the variations in microbial (both fungal and bacterial) diversity, community structure, microbial interaction network and enzymatic activities, which in turn affect the soil C and N pools;
- significant differences were found in the accumulation of soluble forms of N and C in the soil, even though the organic matrices were applied considering their different susceptibility to release available N over time by applying different mineralization coefficients. This resulted in different microbial and biofunctional responses in the soil;
- MOWC and SSC represent sources of recalcitrant C, requiring a more biodiverse community with an augmented degradation potential;
- the application of SP, explored here for the first time as grapevine cultivation amendment by combining soil biochemical and microbial community analysis, resulted instead in an increase in copiotrophs bacteria due to the presence of easily degradable OM, as well as a striking rearrangement in the fungal, accompanied by a higher laccase activity compared to the other amendments. The latter may be aimed at recovering C and P from recalcitrant soil sources to balance the high N availability, without promoting a simultaneous increase in soil microbial biomass (i.e., increased metabolic stress).

These findings, although limited to a case study, provide the basis to suggest that compost and sludge plasters require different and more precise guidelines for their use in the field. The compost-based organic matrices, particularly MOWC, behaved like a soil amendment, whereas SP more like mineral fertilizer. In terms of future development, one could envisage an integrated use of the two matrices, where MOWC would act as a soil conditioner, maintaining soil fertility in the long term, and SP would cover the plants' nutritional needs (mainly N) in the short term. The potential and long-term effects of SP on viticulture need to be further studied, including other geographical areas.

The maintenance of this experimental vineyard will allow to understand the long-term effects of the different amendments, such as their impact on root, leaf and grape-associated microbial communities that, in turn, affect grapevine vigor, grape quality, and wine characteristics.

CRediT authorship contribution statement

Elena Biagi: Writing – original draft, Formal analysis, Methodology, Investigation, Validation, Conceptualization. **Martina Mazzon:** Methodology, Writing – original draft, Formal analysis, Visualization, Conceptualization, Investigation. **Eliana Musmeci:** Formal analysis, Validation, Investigation, Writing – review & editing, Data curation. **Paola Gioacchini:** Data curation, Writing – review & editing, Investigation. **Anna Paesano:** Writing – review & editing, Data curation. **Fabio Fava:** Supervision, Resources. **Claudio Ciavatta:** Supervision, Resources. **Giulio Zanaroli:** Writing – review & editing, Resources, Conceptualization, Supervision. **Claudio Marzadori:** Writing – review & editing, Resources, Conceptualization, Supervision.

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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.apsoil.2025.106406.

Data availability

Data will be made available on request.

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