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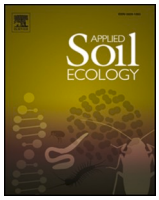

# Long-term effects of biochar on soil chemistry, biochemistry, and microbiota: Results from a 10-year field vineyard experiment

Mohamed Idbella [a,b,*], Silvia Baronti [c], Laura Giagnoni [d], Giancarlo Renella [e], Michelangelo Becagli [g], Roberto Cardelli [g], Anita Maienza [c], Francesco Primo Vaccari [c], Giuliano Bonanomi [a,f]

[a] Department of Agricultural Sciences, University of Naples Federico II, via Università 100, 80055 Portici, NA, Italy
[b] Laboratory of Biosciences, Faculty of Sciences and Techniques, Hassan II University, Casablanca, Morocco
[c] Institute of BioEconomy (IBE), National Research Council (CNR), Via Madonna del Piano 10, 50019 Sesto Fiorentino, Firenze, Italy
[d] Department of Civil, Environmental, Architectural Engineering and Mathematics (DICATAM), University of Brescia, Via Branze 43, 25123 Brescia, Italy
[e] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, Viale dell'Università 16, 35020 Legnaro, Italy
[f] Task Force on Microbiome Studies, University of Naples Federico II, Naples, Italy
[g] Department of Agriculture, Food and Environment (DAFE), University of Pisa, Via del Borghetto, 80, 56124 Pisa, Italy

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ABSTRACT

Application of biochar to soil has been recommended as a carbon sequestration approach that can also improve soil physical and chemical properties. The addition of biochar to soil can change the physicochemical properties of the soil, leading to a subsequent modification of the microbial community. However, the long-term implications of these changes remain insufficiently elucidated. Here, we examined soil chemical and biochemical properties of the bulk soil and employed next-generation sequencing techniques to analyze the microbiological properties of both bulk and rhizosphere soils after 10 years of biochar application. Specifically, we compared these properties between soil treated with two doses of biochar, i.e., SB and DB, and untreated soil, i.e., CK. After 10 years, biochar application increased the soil organic carbon from 12.7 $g.kg^{-1}$ in CK to 17.3 and 23.1 $g.kg^{-1}$, in SB and DB, respectively. Moreover, biochar application led to a slight decrease in soil bulk density, and increased the soil pH value 6.33 in CK to 7.07 in DB. Moreover, our findings revealed a distinct taxonomic signature within bacteria; however, this signature was not observed in terms of diversity. Specifically, we observed an increase in the abundance of oligotrophic bacteria compared to copiotrophic bacteria. The double dose of biochar increased the fungal species richness in the rhizosphere, particularly of *Basidiomycota* yeasts, from a relative abundance of 9.4 % in the CK soil to 17.0 % in the SB soil and 24.8 % in the DB soil and reduced putative plant pathogens like *Phaeoacremonium* and *Aspergillus*. Biochar amendment can significantly improve soil physical, chemical, and biological fertility on the long-term even under intensive viticulture management, with no detectable detrimental effects on microbial diversity and soil functions, and potential of soil organic carbon storage.

## 1. Introduction

Biochar is the solid byproduct of the thermal conversion of biomass at temperatures between 250 °C and 900 °C (I.B.I., 2012). In recent years, biochar production technology has become increasingly robust and sustainable in many regions of the world (Mohan et al., 2014). Incorporating biochar into soil is recognized as a strategy for sequestering soil organic carbon (SOC) and mitigating climate change, as it has the potential to persist for extended periods, ranging from $10^2$ to $10^3$ years (Glaser et al., 2002). This presents opportunities to counterbalance anthropogenic C emissions. Biochar application to agricultural soils also reduces nutrient leaching (Güereña et al., 2013), bioavailability of heavy metals (Park et al., 2011), improves soil structure and soil water holding capacity (Case et al., 2012; Yu et al., 2013) and water availability to plants (Baronti et al., 2014; Baronti et al., 2022), suppresses plant diseases (Elad et al., 2010; Jaiswal et al., 2014), and stimulates soil

* Corresponding author at: Department of Agricultural Sciences/Faculty of Sciences and Techniques, University of Naples Federico II, Via Università 100, Portici, 80055, Napoli, Italy.
*E-mail address:* mohamed.idbella@usmba.ac.ma (M. Idbella).

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microbial activity (Kolb et al., 2009). The specific outcomes depend on various factors such as the type of biochar, application rates, crops, and soil management strategies (Jeffery et al., 2011). In field experiments, different application rates of biochar have been associated with increased plant biomass in various species (Deenik et al., 2010; van de Voorde et al., 2014; Quilliam et al., 2013; Gavili et al., 2019). However, Gavili et al. (2019) reported a negative effect on biomass production and seed yield of soybean at higher biochar application rates (>25 t $ha^{-1}$), contrasting with the positive responses observed in other studies at similar or higher rates.

Soil microorganisms play a fundamental role in SOC mineralization and stabilization, as well as in nutrient availability. Their interactions with plants can have both positive effects, such as enhancing plant growth and nutrient uptake, and negative effects, such as promoting disease susceptibility or inhibiting nutrient absorption (Gul and Whalen, 2016; Idbella et al., 2021). Therefore, analysing soil microbial diversity and microbial activity can provide valuable insights into soil fertility and crop productivity (Calderón et al., 2017). Previous studies have also shown that biochar can alter microbial activity and microbial community structure (Anderson et al., 2011; Lehmann et al., 2011; Giagnoni et al., 2019), with positive effects on microbial activities involved in C and nitrogen (N) dynamics (Kolton et al., 2011; Xu et al., 2014; Chen et al., 2015; Darby et al., 2016; Nguyen et al., 2017). Most of the mentioned results have been obtained from laboratory incubation, greenhouse, or short-term field experiments (Lorenz and Lal, 2014). However, there is still a lack of knowledge regarding the long-term effects of biochar when applied in large-scale farming systems. Results from shorter-term field trials range from 2 years (Solaiman et al., 2010; Anderson et al., 2014) to 3 years (Quilliam et al., 2012), and 3.2 years with Kuzyakov et al. (2009), who reported no significant effects of biochar on microbial biomass and the relative abundance of bacteria and fungi. In contrast, a notable long-term field trial by Nguyen et al. (2018) showed that biochar induced changes in the bacterial communities only after 1 year from application, whereas no significant changes were observed after 9 years. Based on previously referenced studies on the positive effect of biochar application, our hypothesis contends that biochar has the potential to yield persistent beneficial effects on soil physical, chemical, and biological properties, even when the soil is subjected to conventional viticulture management practices. To evaluate our hypothesis, we analysed the soil from a long-term field experiment established in 2009. The primary aim of this experiment was to assess the impacts of biochar addition at one or two doses on soil hydrology, plant physiology, crop yields and vine quality (Baronti et al., 2014). Specific aims of this work were: *i*) to assess the effects of biochar application on soil physical and chemical properties, soil biochemistry, i.e., enzymatic activity, and microbial diversity in vine rhizosphere and bulk soil, and *ii*) to explore the impact of biochar on soil microbiota composition and elucidate its correlation with soil chemical and biochemical properties. Results from this study are important because biochar incorporation into soil at farm scale is an irreversible operation, and clear evidence on the effects of biochar on the long-term is needed to support relevant agricultural policy.

## 2. Material and methods

### 2.1. Study site description

The study was done in the vineyard "La Braccesca Estate" (Marchesi Antinori srl, www.antinori.it) located in Montepulciano (Tuscany, Central Italy, 43°10′15″N, 11° 57′43″E, 290 m a.s.l.). The vineyard was planted in 1995, with plant-rows East-West orientation, and the inter-rows left partially covered with volunteer grass. The vineyard is not irrigated, and an NPK fertilizer (15.0.26) is applied twice a year at a rate of 120 kg $ha^{-1}$. The climate of the study area is typically Mediterranean, characterized by a mean annual temperature of 14.6 °C and cumulative precipitation of 776 mm during the period from 2009 to 2019 (https://www.sir.toscana.it/).

The soil is acidic with a sandy-clay-loam texture (USDA, 2005), with 35 % clay, 20 % silt, 45 % sand, and highly compacted below 0.4 m depth, and main soil properties are reported in supplementary Table S1. A plot experiment with three treatments and five replicates was laid out in 2009, with each of the 15 plots having a surface area of 225 $m^2$ (7.5 m in width and 30 m in length), including 4 vineyard rows and 3 intermediate rows. Treatments were a single application of biochar at a rate of 22 t $ha^{-1}$ on 7th May 2009 (SB), two applications of biochar at a rate of 22 t $ha^{-1}$ each on 7th May 2009 and 3rd February 2010 (DB), and untreated control plots (CK). Commercial biochar used in the experiment was obtained from orchard pruning feedstock (Bagnacavallo, Ravenna, Italy) produced at low temperature (500 °C) with a slow pyrolysis process, and had 77.8 % OC, 0.91 % N, 101 cmol(+) $kg^{-1}$ CEC, 25 % water content, and 2722 $mm^3$ $g^{-1}$ porosity, full biochar chemical and physical properties are reported in Table S2. Biochar was superficially applied to the soil of the vineyards inter-rows with a spreader, and mechanically mixed into the soil to a depth of 30 cm using a chisel plough tiller. Water content of biochar was 25 %, thus each application corresponded to 16.5 t $ha^{-1}$ of dry biochar. The vineyard follows a three-year alternate management practice. Each year, the farm works on one specific inter-row, using a rototiller and ploughing to 0–20 cm. Meanwhile, the two adjacent inter-rows are left uncultivated and covered with volunteer grass, which is mowed twice a year.

### 2.2. Soil and root sampling

In May 2019, i.e., 10 years after the first field biochar application, soils were sampled from vine rhizosphere and bulk soil by a 10 cm diameter soil corer, at a depth of 0–20 cm after removal of above-ground litter, in three points of each replicate plot to form a representative composite sample from each plot. A total of five replicate samples were selected for each treatment, resulting in 15 samples for rhizosphere and 15 samples for bulk soil. Bulk soil was collected from the same plots but in areas not colonized by grape roots. The 15 rhizosphere and 15 bulk soil samples were sieved in the laboratory at field moisture (2 mm mesh), and then divided into three portions: one kept at 4 °C for the analysis of soil biochemical activities, a portion was stored at −80 °C for DNA extraction, and another portion was air-dried for chemical analysis.

Fine roots with a diameter of <2 mm were also collected from topsoil (0–30 cm). Grape roots were distinguished from roots of herbaceous species by their colour and shape. Furthermore, larger grape roots were systematically traced to the base of the plants, ensuring a reliable method for identification. Rhizosphere soil samples were obtained by gently shaking the roots with a sterile clamp to separate soil adhering to them and remove the remnant.

### 2.3. Analysis of soil chemical properties

Soil organic C was determined by the method of Ciavatta et al. (1989) based on wet oxidation of SOM with 2 N $K_2Cr_2O_7$ in concentrated $H_2SO_4$ at 160 °C followed by titration of the dichromate excess with $FeSO_4$. Soil $NO_3$-N concentration was determined through extraction with distilled water (w:v 1:5) followed by quantification using ion chromatography (Dionex DX120). Soil $NH_4^+$-N concentration was determined after extraction from soil with 2 M KCl and quantified colorimetrically by Na-salycilate/Na-dichloroisocyanurate method (Kandeler and Gerber, 1988). Soil available P was extracted using the method of Bray and Kurtz (1945), and P concentrations in the extracts were quantified by UV spectrophotometry at 880 nm after reaction with the sulfo-molybdic acid reagent (Murphy and Riley, 1986). Soil pH value was measured, using a pH meter (XS Instruments, Carpi, MO, Italy), in a soil: distilled water suspension (1:2.5 w:v) stirred for 30 min, settled for 1 h, then centrifuged for 10 min. Bulk density of the upper soil layer (0–10 cm) was determined with the core method (Grossman and Reinsch, 2002) using metallic cylinders of 100 $cm^3$ volume 50 mm × 54

mm cylindrical cores weighed at field conditions, dried at 105 °C in oven for 48 h, and reweighed for calculating the moisture content. Bulk density was calculated as ratio between the dry weight and the volume of the sample.

### 2.4. DNA extraction and high-throughput Miseq sequencing

The DNA was extracted from all soil samples using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. After extraction, the purity and concentration of the DNA were quantified using a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA). Subsequently, the DNA was diluted in sterile water at a ratio of 1:10. The DNA extraction, PCR and sequencing were performed as described in Idbella et al. (2022). In details, the V3-V4 regions of the bacterial 16S rRNA gene and ITS1–2 regions of the fungal internal transcribed spacer (ITS) were amplified with the primer sets S-D-Bact-0341-b-S-17/S-D-Bact-0785-a-A-21 (Berni Canani et al., 2017) and BITS1fw/B58S3-ITS2rev (Bokulich and Mills, 2013), respectively, as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 45 s, and a final single extension at 72 °C for 10 min. The amplicons (paired end, 2 × 250 bp) were then sequenced using the Illumina MiSeq platform, and the library preparation was performed according to the Illumina metagenomic workflow (IlluminaTechnicalSupport, n.d.).

### 2.5. Bioinformatics processing of the sequences

The resulting demultiplexed paired-end reads were merged, stripped of primer sequences, aligned, quality filtered, and then pooled using the DADA2 package (Callahan et al., 2016) in R software (4.2.2) (R Core Team, 2022). This pipeline generates amplicon sequence variants (ASVs), which have demonstrated more sensitivity and accuracy in discriminating ecological patterns than operational taxonomic units (OTUs) (Callahan et al., 2016) because they calculate sequencing error rates rather than clustering to 97 % similarity (Hugerth and Andersson, 2017), thus resolving to the level of differences at single nucleotides level in the sequenced gene region. Quality filtering of the raw reads was performed using Cutadapt software (Martin, 2011), discarding both adapter sequences and low-quality ends (<Q20). Taxonomy was then assigned based on the UNITE database for fungi (Nilsson et al., 2019) and SILVA database for bacteria (Quast et al., 2013).

### 2.6. Analysis of soil respiration, microbial biomass and soil enzymatic activities

Soil basal respiration was determined with the alkali titration method (Anderson and Domsch, 1978), using 20 g of soil placed in sealed glass jars for 3 d at 25 °C in the dark, in the presence of 1 M NaOH. After incubation, the beakers NaOH was added with 0.75 N $BaCl_2$ and phenolphthalein indicator, and then the solution was titrated against 0.1 M HCl. Soil microbial biomass was estimated by the quantification of the adenosyne triphosphate (ATP) soil content with the method of Ciardi and Nannipieri (1990). The arylesterase activity was determined according to Zornoza et al. (2009), the β-glucosidase activity according to Tabatabai (1982), the cellulase activity was determined by the rate of hydrolysis of 4-nitrophenyl-β-d-cellobioside as reported by Imparato et al. (2016). Concerning the N mineralizing enzymes, the protease activity was determined using Na-caseinate as substrate (Ladd and Butler, 1972), and urease activity was determined according to Nannipieri et al. (1974). The tyrosine released by protease activity was determined colorimetrically with the Folin-Ciocalteu reagent at 700 nm, whereas the $NH_4^+$ produced by urease activity was colorimetrically quantified at 436 nm after reaction with the Nessler reagent using a calibration curve formed using ammonia standards. Concerning the P and S mineralizing enzyme activities, the acid and alkaline phosphomonoesterase activities were determined according to Tabatabai and Bremner (1969), the phosphodiesterase activity was determined with the method of Browman and Tabatabai (1978), and the arylsulfatase activity was determined according to Tabatabai and Bremner (1970). Concentrations of 4-nitrophenol (p-NP) produced in the assays of acid and alkaline phosphomonoesterase, phosphodiesterase, arylesterase, β-glucosidase and cellulase activities were quantified from a p-NP calibration curve after subtraction of the absorbance of the respective controls at 400 nm wavelength.

### 2.7. Data visualization and statistic

Alpha-diversity metrics were calculated and the corresponding boxplots along with low-taxonomic heatmaps and nonmetric multidimensional scaling (nMDS) plots were generated using Primer7 software (Primer-E Ltd, Plymouth; UK). The PERMANOVA (permutational analysis of variance) test (999 permutations) was performed to evaluate the significance of variation in bacterial and fungal community composition, with treatments (i.e., control, biochar, and double biochar) and sampling location (i.e., bulk or rhizosphere soil) as fixed factors. The ANOVA test was conducted to assess the significance of variance between alpha diversity metrics and soil chemical and biochemical properties, and means were pairwise separated using the post hoc Tukey test. The statistical analyses were performed using R software (4.2.2) (R Core Team, 2022) at a significance level of $p < 0.05$.

To investigate the functionality of fungi, FUNGuild annotation tool (Nguyen et al., 2016) was used to identify putative fungal functional groups/guilds. In addition, a heatmap based on the Pearson correlation matrix between bulk soil chemical properties was constructed using the ComplexHeatmap package in R (Gu et al., 2016). Co-occurrence network analyses were performed for the communities in the six differently treated soils. For each bacterial and fungal community, only the 50 most abundant ASVs were analysed to focus on the most abundant ASVs and reduce the effects of rare ones. Pairwise correlations between ASVs were calculated using Spearman correlation in R (Hmisc package 4.0–1). Based on the statistical analysis, only strong and significant (Spearman's $r > 0.6$ or $r < -0.6$ and $P < 0.05$) correlations were considered. The network was visualised using Cytoscape (version 3.8.3, Shannon et al., 2003) for comparison of co-occurrence between the bulk and rhizosphere soils and Gephi (version 0.9.2, Bastian and Jacomy, 2009) for comparison of co-occurrence in each treatment within the bulk and rhizosphere soils. Each edge represents a robust and significant correlation, and each node represents an ASV. A series of integrative metrics were calculated and compared to describe the network topology.

## 3. Results

### 3.1. Biochar application enhances soil physical and chemical properties, stimulates enzymatic activity, increases soil respiration, and promotes microbial biomass growth

Compared to CK soil, the application of DB significantly increased the soil pH value from 6.33 to 7.07 and the $NO_3^-$-N concentration from 1.51 to 5.88. Additionally, both SB and DB applications led to a significant increase in the SOC concentration from 12.7 $g.kg^{-1}$ in CK to 17.3 $g.kg^{-1}$ and 23.1 $g.kg^{-1}$ in SB and DB, respectively. Similarly, SB and DB exhibited a significant increase in $NH_4^+$-N content from 12.2 $mg.kg^{-1}$ in CK to 14.3 $mg.kg^{-1}$ in DB, as well as a significant increase in P concentrations from 147.4 $mg.kg^{-1}$ in CK to 262 $mg.kg^{-1}$ and 313 $mg.kg^{-1}$ in SB and DB, respectively. Conversely, SB and DB resulted in a significant reduction in bulk density from 1.63 $g.(cm^3)^{-1}$ in CK to 1.59 $g.(cm^3)^{-1}$ and 1.53 $g.(cm^3)^{-1}$ in SB and DB, respectively (Table 1). Among the measured enzyme activities, the alkaline phosphatase, β-glucosidase and arylsulfatase were not changed by any biochar amendments; however, the cellulase activity was lower in the SB and DB soils compared to CK soil, although the differences were not significant. SB and DB

**Table 1**
Physical, chemical, and biochemical parameters in the untreated control (CK), single biochar application (SB), and double biochar applications (DB). Different letters within each row indicate significant differences (Duncan test, $p < 0.05$).

| Soil parameters | Treatments | | |
|---|---|---|---|
| | CK Mean ± s.d. | SB Mean ± s.d. | DB Mean ± s.d. |
| *Physical & Chemical properties* | | | |
| pH | 6.33 ± 0.06 c | 6.83 ± 0.11 b | 7.07 ± 0.10 a |
| Bulk density (g.(cm$^3$)$^{-1}$) | 1.63 ± 0.03 a | 1.59 ± 0.02 b | 1.53 ± 0.02 c |
| Organic carbon (g.Kg$^{-1}$) | 12.7 ± 0.67 c | 17.3 ± 1.06 b | 23.1 ± 1.15 a |
| $NO^{3-}$-N (mg.Kg$^{-1}$) | 1.51 ± 0.16 b | 1.78 ± 0.12 b | 5.88 ± 0.66 a |
| $NH_4^+$-N (mg.Kg$^{-1}$) | 12.2 ± 0.42 b | 13.4 ± 0.30 a | 14.3 ± 1,29 a |
| P (mg.kg$^{-1}$) | 147.4 ± 12.1 c | 262 ± 32.2 b | 313 ± 31.4 a |
| *Biochemical parameters* | | | |
| Soil respiration (mg $CO_2$/g d. w./d) | 0.35 ± 0.02 b | 0.18 ± 0.01 b | 1.04 ± 0.04 a |
| ATP (µg kg$^{-1}$) | 8595 ± 266 a | 8235 ± 175 a | 8553 ± 172a |
| Arylesterase (mg kg$^{-1}$ h$^{-1}$) | 3555 ± 875 c | 4578 ± 1177 b | 6186 ± 944a |
| Cellulase (mg kg$^{-1}$ h$^{-1}$) | 1428 ± 235 a | 1200 ± 150.1 a | 1044 ± 236a |
| Acid phosphatase (mg kg$^{-1}$ h$^{-1}$) | 5305 ± 467 a | 4346 ± 145.4 b | 3322 ± 288c |
| Alkaline phosphatase (mg kg$^{-1}$ h$^{-1}$) | 3557 ± 695 ab | 2994 ± 312 b | 3988 ± 451a |
| Arylsulfatase (mg kg$^{-1}$ h$^{-1}$) | 254 ± 39.3 a | 178 ± 57.8 a | 208 ± 45.3a |
| β-Glucosidase (mg kg$^{-1}$ h$^{-1}$) | 1441 ± 280 a | 1604 ± 178 a | 1436 ± 316.3 a |
| Protease (mg kg$^{-1}$ h$^{-1}$) | 0.47 ± 0.07 b | 0.73 ± 0.09 a | 0.67 ± 0.18 a |

significantly increased the arylesterase to 4578 mg.kg$^{-1}$.h$^{-1}$ and 6186 mg.kg$^{-1}$.h$^{-1}$, respectively, compared to CK, which was 3555 mg.kg$^{-1}$.h$^{-1}$. Moreover, protease was significantly higher in the SB and DB soils with values of 0.73 mg.kg$^{-1}$.h$^{-1}$ and 0.67 mg.kg$^{-1}$.h$^{-1}$, respectively, compared to the CK soil, which had a value of 0.47 mg.kg$^{-1}$.h$^{-1}$. In contrast, the acid phosphatase was significantly lower in the SB and DB soils, measuring 4346 mg.kg$^{-1}$.h$^{-1}$ and 3322 mg.kg$^{-1}$.h$^{-1}$, respectively, compared to the CK soil, which was 5305 mg.kg$^{-1}$.h$^{-1}$ (Table 1). Soil respiration was significantly higher in DB soil while microbial biomass showed no significant differences among treatments (Table 1).

Correlation analysis showed that bulk density was negatively correlated with SOC, $NH_4^+$-N, $NO_3^-$-N, P, alkaline phosphomonoesterase activity, and soil respiration. Nevertheless, bulk density showed a positive correlation solely with acid phosphatase. Cellulase, protease, arylesterase, alkaline phosphatase, soil respiration and ATP content exhibited positive correlations with each other. Acid phosphatase showed negative correlations with other enzymatic activities, soil pH, SOC, $NO_3^-$-N, and P (Fig. S1).

### *3.2. Biochar application influences microbial diversity and community structure in both the vine rhizosphere and bulk soil, leading to significant shifts in microbial composition and abundance*

Microbial community diversity showed no significant difference in ASVs number, species richness, and Shannon indices either for bacterial or fungal diversity (Fig. 1). The only significant difference found was in the fungal species richness between the rhizosphere of the CK treatment and the bulk soil across treatments (Fig. 1). Taxonomic classification of bacterial community showed that all soils were dominated by *Proteobacteria* with 31.4–32.2 % of all sequences among treatments, followed by *Acidobacteria* and *Actinobacteria* with 18.2–23.6 % and 9.9–12.9 %, respectively in all treatments (Fig. 2). Abundances of *Bacteroidetes*, *Cyanobacteria* and *Gemmatimonadetes* were higher in the bulk soil than in the rhizosphere regardless of the treatments, and there were no significant differences in the most dominant phyla across treatments.

Fungal community composition showed significant difference between treatments and between bulk and rhizosphere soil (Fig. 2). In the CK soil, *Ascomycota* phylum showed a relative abundance of 49.8 % in the bulk and 73.8 % in the rhizosphere soil, the *Basidiomycota* phylum showed an increasing range within the rhizosphere, starting from 9.4 % in the CK soil to 17.0 % in the SB soil and 24.8 % in the DB soil. *Mortierellomycota* showed high abundance in the rhizosphere of CK and SB soil (12.7 % and 11.7 %, respectively), while their abundance was lower in the DB soil (Fig. 2). *Glomeromycota* and *Chytridiomycota* showed different trends in the rhizosphere with the former showing higher abundance in the CK soil, the latter showing higher abundance in SB and DB soils, while both groups showed low relative abundance in the bulk soil (Fig. 2).

The PERMANOVA analysis revealed significant differences in the bacterial and fungal community structure between the rhizosphere and bulk soils ($P$ value <0.05, Table S3). In addition, within the bulk soils, the bacterial community exhibited significant differences between the control and DB soils (Table S3).

At the lowest taxonomic level, the main bacterial groups driving the ASVs were *Rubrobacter*, *Flavobacterium*, *Gemmatirosa*, *Longimicrobiaceae*, *Burkholderiaceae* and *Isophaeraceae*, which were more abundant in the bulk soil compared to the rhizosphere (Fig. S2). The group consisting of *Elsterales*, *TK10*, *Gemmataceae*, *Xanthobacteraceae*, *IMCC26256* strain, *Brevundimonas*, *Pseudomonas* and *Gaiellales* were more abundant in the rhizosphere than in the bulk soil. In addition to this list, *Acidobacteriales* and *Candidatus_udaeobacter* were most abundant in the plant rhizosphere, with a decreasing trend from control to SB to DB soils (Fig. S2). Moreover, the ASVs *WD21021_soil_group* and *Gemmatimonas* were more abundant in the CK and SB soils than in DB soils, especially in the bulk soil, whereas the ASVs *RCP2-54*, *Pirellula* and *TRA3-20* were more abundant in the DB soils than in the CK and SB soils. Finally, *AD3* and *Burkholderia-Caballeronia-Paraburkholderia* ASVs were present only in the CK rhizosphere soil and progressively decreased in the SB and DB soils, while they were completely absent in the bulk soil (Fig. S2).

Regarding the fungal community at the lowest taxonomic level, a more diverse pattern was observed, including specific ASV signatures within each soil. For example, both *Chytridiaceae* and *Hypocreales* were more abundant in the rhizosphere of plants grown on SB and DB soils, whereas they were absent in the remaining soils (Fig. 3). In the DB soil, the most abundant *Basidiomycota* in the rhizosphere were *Saitozyma* and *Solicoccozyma*. The *Robillarda* ASV showed high abundance only in the rhizosphere of plants on DB soil, while *Lachnella* was abundant only in the rhizosphere of the SB soil (Fig. 3). Fungi belonging to genera *Penicillium*, *Talaromyces*, *Aspergillus*, *Mortierella*, and *Chaetomium* were more abundant in the rhizosphere of CK and SB soils, while they were less abundant in the DB soil, and the ASV *Ilyonectria* was only present in the rhizosphere of CK and DB soils (Fig. 3). The main ASVs in the bulk soil were *Solicoccozyma* and *Saitozyma*, whereas *Alternaria*, *Boeremia* and *Cladosporium* were more abundant in the SB and DB bulk soil, while *Subulicystidium* was more abundant under soil CK. Notably, *Phaeoacremonium* was detectable only in rhizosphere of CK soil.

The FUNGuild analysis revealed that most of the detected fungi were saprotrophic, followed by pathotrophic, while symbiotrophic fungi were the least abundant (Fig. 4). Distribution patterns of ecological guild functions differed between bulk and rhizosphere soils, and between biochar treatments. For example, in the rhizosphere the abundance of woody and foliar saprotrophs increased whereas abundance of litter saprotrophs decreased from CK to DB. In addition, the parasite abundance increased biochar-amended compared to control soil, the arbuscular mycorrhizal fungi were more abundant in CK soil compared to SB and DB, while ectomycorrhizal fungi were more abundant in SB than in

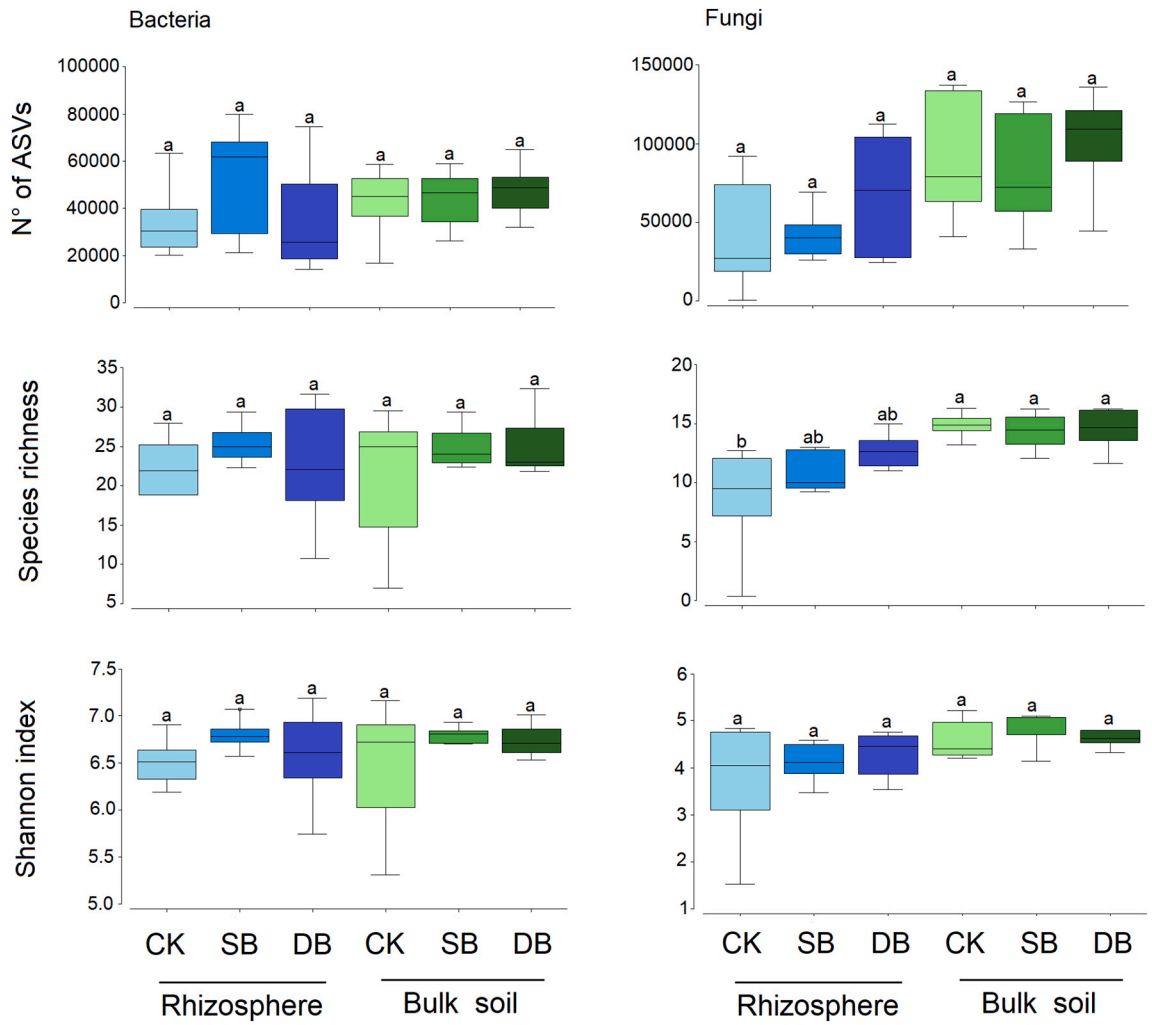


**Fig. 1.** Box plots showing the variation in the numbers of ASVs, Shannon diversity and species richness indices for bacterial and fungal communities for each treatment in both the rhizosphere and bulk soil. Notes: CK, SB, and DB represent untreated control, single application of biochar at a rate of 22 t $ha^{-1}$ (SB), two applications of biochar at a rate of 22 t $ha^{-1}$ (DB), respectively. Different letters indicate significant ($P < 0.05$) differences in the indices. The lower and upper bounds of the boxplots show the first and third quartiles (the 25th and 75th percentiles), the middle line shows the median, whiskers above and below the boxplot indicate inter-quartile range.

other soils. Compared to CK and DB bulk soils, the bulk soil of the SB treatment had higher abundance of ectomycorrhiza, soil and litter saprotrophs were less abundant, and higher parasite fungi, especially plant and lichen parasites. Finally, the bulk soil was characterized by higher abundance of plant saprotrophs, less epiphytes and absence of arbuscular mycorrhiza compared to rhizosphere soil.

### *3.3. Biochar application significantly alters microbial co-occurrence network, leading to increased connectivity and stability*

We constructed two co-occurrence networks, one separating the rhizosphere from the bulk soil (Fig. S3) and the other separating the rhizosphere and bulk soil for each treatment (Fig. 5), and calculated seven topological parameters to evaluate the interactions between ASVs in the two networks (Tables S4 and S5). The rhizosphere microbial network contained 193 nodes and 2389 edges, whereas the bulk soil network contained 186 nodes and 935 edges (Fig. S3). The values of network centralization, network heterogeneity and network density were significantly higher in the rhizosphere than in the bulk soil (Table S4). However, the characteristic path length was higher in the bulk soil than in the rhizosphere (Table S4). As for the co-occurrence networks of the treatments within the rhizosphere, the number of nodes for CK, SB, and DB soils were 93, 96, and 94, respectively, while the number of edges were 337, 333, and 401, respectively. Percentage of positive ASVs correlations in the microbial networks was higher for SB (70.6 %) and DB (78.8 %) soils. Values for network centralization, network heterogeneity and network density showed a significant increase from CK to SB to DB soils, while the characteristic path length and clustering coefficient showed no significant changes. In contrast, for the bulk soil, the number of nodes for CK, SB, and DB soils were 94, 93, and 95, respectively, while the number of edges were 433, 300, and 263, respectively (Fig. 5). The proportions of positive ASVs correlations in the microbial networks was higher, were 63.9 % for the DB and 79.2 % for the CK soil. Values of network centralization, network heterogeneity, and network density showed a significant decrease from CK to SB to DB soils, while the characteristic path length and clustering coefficient showed no significant changes (Table S5).

Nodes with high degrees, high closeness centrality, and low betweenness centrality were considered keystone taxa. In all the treatments, >30.6 % of the top 13 keystone taxa were *Ascomycota*, >15.4 % were *Proteobacteria*, and >10.8 % were *Basidiomycota*.

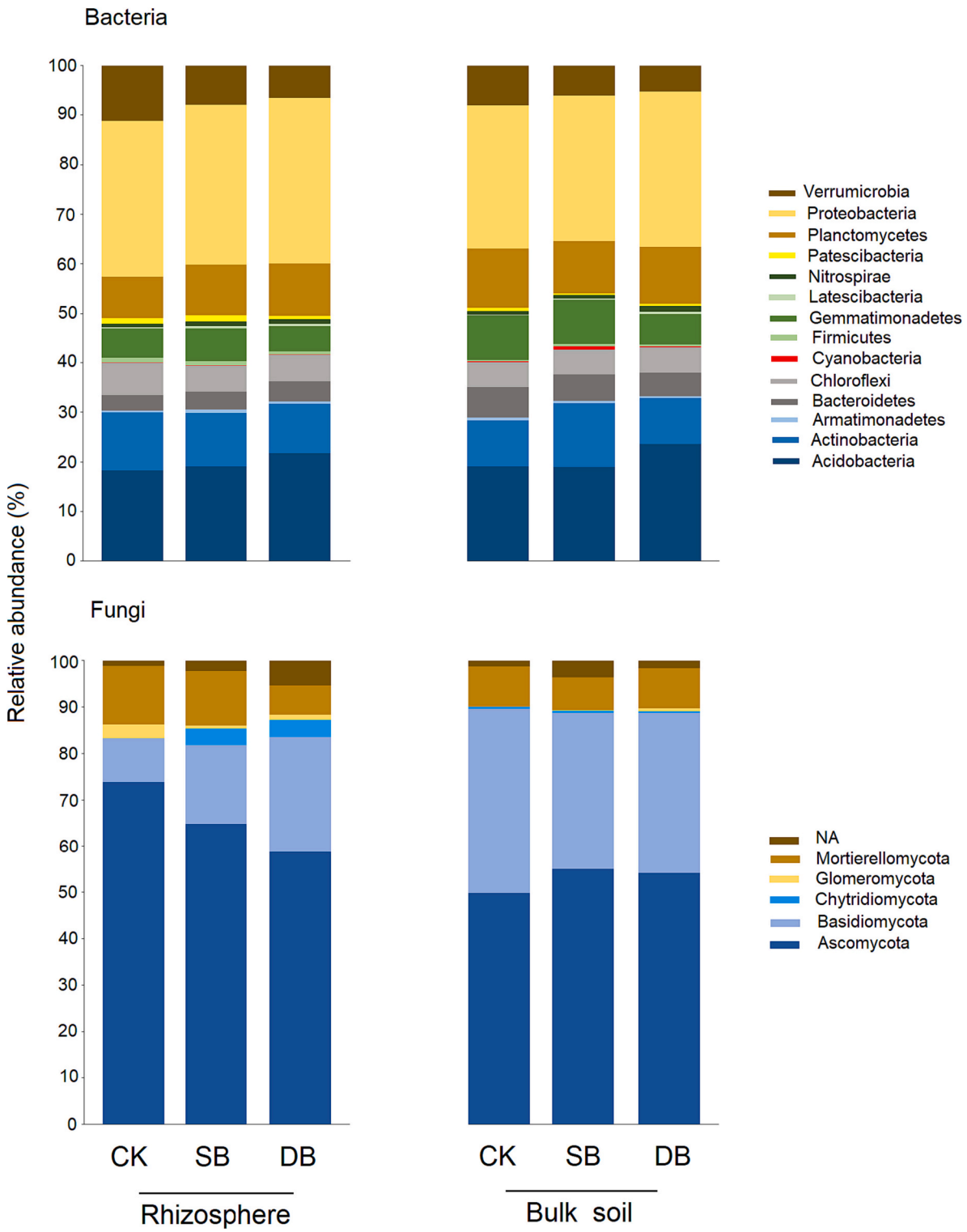


**Fig. 2.** The relative abundance of bacterial (upper panel) and fungal (lower panel) phyla for each treatment in both the rhizosphere and bulk soil. Notes: CK, SB, and DB represent untreated control, single application of biochar at a rate of 22 t $ha^{-1}$ (SB), two applications of biochar at a rate of 22 t $ha^{-1}$ (DB), respectively.

### *3.4. Biochar-induced modifications in microbiota are positively correlated with increased biochemical activity*

The nMDS analysis of the bulk microbial community distribution according to the chemical parameters showed a defined pattern associated with each treatment (Fig. 6). In the DB soil bacterial community, a positive correlation with arylesterase activity, P availability, SOC concentration, and $NO_3^-$-N concentration was observed, whereas the bacterial community of the SB soil was positively correlated with β-glucosidase, cellulase, and alkaline phosphatase, and negatively correlated with arylsulfatase activity. Bacterial community of the CK soil showed a scattered distribution of the replicates based on many parameters including SOC, and β-glucosidase and arylsulfatase activities.

Regarding the fungal community, the distribution showed a large

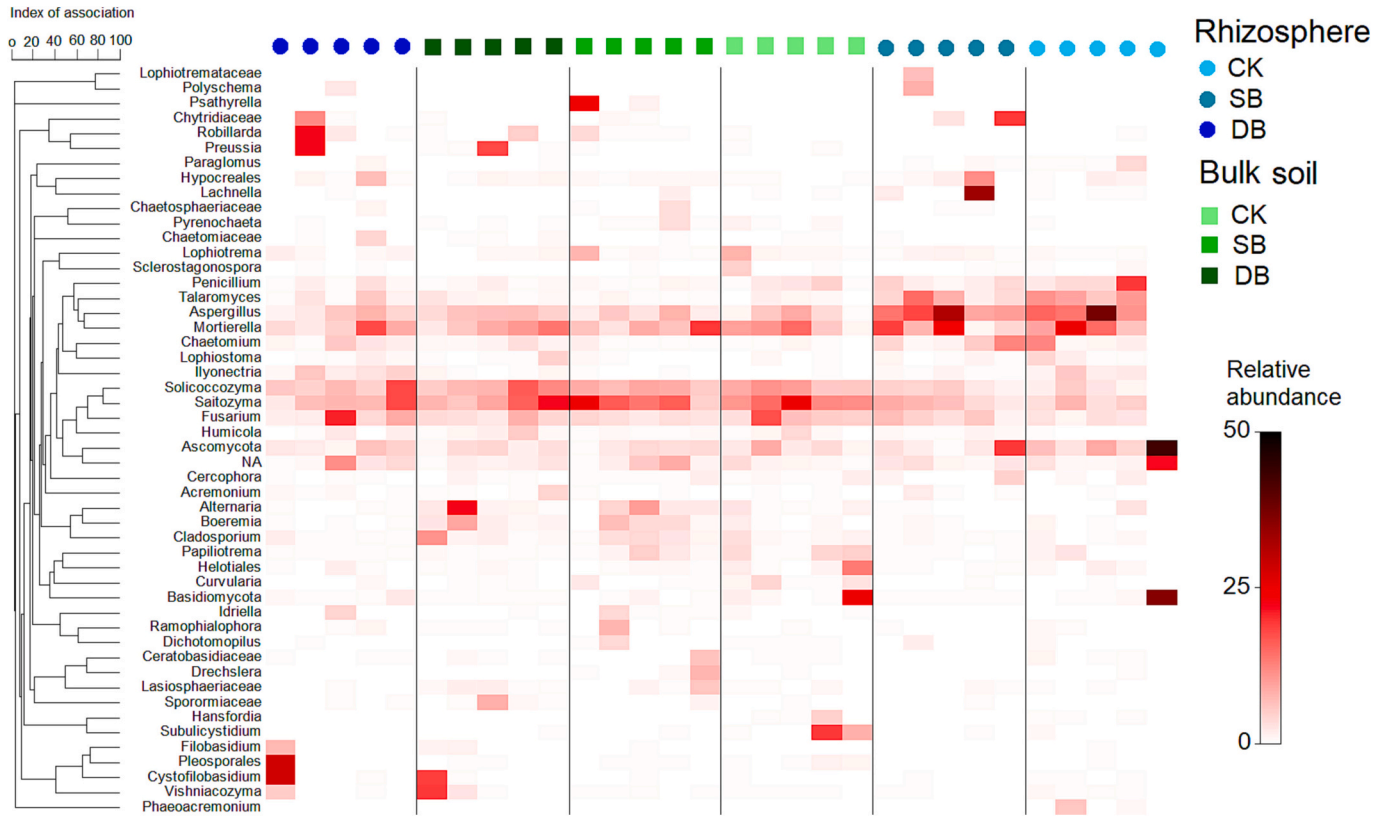


**Fig. 3.** Heatmap showing relative abundance of the 50 most frequent Amplicon Sequence Variants (ASVs) in the fungal community for each treatment in both the rhizosphere and bulk soil. The hierarchical clustering of variables is based on Whittaker's association index. Notes: CK, SB, and DB represent untreated control, single application of biochar at a rate of 22 t ha$^{-1}$ (SB), two applications of biochar at a rate of 22 t ha$^{-1}$ (DB), respectively.

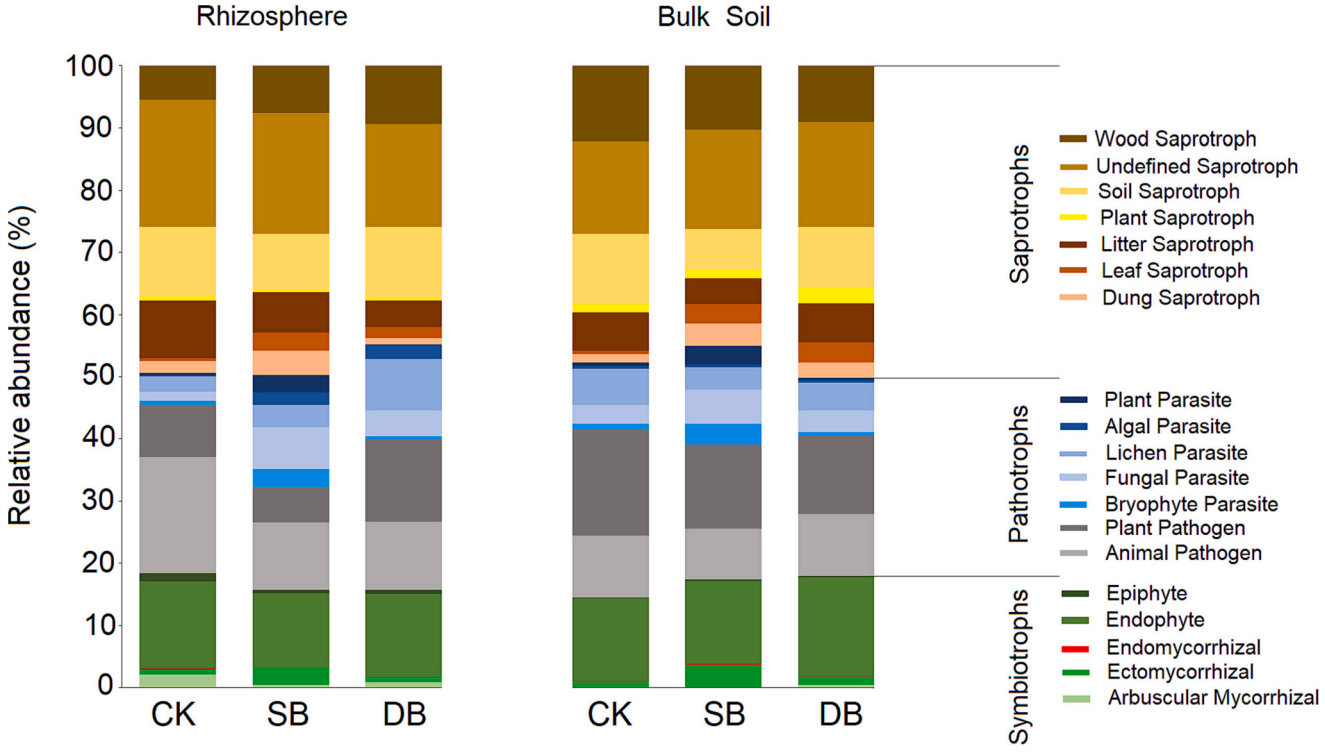


**Fig. 4.** Relative abundance of fungal functional guild for each treatment in both the rhizosphere and bulk soil. Notes: CK, SB, and DB represent untreated control, single application of biochar at a rate of 22 t ha$^{-1}$ (SB), two applications of biochar at a rate of 22 t ha$^{-1}$ (DB), respectively.

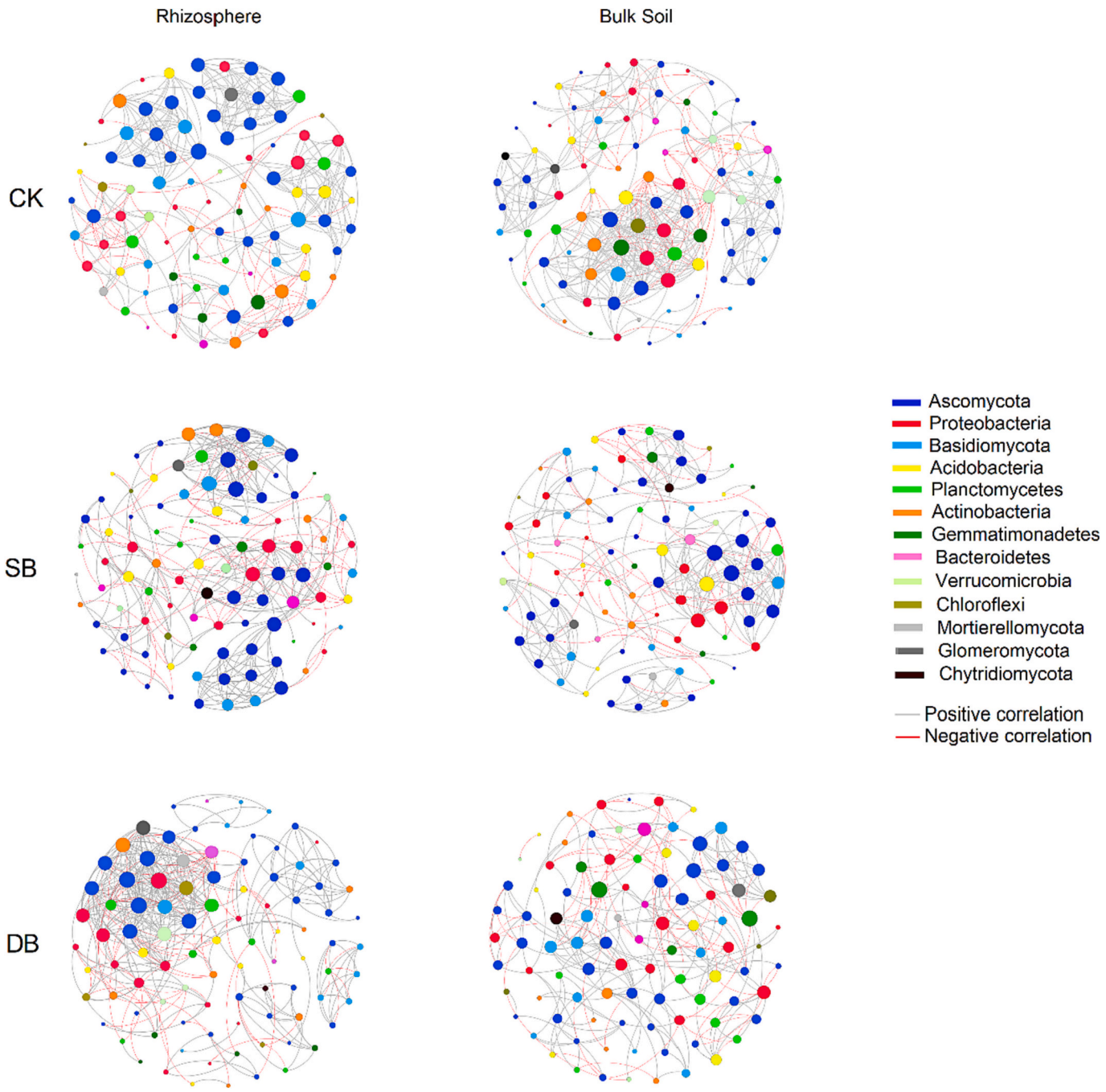


**Fig. 5.** Correlation base network analysis showing potential interactions between bacterial and fungal families for each treatment in both the rhizosphere and bulk soil. The lines connecting nodes (edges) represent positive (grey) or negative (red) co-occurrence relationship. The intensity of the colour and the length of the edges represent the strength of correlation. The connection stands for a strong (Spearman's $\rho > 0.6$ and $\rho < -0.6$) and significant ($P$-value$<0.05$) correlation. The size of each node is proportional to the ASV relative abundance, only the top 50 ASVs were kept. The nodes were coloured by phylum level. Notes: CK, SB, and DB represent untreated control, single application of biochar at a rate of 22 t ha$^{-1}$ (SB), two applications of biochar at a rate of 22 t ha$^{-1}$ (DB), respectively.

variability depending on a set of parameters. Soil available P, soil pH value, SOC concentration, and protease activity had the highest positive correlation with the fungal community in the SB and DB soils, whereas in the CK soil, fungal diversity was mainly correlated to available P, and to acid phosphomonoesterase, arylesterase, and cellulase activities (Fig. 6).

## 4. Discussion

### *4.1. Biochar application enhances soil physical and chemical properties, stimulates enzymatic activity, increases soil respiration, and promotes microbial activity*

Our results showed significant differences in key soil physico-chemical properties between the soils where biochar was added 10 years ago and soils without biochar. These differences included a significant increase in the SOC concentration, an increase of the acidic pH value, and a decrease of bulk density. The SOC increase in the DB

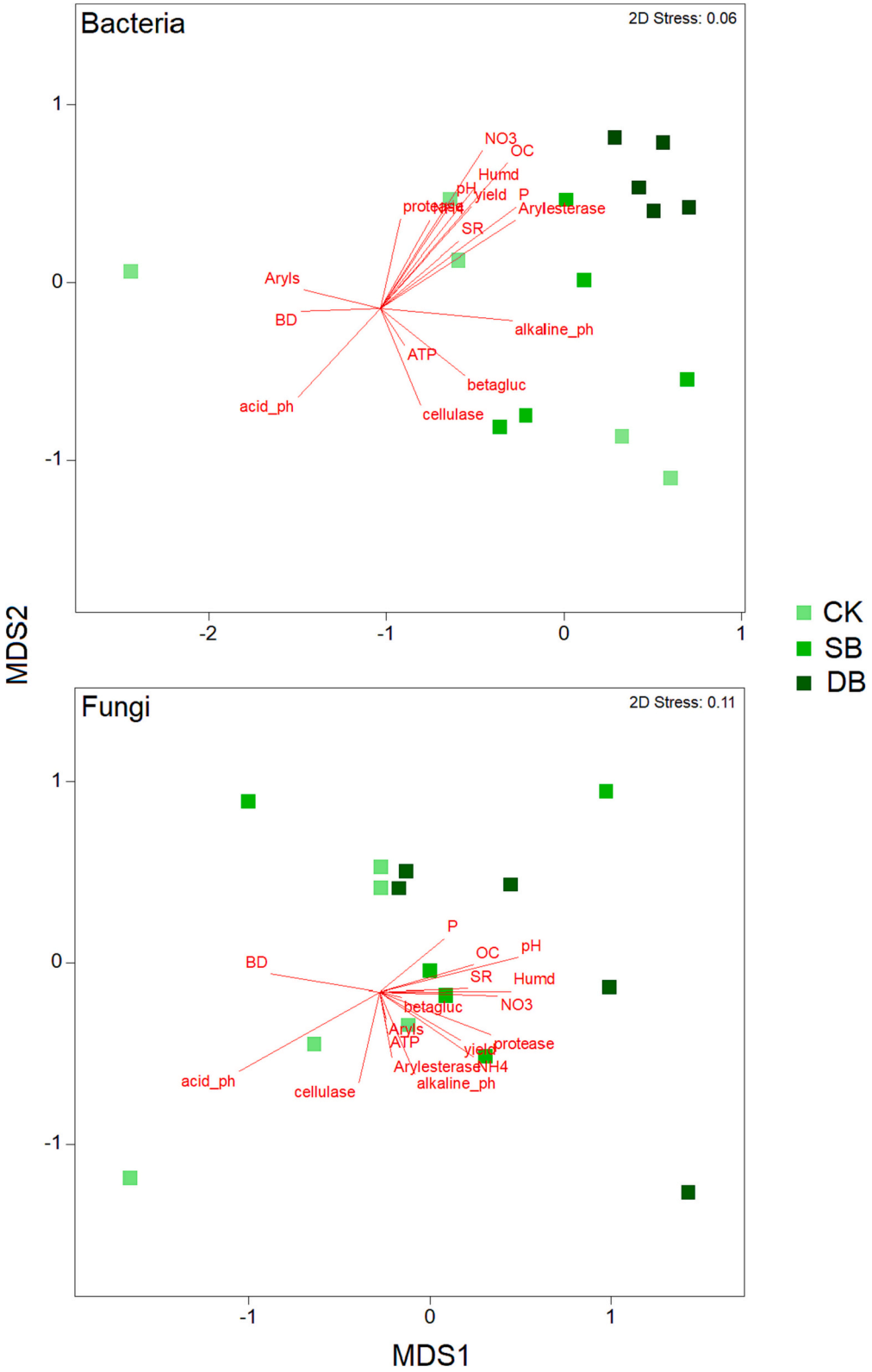


**Fig. 6.** Nonmetric multidimensional scaling (NMDS) plots of bacteria and fungi communities for each treatment in the bulk soil. MDS axis 1 and MDS axis 2 represent the two axes of the two-dimensional ordination space. Each point represents the microbiome of one replicate of the treatment. Notes: CK, SB, and DB represent untreated control, single application of biochar at a rate of 22 t $ha^{-1}$ (SB), two applications of biochar at a rate of 22 t $ha^{-1}$ (DB), respectively. The stress-level shown in each plot indicates how well the individual distances between objects are represented (between 0 and 1; the closer to 0, the better are original data points represented in the ordination space). Vectors represent soil environmental variables, which significantly correlated using Pearson's correlation with the ordination ($P < 0.05$ based on 999 permutations).

treatment could explain the higher respiration rate than that of the SB and CK soil. To date, short-term increase of soil respiration after biochar addition have been reported and attributed either to the inputs of labile C (Smith et al., 2010) or priming effect (Keith et al., 2015). Labile C component of biochar is usually depleted within weeks or months and cannot explain the higher respiration observed after 10 y, which we attribute more to a greater availability of non-labile C, which gradually becomes accessible to soil microorganisms over time. These results could be explained by the fact that soil amendment with biochar likely exerted a protective function on SOC (Ventura et al., 2015). This protective effect is attributed to the stabilizing influence of biochar on plant organic inputs, gradually enhancing their persistence as SOC over time. Hagemann et al. (2017) reported that a complex, nutrient-rich organic coating forms above and within the biochar particles during the aging of the biochar, which could contribute to the stabilization of the labile C inputs. The significant increase in SOC concentration by 37 % in SB and 82 % in DB, compared to the CK soil, played a pivotal role in fostering a more active microbial community. This increase in microbial activity aligns with the 'C pump' mechanism, which suggests that microbial activity is a driving force in the stabilization of SOC (Miltner et al., 2012). Biochar can also protect native and exogenous organic inputs by improving soil aggregation. However, evidence on the effects of biochar on aggregate stability is limited and often contradictory (Sarker et al., 2022). A recent meta-analysis based on 641 comparisons found a 16.4 % improvement in soil aggregation, with the effect depending on the type of biochar and soil, being greater for acidic soils (Islam et al., 2021). While it has been hypothesized that greater occlusion of SOC within aggregates could potentially contribute to higher soil respiration, direct measurements of aggregate stability and occluded organic C concentrations have not been conducted in this study. Nevertheless, our results indicated that both SB and DB applications led to significant improvements in several soil physico-chemical properties and microbial activities compared to the control soil. However, it is worth noting that the DB application resulted in more pronounced changes in soil properties compared to the SB application. It is important to consider that the effects of biochar application rates may vary depending on the specific characteristics of the biochar used, soil properties, and environmental conditions (Iacomino et al., 2022). In this study, the biochar used had an initial high pH value, which could have contributed to the long-term soil pH increase effect. Furthermore, the persistence of biochar basic oxides and the sorption capacity of biochar for $NO_3^-$-N ions could have influenced the observed effects.

Similar values of microbial biomass between control and biochar amended soils 10 y after the soil amendment parallel those of previous studies reporting results from other short- and long-term studies (e.g., Kuzyakov et al., 2009; Nguyen et al., 2018). These results indicate that labile C in biochar is rapidly consumed within weeks or months after incorporation into the soil, and its effect is therefore not relevant on the long-term. No effects of biochar on β-glucosidase activity, a slight reduction in cellulase activity, and an increase in arylesterase activity in SB and DB soils were in line with previous reports (e.g. Jin, 1989). These results suggest that the presence of biochar in these soils may lead to a higher proportion of carboxylic and phenolic compounds in the SOC available to microorganisms in SB and DB soils compared to in CK soil. These compounds are often recalcitrant or complex forms of carbon that require specific enzymes for decomposition such as arylesterase (Ling et al., 2012). In this study, decreased or unchanged activities of several extracellular enzymes involved in the C cycle, i.e., β-glucosidase and cellulase, could be reflected in the slowing of C mineralization and lead to greater soil C storage. Several studies have documented the significant effect of biochar addition on soil enzymes, especially those involved in the C cycle (Bailey et al., 2011; Chen et al., 2013; Elzobair et al., 2016; Moreno et al., 2022). Our observations are consistent with the results of Wang et al. (2015) and Tian et al. (2016), who showed the significant reduction some enzymes involved in the C cycle, such as β-glucosidase and cellulase. In addition, our results showed a significant increase in protease activity and a significant decrease in acid phosphatase activity upon biochar application. The shift towards a more alkaline pH induced by biochar may promote the growth of protease-producing microorganisms, as protease enzymes typically exhibit optimal activity in alkaline conditions (Kumar and Takagi, 1999). Conversely, Acid phosphatase is most effective under acidic conditions, and the rise in soil pH caused by biochar application can impede its enzymatic activity, as it tends to function less efficiently under alkaline conditions.

Increase of microbial activity could be also related to the long-term increase of the soil pH value. The long-term increase effect confirms those observed shortly after biochar amendment of acidic and sub-acidic soils (Lehmann and Joseph, 2009) and those reported from soils of kiln sites in both tropical (Coomes and Miltner, 2017) and temperate areas (Hardy et al., 2017). The long-term increase of the soil pH value by 0.50 in SB and 0.73 pH units in DB soils 10 y after the biochar application even under conventional vineyard management could be due not only to the initial high pH value (9.8) of the used biochar (Genesio et al., 2015), but also to base cations in the soil and the persistence of biochar basic oxides (Yuan et al., 2010). Other mechanisms of pH increase could involve processes related to the sorption of $NO_3^-$N by plants, which can lead to an increase in pH due to the production of $OH^-$ ions. This effect contrasts with the uptake of $NH_4^+$, which produces $H^+$ ions and contributes to soil acidification (Neumann and Ludewig, 2023). Moreover, sorption processes can occur through base functional groups or H-bonding between $NO_3^-$-N ions and the biochar surface. Additionally, capillary forces within biochar micropores have the potential to mitigate the acidifying potential of such ions in the soil solution. As such ions come into contact with biochar micropores, they may experience reduced diffusion and adsorption onto biochar surfaces due to capillary effects. This effect diminishes the immediate availability of ions for reactions that contribute to soil acidification. As a result, the presence of biochar may attenuate the acidifying impact of certain ions in the soil solution (Neumann and Ludewig, 2023). Becagli et al. (2021) reported that $NO_3^-$-N is less leached in biochar-amended soils. The authors suggested that biochar capture the $NO_3^-$-N in its multi-layered structure and porosity or facilitate the adsorption of the nutrient by plants through both direct and indirect effects.

### 4.2. *Biochar application influences bacterial community structure and composition*

Temperature, available oxygen, pH, and quality of available organic C are key factors affecting bacterial richness and community structure (Fierer, 2017), and supplementing soil with biochar significantly alters these ecological factors. Our results showed that 10 y after biochar application, the composition of the soil microbial community was significantly different from that of the CK soils, while the diversity remained unchanged. To date, effects of biochar on bacterial diversity have been ascribed to the pH increase and labile organic inputs provided by biochar. For example, a decrease in *Acidobacteria* has been linked to soil alkalinization (Jenkins et al., 2017; Chen et al., 2019), whereas an increase in *Proteobacteria*, *Gemmatimonadetes*, and *Bacteroidetes* in acidic ferralsol (Sheng and Zhu, 2018) has been associated with their copiotrophic metabolism, which is stimulated by the greater availability of labile C, particularly when biochar is mixed with compost or plant residues (Bonanomi et al., 2020). Differently from these results of short-term studies, we observed an increase in the relative abundance of *Acidobacteria* and slight reduction or no effects on the abundance of the copiotrophic bacterial phyla. Soil microbiota dominated by fast-growing opportunistic bacteria (e.g., especially *Proteobacteria*) but depleted in oligotrophic bacteria (e.g., *Acidobacteria*) is typical of intensive agricultural systems subject to physical disturbance and large fluctuations in organic C availability. This results in low stability in the functionality of the soil microbiota, which also favors the spread of phytopathogens (Bonanomi et al., 2021). The DB soil was rich in oligotrophic bacteria despite the high SOM and respiration rate suggesting that the bacterial

community of soils 10 y after amendment with biochar reached a steady state despite the larger SOC concentration. This result parallels those previously reported for soils under long-term conservative agricultural management such as organic farming (Bonanomi et al., 2016). These considerations are also supported by the observed increase in the stability, density and the heterogeneity of the microbial co-occurrence and interactions in the bacterial communities of the biochar-amended soils.

In this study, we observed a slight reduction in the relative abundance of *Actinobacteria* in DB soil compared to the control soil. This finding contradicts previous studies that reported an increase in *Actinobacteria* abundance with the addition of biochar (Kolton et al., 2011; Sheng and Zhu, 2018). We hypothesize that the near neutral pH conditions in the DB soils might have influenced the *Actinobacteria* abundance. Furthermore, we propose two potential factors contributing to the reduction in *Actinobacteria* relative abundance: the prolonged increase in soil pH over a period of 10 years, and a decrease in the availability of phenolic substrates. These phenolic substrates could have provided competitive advantages to *Actinobacteria* over other bacterial groups. The ability of biochar to adsorb pesticides and fungicides (Brtnicky et al., 2021) could be another ecological factor influencing the *Actinobacteria* in the DB soils. *Actinobacteria* may utilize these organic compounds as C sources, and their reduction in abundance could be attributed to this aspect. For example, Bonanomi et al. (2016) reported that *Actinobacteria* were less abundant in soils under organic farming compared to conventional farms with intensive use of agrochemicals.

When linking microbial community to the soil parameters, bacteria in the SB soils were positively correlating with β-glucosidase, cellulase, and alkaline phosphatase activities, while negatively correlating with arylsulfatase activity. This suggests that long-term biochar application may elicit bacteria with a preference for cellulose decomposition and phosphate mineralization, possibly due to the influx of labile carbon compounds provided by the biochar (Ngo et al., 2013). The negative correlation with arylsulfatase activity might indicate a lesser emphasis on sulfur cycling processes within the bacterial community (Sun et al., 2014). However, in the DB soils, we observed a bacterial community that exhibited a positive correlation with several key parameters, including arylesterase activity, P availability, SOC concentration, and $NO_3$-N concentration. This pattern suggests that biochar application over a decade has fostered a bacterial community closely associated with the degradation of organic compounds, enhanced nutrient availability, and increased carbon content. These findings align with the notion that biochar can act as a long-term stabilizer of soil organic carbon, promoting a nutrient-rich environment conducive to microbial activity (Mitchell et al., 2015; Yang et al., 2020; Duan et al., 2022). In contrast, the CK soil exhibited a more scattered distribution of replicates based on a broad spectrum of parameters, including SOC concentration, β-glucosidase, and arylsulfatase activities. This lack of a well-defined pattern suggests a higher degree of microbial community variability in the absence of biochar. Soil microbial communities in the CK treatment may be subject to greater fluctuations in response to changing environmental conditions and resource availability.

### 4.3. Biochar effects on fungal communities and symbiotic relationships in vineyards

In the DB soils, abundance of fungal species richness in the rhizosphere of grapevine increased. Previous studies reported either positive (Tarin et al., 2021), neutral (Yao et al., 2017) or negative effects on fungal diversity (Dai et al., 2018). Fungal hyphae can colonize biochar particles which provide protection from desiccation and/or predation (Warnock et al., 2007), and these features likely provided physical space and spatial heterogeneity that would allow good differentiation and coexistence for a larger number of fungal species in the rhizosphere on the long-term. At the community level, biochar caused a relative decrease in *Ascomycota* and an increase in *Basidiomycota* and *Chytridiomycota* in the vine rhizosphere. A relative increase of *Basidiomycota* in biochar amended soils was previously reported (Noyce et al., 2016; Tarin et al., 2021); this result is important because *Basidiomycota* are among the major drivers of litter and wood decomposition in agricultural and forest ecosystems. In our study, the most abundant *Basidiomycota* in grape rhizosphere were *Saitozyma* and *Solicoccozyma*, two ASVs that were significantly enriched in biochar-amended-soils, and these yeasts are commonly isolated from soils worldwide (e.g., Takashima et al., 2012), and are considered able to incorporate carbon from cellulose (Štursová et al., 2012), which indicates their involvement in the decomposition of dead plant biomass. In general, yeast fungi of the genus *Basidiomycota* are widely distributed in soil (Yurkov, 2018), but their distribution and functional role are not well understood, and further studies are needed to decipher the effects of these fungi on grape root growth and health. Moreover, vine rhizosphere in the SB and especially in the DB soils was enriched with two *Chytridiomycota* ASVs Basidiomycota yeasts, one unidentified and one *Rhizophlyctis* species. *Chytridiomycota* are fungi that thrive in aquatic ecosystems and damp soil but can also survive dry spells (Gleason et al., 2004). The high abundance of *Chytridiomycota* in SB and DB soils could be related to their higher water retention as compared to the CK soil (Baronti et al., 2014). Moreover, *Chytridiomycota* play a crucial role in decomposing organic matter and facilitating the efficient recycling of nutrients within the ecosystem.

At the functional level, no increase in the saprotrophic guild was observed 10 y after the biochar application. This result contrasts with short-term incubation studies that have shown a promotion of saprotrophic fungi in the presence of biochar, especially when the biochar is rich in labile C fractions (Dai et al., 2018) or when the soil has been amended with other organic matter such as cellulose or lignin (Clocchiatti et al., 2020). While the rhizosphere of grapes growing in biochar-amended soil showed an enrichment of plant pathogens compared to the control, the overall higher biomass production, bunch yield and wine quality in biochar-amended soils, along with the absence of pathogenicity (Genesio et al., 2015), could be explained by the fact that FUNGuild annotation tool included all plant pathogens, even those that do not attack the grape (e.g., *Fusarium*). The enhanced protection for vine plants grown in soils enriched with biochar may also be attributed to the higher relative abundance of the plant beneficial fungus *Trichoderma*, which has antagonistic properties against soil-borne pathogens by inducing systemic resistance (Lorito and Woo, 2015), compared to plants grown in the control soil. On the other hand, the rhizosphere of vines grown in the control soil was enriched with *Aspergillus*, *Penicillium* and *Phaeoacremonium* groups. These groups comprise fast-growing opportunistic species and several plant pathogen species producing ochratoxin A (e.g., *A. carbonarius*), causing serious problems in grape production in arid and Mediterranean climates (Visconti et al., 2008). Notably, the causal agent of the "Esca disease complex", *Phaeoacremonium*, was detected only in the rhizosphere of plants grown in the control soil, not the biochar-enriched soils (Graniti et al., 2000).

Grapevine forms symbiotic associations with arbuscular mycorrhizal fungi (AMF) that improve plant mineral nutrition, water uptake, and induce disease resistance (Trouvelot et al., 2015). Several AMFs form symbiotic relationships with grapevine, including *Acaulospora*, *Diversispora*, *Glomus*, *Gigaspora*, *Funneliformis*, *Paraglomus*, *Rhizophagus*, and *Claroideoglomus*. In the studied soils, long-term biochar amendment increased *Glomeromycota* compared to the CK soil, likely in relation to the improvement of soil physico-chemical properties, such as enhanced nutrient availability (Warnock et al., 2007). Dominance of *Glomerales* and *Paraglomerales* AMF in the rhizosphere of Italian vineyard has been reported (Lumini et al., 2010; Balestrini et al., 2010). In contrast, the AMF in the rhizosphere of control soil was dominated by *Paraglomus*, *Funneliformis* and *Diversispora*. The establishment of plant-fungal interactions in AMF is strongly modulated by complex chemical communication, specifically by plants release of strigolactones that stimulate AMF spore germination and hyphal branching propaedeutic to symbiosis establishment (Akiyama et al., 2005). We hypothesize that biochar

could enhance plant-fungal signaling by adsorbing specific chemical compounds, as observed in the modulation of bacterial quorum sensing (Masiello et al., 2013) or plant-pathogen interactions (Jaiswal et al., 2018; Bonanomi et al., 2022). By adsorbing such chemicals, biochar could potentially facilitate the establishment of mycorrhizal symbioses. Given the importance of the interaction between AMF and grapevines for grape yield and wine quality (Torres et al., 2018), further research is needed to assess the functional impact of changes in the symbiotrophic microbiota in the vineyard.

Our nMDS analysis showed a complex interplay of parameters influencing fungal community distribution. In both SB and DB soils, fungi exhibited a positive correlation with P, pH, SOC concentration, and protease activity. These correlations suggest that biochar-amended soils create conditions favorable for fungal proliferation, including improved nutrient availability, pH, and increased carbon content (Prayogo et al., 2014; Wang et al., 2020). The elevated protease activity indicates potential for increased decomposition of organic matter and nutrient cycling within these fungal communities. In contrast, in the CK soil, fungal diversity appeared to be mainly correlated with available P and enzymatic activities, specifically acid phosphomonoesterase, arylesterase, and cellulase. This suggests that in the absence of biochar, fungal communities may rely more heavily on nutrient availability and a suite of enzymatic processes to drive their activity and diversity.

### *4.4. Biochar impact on microbial community networks*

Regarding the co-occurrence network analysis, our results revealed that the rhizosphere network had higher values of network centralization, network heterogeneity, and network density compared to the bulk soil network. This suggests that the microbial interactions in the rhizosphere are more connected, diverse, and dense, indicating a potentially more complex microbial community (Gao et al., 2022). However, the characteristic path length, which represents the average number of steps between any two nodes, was found to be higher in the bulk soil, indicating a longer distance for microbial interactions. Moreover, the percentage of positive correlations between different microbial groups, within the rhizosphere, was higher in the SB and DB soils, suggesting a more cooperative and interdependent microbial community in those treatments. Additionally, the values of network centralization, network heterogeneity, and network density increased significantly from CK to SB to DB soils, indicating a progression towards more complex and interconnected microbial networks. On the other hand, for the bulk soil, the proportion of positive correlations between ASVs was higher in the CK soil compared to the DB soil. Furthermore, the values of network centralization, network heterogeneity, and network density decreased significantly from CK to SB to DB soils, suggesting a reduction in complexity and connectivity of microbial interactions in the bulk soil (Guseva et al., 2022).

## 5. Conclusions

In conclusion, our long-term study underscores the value of biochar amendment in improving soil fertility in vineyard systems. The benefits extend to enhanced soil carbon storage, favorable changes in microbial communities, and shifts in microbial metabolism. These findings have significant agricultural and environmental implications, highlighting the potential of biochar to promote sustainable vineyard management practices that contribute to both soil health and ecosystem resilience.

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## CRediT authorship contribution statement

**Mohamed Idbella:** Data curation, Formal analysis, Methodology, Software, Visualization, Writing – original draft. **Silvia Baronti:** Conceptualization, Investigation, Methodology, Validation, Writing – review & editing. **Laura Giagnoni:** Investigation, Methodology, Writing – review & editing. **Giancarlo Renella:** Conceptualization, Investigation, Methodology, Validation, Writing – review & editing. **Michelangelo Becagli:** Investigation, Methodology, Validation. **Roberto Cardelli:** Investigation, Methodology, Validation. **Anita Maienza:** Investigation, Methodology, Validation, Visualization. **Francesco Primo Vaccari:** Conceptualization, Investigation, Methodology, Project administration, Resources, Writing – review & editing. **Giuliano Bonanomi:** Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft.

## Declaration of competing interest

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

## Data availability

Reads of the sequence data have been deposited in the NCBI Sequence Read Archive (SRA) under the bioproject “Braccesca & Biochar” with accession no. PRJNA842337.

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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.2023.105217.

## References

Akiyama, K., Matsuzaki, K., Hayashi, H., 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435, 824–827.
Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol. Biochem. 10, 215–221.
Anderson, C.R., Condron, L.M., Clough, T.J., Fiers, M., Stewart, A., Hill, R.A., Sherlock, R.R., 2011. Biochar induced soil microbial community change: implications for biogeochemical cycling of carbon, nitrogen and phosphorus. Pedobiologia 54, 309–320.
Anderson, C.R., Hamonts, K., Clough, T.J., Condron, L.M., 2014. Biochar does not affect soil N-transformations or microbial community structure under ruminant urine patches but does alter relative proportions of nitrogen cycling bacteria. Agric. Ecosyst. Environ. 191, 63–72.
Bailey, V.L., Fansler, S.J., Smith, J.L., Bolton Jr., H., 2011. Reconciling apparent variability in effects of biochar amendment on soil enzyme activities by assay optimization. Soil Biol. Biochem. 43, 296–301.
Balestrini, R., Magurno, F., Walker, C., et al., 2010. Cohorts of arbuscular mycorrhizal fungi (AMF) in *Vitis vinifera*, a typical Mediterranean fruit crop. Environ. Microbiol. Rep. 2, 594–604.
Baronti, S., Vaccari, F.P., Miglietta, F., Calzolari, C., Lugato, E., Orlandini, S., Genesio, L., 2014. Impact of biochar application on plant water relations in *Vitis vinifera* (L.). Eur. J. Agron. 53, 38–44.
Baronti, S., Magno, R., Maienza, A., Montagnoli, A., Ungaro, F., Vaccari, F.P., 2022. Long term effect of biochar on soil plant water relation and fine roots: results after 10 years of vineyard experiment. Sci. Total Environ. 851, 158225.
Bastian, M., Jacomy, M., 2009. Gephi: an open-source software for exploring and manipulating networks. In: International AAAI Conference on Weblogs and Social Media: San Jose, CA, USA.
Becagli, M., Guglielminetti, L., Cardelli, R., 2021. Effects of combined biochar and vermicompost solution on leachate characterization and nitrogen balance from a greenhouse tomato (*Solanum lycopersicum*) cultivation soil. Commun. Soil Sci. Plant Anal. 52, 1879–1893.

Berni Canani, R., De Filippis, F., Nocerino, R., Laiola, M., Paparo, L., Calignano, A., De Caro, C., Coretti, L., Chiariotti, L., Gilbert, J.A., 2017. Specific signatures of the gut microbiota and increased levels of butyrate in children treated with fermented cow's milk containing heat-killed *Lactobacillus paracasei* CBA L74. Appl. Environ. Microbiol. 83, 10.
Bokulich, N.A., Mills, D.A., 2013. Improved selection of internal transcribed spacer-specific primers enables quantitative, ultra-highthroughput profiling of fungal communities. Appl. Environ. Microbiol. 79, 2519–2526.
Bonanomi, G., De Filippis, F., Cesarano, G., La Storia, A., Ercolini, D., Scala, F., 2016. Organic farming induces changes in soil microbiota that affect agro-ecosystem functions. Soil Biol. Biochem. 103, 327–336.
Bonanomi, G., De Filippis, F., Zotti, M., Idbella, M., Cesarano, G., Al-Rowaily, S., Abd-ElGawad, A., 2020. Repeated applications of organic amendments promote beneficial microbiota, improve soil fertility and increase crop yield. Appl. Soil Ecol. 156, 103714.
Bonanomi, G., Idbella, M., Abd-ElGawad, A.M., 2021. Microbiota management for effective disease suppression: a systematic comparison between soil and mammals gut. Sustainability 13 (14), 7608.
Bonanomi, G., Zotti, M., Idbella, M., Cesarano, G., Al-Rowaily, S.L., Abd-ElGawad, A.M., 2022. Mixtures of organic amendments and biochar promote beneficial soil microbiota and affect *Fusarium oxysporum* f. sp. lactucae, *Rhizoctonia solani* and *Sclerotinia minor* disease suppression. Plant Pathol. 71, 818–829.
Bray, R.H., Kurtz, L.T., 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Sci. 59, 39–45.
Browman, M.G., Tabatabai, M.A., 1978. Phosphodiesterase activity of soils. Soil Sci. Soc. Am. J. 42, 284–290.
Brtnicky, M., Datta, R., Holatko, J., Bielska, L., Gusiatin, Z.M., Kucerik, J., Pecina, V., 2021. A critical review of the possible adverse effects of biochar in the soil environment. Sci. Total Environ. 148756.
Calderón, K., Spor, A., Breuil, M.C., et al., 2017. Effectiveness of ecological rescue for altered soil microbial communities and functions. ISME J. 11, 272–283.
Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583.
Case, D.C.C., McNamara, N.P., Reay, D.S., Whitaker, J., 2012. The effect of biochar addition on $N_2O$ and $CO_2$ emissions from a sandy soil – the role of soil aeration. Soil Biol. Biochem. 51, 125–134.
Chen, J., Liu, X., Zheng, J., Zhang, B., Lu, H., Chi, Z., Pan, G., Li, L., Zheng, J., Zhang, X., Wang, J., Yu, X., 2013. Biochar soil amendment increased bacterial but decreased fungal gene abundance with shifts in community structure in a slightly acid rice paddy from Southwest China. Appl. Soil Ecol. 71, 33–44.
Chen, J., Liu, X., Li, L., Zheng, J., Qu, J., Zheng, J., Zhang, X., Pan, G., 2015. Consistent increase in abundance and diversity but variable change in community composition of bacteria in topsoil of rice paddy under short term biochar treatment across three sites from South China. Appl. Soil Ecol. 91, 68–79.
Chen, L., Jiang, Y., Liang, C., Luo, Y., Xu, Q., Han, C., Sun, B., 2019. Competitive interaction with keystone taxa induced negative priming under biochar amendments. Microbiome 7, 1–18.
Ciardi, C., Nannipieri, P., 1990. A comparison of methods for measuring ATP in soil. Soil Biol. Biochem. 22, 725–727.
Ciavatta, C., Vittori Antisari, L., Sequi, P., 1989. Determination of organic carbon in soils and fertilizers. Commun. Soil Sci. Plant Anal. 20, 759–773.
Clocchiatti, A., Hannula, S.E., van den Berg, M., Korthals, G., De Boer, W., 2020. The hidden potential of saprotrophic fungi in arable soil: patterns of short-term stimulation by organic amendments. Appl. Soil Ecol. 147, 103434.
Coomes, O.T., Miltner, B.C., 2017. Indigenous charcoal and biochar production: potential for soil improvement under shifting cultivation systems. Land Degrad. Dev. 28, 811–821.
Dai, Z., Enders, A., Rodrigues, J.L., Hanley, K.L., Brookes, P.C., Xu, J., Lehmann, J., 2018. Soil fungal taxonomic and functional community composition as affected by biochar properties. Soil Biol. Biochem. 126, 159–167.
Darby, I., Xu, C.Y., Wallace, H.M., Joseph, S., Pace, B., Bai, S.H., 2016. Short-term dynamics of carbon and nitrogen using compost, compost-biochar mixture and organo-mineral biochar. Environ. Sci. Pollut. Res. 23, 11267–11278.
Deenik, J.L., McClellan, T., Uehara, G., Antal, M.J., Campbell, S., 2010. Charcoal volatile matter content influences plant growth and soil nitrogen transformations. Soil Sci. Soc. Am. J. 74, 1259–1270.
Duan, Y., Zhang, L., Yang, J., Zhang, Z., Awasthi, M.K., Li, H., 2022. Insight to bacteria community response of organic management in apple orchard-bagasse fertilizer combined with biochar. Chemosphere 286, 131693.
Elad, Y., Rav David, D., Meller Harel, Y., Borenshtein, M., Ben Kalifa, H., Silber, A., Graber, E.R., 2010. Induction of systemic resistance in plants by biochar, a soilapplied carbon sequestering agent. Phytopathology 100, 913–921.
Elzobair, K.A., Stromberger, M.E., Ippolito, J.A., 2016. Stabilizing effect of biochar on soil extracellular enzymes after a denaturing stress. Chemosphere 142, 114–119.
Fierer, N., 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. Nat. Rev. Microbiol. 15, 579–590.
Gao, C., Xu, L., Montoya, L., Madera, M., Hollingsworth, J., Chen, L., Purdom, E., Singan, V., Vogel, J., Hutmacher, R.B., 2022. Co-occurrence networks reveal more complexity than community composition in resistance and resilience of microbial communities. Nat. Commun. 13, 1–12.
Gavili, E., Moosavi, A.A., Zahedifar, M., 2019. Integrated effects of cattle manure-derived biochar and soil moisture conditions on soil chemical characteristics and soybean yield. Arch. Agron. Soil Sci. 65, 1758–1774.
Genesio, L., Miglietta, F., Baronti, S., Vaccari, F.P., 2015. Biochar increases vineyard productivity without affecting grape quality: results from a four years field experiment in Tuscany. Agric. Ecosyst. Environ. 201, 20–25.
Giagnoni, L., Maienza, A., Baronti, S., Vaccari, F.P., Genesio, L., Taiti, C., Martellini, T., Scodellini, R., Cincinelli, A., Costa, C., Mancuso, S., Renella, G., 2019. Long-term soil biological fertility, volatile organic compounds and chemical properties in a vineyard soil after biochar amendment. Geoderma 344, 127–136.
Glaser, B., Lehmann, J., Zech, W., 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal – a review. Biol. Fertil. Soils 35, 219–230.
Gleason, F.H., Letcher, P.M., McGee, P.A., 2004. Some Chytridiomycota in soil recover from drying and high temperatures. Mycol. Res. 108, 583–589.
Graniti, A., Mugnai, L., Surico, G., 2000. Esca of grapevine: a disease complex or a complex of diseases. In: Esca of Grapevine, pp. 1000–1005.
Grossman, R.B., Reinsch, T.G., 2002. 2.1 Bulk density and linear extensibilityMethods of Soil Analysis: Part 4 Physical Methods, SSSA Book Series. Soil Science Society of America, Madison, WI, pp. 201–228.
Gu, Z., Eils, R., Schlesner, M., 2016. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics 32, 2847–2849.
Güereña, D., Lehmann, J., Hanley, K., Enders, A., Hyland, C., Riha, S., 2013. Nitrogen dynamics following field application of biochar in a temperate North American maize-based production system. Plant Soil 365, 239–254.
Gul, S., Whalen, J.K., 2016. Biochemical cycling of nitrogen and phosphorus in biochar-amended soils. Soil Biol. Biochem. 103, 1–15.
Guseva, K., Darcy, S., Simon, E., Alteio, L.V., Montesinos-Navarro, A., Kaiser, C., 2022. From diversity to complexity: microbial networks in soils. Soil Biol. Biochem. 169, 108604.
Hagemann, N., Joseph, S., Schmidt, H.P., Kammann, C.I., Harter, J., Borch, T., Kappler, A., 2017. Organic coating on biochar explains its nutrient retention and stimulation of soil fertility. Nat. Commun. 8 (1), 1–11.
Hardy, B., Cornelis, J.T., Houben, D., Leifeld, J., Lambert, R., Dufey, J.E., 2017. Evaluation of the long-term effect of biochar on properties of temperate agricultural soil at pre-industrial charcoal kiln sites in Wallonia, Belgium. Eur. J. Soil Sci. 68 (1), 80–89.
Hugerth, L.W., Andersson, A.F., 2017. Analysing microbial community composition through amplicon sequencing: from sampling to hypothesis testing. Front. Microbiol. 8, 1–22.
I.B.I., 2012. Standardized product definition and product testing guidelines for biochar that is used in soil. Available at. https://biochar-international.org/.
Iacomino, G., Sarker, T.C., Ippolito, F., Bonanomi, G., Vinale, F., Staropoli, A., Idbella, M., 2022. Biochar and compost application either alone or in combination affects vegetable yield in a volcanic Mediterranean soil. Agronomy 12 (9), 1996.
Idbella, M., Bonanomi, G., De Filippis, F., et al., 2021. Contrasting effects of *Rhizophagus irregularis* versus bacterial and fungal seed endophytes on *Trifolium repens* plant-soil feedback. Mycorrhiza 31, 103–115.
Idbella, M., De Filippis, F., Zotti, M., Sequino, G., Abd-ElGawad, A.M., Fechtali, T., Mazzoleni, S., Bonanomi, B., 2022. Specific microbiome signatures under the canopy of Mediterranean shrubs. Appl. Soil Ecol. 173, 104407.
Imparato, V., Hansen, V., Santos, S., Nielsen, T.K., Giagnoni, L., Hauggaard-Nielsen, H., Johansen, A., Renella, G., Winding, A., 2016. Gasification biochar has limited effects on functional and structural diversity of soil microbial communities in a temperate agroecosystem. Soil Biol. Biochem. 99, 128–136.
Islam, M.U., Jiang, F., Guo, Z., Peng, X., 2021. Does biochar application improve soil aggregation? A meta-analysis. Soil Tillage Res. 209, 104926.
Jaiswal, A.K., Elad, Y., Graber, E.R., Frenkel, O., 2014. *Rhizoctonia solani* suppression and plant growth promotion in cucumber as affected by biochar pyrolysis temperature, feedstock and concentration. Soil Biol. Biochem. 69, 110–118.
Jaiswal, A.K., Frenkel, O., Tsechansky, L., Elad, Y., Graber, E.R., 2018. Immobilization and deactivation of pathogenic enzymes and toxic metabolites by biochar: a possible mechanism involved in soilborne disease suppression. Soil Biol. Biochem. 121, 59–66.
Jeffery, S., Verheijen, F.G.A., van der Velde, M., Bastos, A.C., 2011. A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. Agric. Ecosyst. Environ. 144, 175–187.
Jenkins, J.R., Viger, M., Arnold, E.C., Harris, Z.M., Ventura, M., Miglietta, F., Taylor, G., 2017. Biochar alters the soil microbiome and soil function: results of next-generation amplicon sequencing across Europe. GBC Bioenergy 9, 591–612.
Jin, H., 1989. Thesis: characterization of microbial life colonizing biochar and biochar-amended soils. J. Chem. Inf. Model. 53, 160.
Kandeler, E., Gerber, H., 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. Biol. Fertil. Soils 6, 68–72.
Keith, A., Singh, B., Dijkstra, F.A., 2015. Biochar reduces the rhizosphere priming effect on soil organic carbon. Soil Biol. Biochem. 88, 372–379.
Kolb, S.E., Fermanich, K.J., Dornbush, M.E., 2009. Effect of charcoal quantity on microbial biomass and activity in temperate soils. Soil Sci. Soc. Am. J. 73, 1173–1181.
Kolton, M., Harel, Y.M., Pasternak, Z., Graber, E.R., Elad, Y., Cytryn, E., 2011. Impact of biochar application to soil on the root-associated bacterial community structure of fully developed greenhouse pepper plants. Appl. Environ. Microbiol. 77, 4924–4930.
Kumar, C.G., Takagi, H., 1999. Microbial alkaline proteases: from a bioindustrial viewpoint. Biotechnol. Adv. 17, 561–594.
Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., Xu, X., 2009. Black carbon decomposition and incorporation into soil microbial biomass estimated by $^{14}C$ labeling. Soil Biol. Biochem. 41, 210–219.
Ladd, J.N., Butler, J.H.A., 1972. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Biol. Biochem. 4, 19–30.

Lehmann, J., Joseph, S., 2009. Biochar for environmental management: an introduction. In: Lehmann, J., Joseph, S. (Eds.), Biochar for Environmental Management. Earthscan, London, pp. 1–12.
Lehmann, J., Rillig, M., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota: a review. Soil Biol. Biochem. 43, 1812–1836.
Ling, W., Lu, X., Gao, Y., Liu, J., Sun, Y., 2012. Polyphenol oxidase activity in subcellular fractions of tall fescue contaminated by polycyclic aromatic hydrocarbons. J. Environ. Qual. 41, 807.
Lorenz, K., Lal, R., 2014. Biochar application to soil for climate change mitigation by soil organic carbon sequestration. J. Plant Nutr. Soil Sci. 177, 651–670.
Lorito, M., Woo, S.L., 2015. *Trichoderma*: a multi-purpose tool for integrated pest management. In: Principles of Plant-Microbe Interactions. Springer, Cham, pp. 345–353.
Lumini, E., Orgiazzi, A., Borriello, R., et al., 2010. Disclosing arbuscular mycorrhizal fungal biodiversity in soil through a land-use gradient using a pyrosequencing approach. Environ. Microbiol. 12, 2165–2179.
Martin, M., 2011. Cutadapt removes adaptor sequences from high-throughput sequencing reads. EMBnet J. 17, 10–12.
Masiello, C.A., Chen, Y., Gao, X., Liu, S., Cheng, H.Y., Bennett, R., Rudgers, J.A., Wagner, D.S., Zygourakis, K., Silberg, J.J., 2013. Biochar and microbial signaling: production conditions determine effects on microbial communication. Environ. Sci. Technol. 47, 11496–11503.
Miltner, A., Bombach, P., Schmidt-Brücken, B., Kästner, M., 2012. SOM genesis: microbial biomass as a significant source. Biogeochemistry 111, 45–55.
Mitchell, P.J., Simpson, A.J., Soong, R., Simpson, M.J., 2015. Shifts in microbial community and water-extractable organic matter composition with biochar amendment in a temperate forest soil. Soil Biol. Biochem. 81, 244–254.
Mohan, D., Sarswat, A., Ok, Y.S., Pittman Jr., C.U., 2014. Organic and inorganic contaminants removal from water with biochar, a renewable, low cost and sustainable adsorbent – a critical review. Bioresour. Technol. 160, 191–202.
Moreno, J.L., Bastida, F., Díaz-López, M., Li, Y., Zhou, Y., López-Mondéjar, R., Benavente-Ferraces, I., Rojas, R., Rey, A., García-Gil, J.C., Plaza, C., 2022. Response of soil chemical properties, enzyme activities and microbial communities to biochar application and climate change in a Mediterranean agroecosystem. Geoderma 407, 115536.
Murphy, J., Riley, J.P., 1986. Citation-classic—a modified single solution method for the determination of phosphate in natural-waters. Current contents. J. Agric. Biol. Environ. Stat. 12, 16.
Nannipieri, P., Ceccanti, B., Cervelli, S., Sequi, P., 1974. Use of 0.1 m pyrophosphate to extract urease from a podzol. Soil Biol. Biochem. 359–362.
Neumann, G., Ludewig, U., 2023. Chapter 14 - rhizosphere chemistry influencing plant nutrition. In: Rengel, Zed, Cakmak, Ismail, White, Philip J. (Eds.), Marschner's Mineral Nutrition of Plants, fourth edition. Academic Press, pp. 545–585.
Ngo, P.T., Rumpel, C., Ngo, Q.A., Alexis, M., Vargas, G.V., M.d.l.L. Mora Gil, M.D.L., Dang, D.K., Jouquet, P., 2013. Biological and chemical reactivity and phosphorus forms of buffalo manure compost, vermicompost and their mixture with biochar. Bioresour. Technol. 148, 401–407.
Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: an open annotation tool for parsing fungal community data sets by ecological guild. Fungal Ecol. 20, 241–248.
Nguyen, T.T.N., Xu, C.Y., Tahmasbian, I., Che, R., Xu, Z., Zhou, X., Wallace, H.M., Bai, S. H., 2017. Effects of biochar on soil available inorganic nitrogen: a review and meta analysis. Geoderma 288, 79–96.
Nguyen, T.T.N., Wallace, H.M., Xu, C.Y., Van Zwieten, L., Weng, Z.H., Xu, Z., Che, R., Tahmasbian, I., Hu, H.W., Bai, S.H., 2018. The effects of short term, long term and reapplication of biochar on soil bacteria. Sci. Total Environ. 636, 142–151.
Nilsson, R.H., Larsson, K.H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F.O., Tedersoo, L., Saar, I., Kõljalg, U., Abarenkov, K., 2019. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Res. 47, 259–264.
Noyce, G.L., Winsborough, C., Fulthorpe, R., Basiliko, N., 2016. The microbiomes and metagenomes of forest biochars. Sci. Rep. 6, 1–12.
Park, J.H., Choppala, G.K., Bolan, N.S., Chung, J.W., Chuasavathi, T., 2011. Biochar reduces the bioavailability and phytotoxicity of heavy metals. Plant Soil 348, 439–451.
Prayogo, C., Jones, J.E., Baeyens, J., Bending, G.D., 2014. Impact of biochar on mineralization of C and N from soil and willow litter and its relationship with microbial community biomass and structure. Biol. Fertil. Soils 50, 695–702.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, 590–600.
Quilliam, R.S., Marsden, K.A., Gertler, C., Rousk, J., DeLuca, T.H., Jones, D.L., 2012. Nutrient dynamics, microbial growth and weed emergence in biochar amended soil are influenced by time since application and reapplication rate. Agric. Ecosyst. Environ. 158, 192–199.
Quilliam, R.S., Glanville, H.C., Wade, S.C., Jones, D.L., 2013. Life in the 'charosphere' – does biochar in agricultural soil provide a significant habitat for microorganisms? Soil Biol. Biochem. 65, 287–293.
R Core Team, 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.R Core Team., 2016. R: A language and environment for statistical computing. R Found. Stat. Comput. https://www.R-project.org/.
Sarker, T.C., Zotti, M., Fang, Y., Giannino, F., Mazzoleni, S., Bonanomi, G., Chang, S.X., 2022. Soil aggregation in relation to organic amendment: a synthesis. J. Soil Sci. Plant Nutr. 1–22.
Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13, 2498–2504.
Sheng, Y., Zhu, L., 2018. Biochar alters microbial community and carbon sequestration potential across different soil pH. Sci. Total Environ. 622, 1391–1399.
Smith, J.L., Collins, H.P., Bailey, V.L., 2010. The effect of young biochar on soil respiration. Soil Biol. Biochem. 42, 2345–2347.
Solaiman, Z.M., Blackwell, P., Abbott, L.K., Storer, P., 2010. Direct and residual effect of biochar application on mycorrhizal root colonisation, growth and nutrition of wheat. Soil Res. 48, 546–554.
Štursová, M., Žifčáková, L., Leigh, M.B., et al., 2012. Cellulose utilization in forest litter and soil: identification of bacterial and fungal decomposers. FEMS Microbiol. Ecol. 80, 735–746.
Sun, Z., Bruun, E.W., Arthur, E., Jonge, L.W.D., Moldrup, P., Nielsen, H.H., Elsgaard, L., 2014. Effect of biochar on aerobic processes, enzyme activity, and crop yields in two sandy loam soils. Biol. Fertil. Soils 50, 1087–1097.
Tabatabai, M.A., 1982. Soil enzymes. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, 2nd ed. American Society of Agronomy/Soil Science Society of America, Madison, WI, USA, pp. 903–947.
Tabatabai, M., Bremner, J., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1, 301–307.
Tabatabai, M.A., Bremner, J.M., 1970. Arylsulfatase activity of soils. Soil Sci. Soc. Am. J. 34, 225–229.
Takashima, M., Sugita, T., Van, B.H., et al., 2012. Taxonomic richness of yeasts in Japan within subtropical and cool temperate areas. PLoS One 7, e50784.
Tarin, M.W.K., Fan, L., Xie, D., Tayyab, M., Rong, J., Chen, L., Zheng, Y., 2021. Response of soil fungal diversity and community composition to varying levels of bamboo biochar in red soils. Microorganisms 9, 1385.
Tian, J., Wang, J., Dippold, M., Gao, Y., Blagodatskaya, E., Kuzyakov, Y., 2016. Biochar affects soil organic matter cycling and microbial functions but does not alter microbial community structure in a paddy soil. Sci. Total Environ. 556, 89–97.
Torres, N., Antolín, M.C., Goicoechea, N., 2018. Arbuscular mycorrhizal symbiosis as a promising resource for improving berry quality in grapevines under changing environments. Front. Plant Sci. 9, 897.
Trouvelot, S., Bonneau, L., Redecker, D., Van Tuinen, D., Adrian, M., Wipf, D., 2015. Arbuscular mycorrhiza symbiosis in viticulture: a review. Agron. Sustain. Dev. 35, 1449–1467.
USDA, 2005. National Soil Survey Handbook. Natural Resources Conservation Service (United States Department of Agriculture).
van de Voorde, T., Bezemer, T., Groenigen, J., Jeffery, S., Mommer, L., 2014. Soil biochar amendment in a nature restoration area: effects on plant productivity and community composition. Ecol. Appl. 24, 1167–1177.
Ventura, M., Alberti, G., Viger, M., Jenkins, J.R., Girardin, C., Baronti, S., Tonon, G., 2015. Biochar mineralization and priming effect on SOM decomposition in two European short rotation coppices. GCB Bioenergy 7, 1150–1160.
Visconti, A., Perrone, G., Cozzi, G., Solfrizzo, M., 2008. Managing ochratoxin a risk in the grape-wine food chain. Food Addit. Contam. 25, 193–202.
Wang, X., Song, D., Liang, G., Zhang, Q., Ai, C., Zhou, W., 2015. Maize biochar addition rate influences soil enzyme activity and microbial community composition in a fluvo-aquic soil. Appl. Soil Ecol. 96, 265–272.
Wang, G.F., Ma, Y., Chenia, H.Y., Govinden, R., Luo, J., Ren, G.D., 2020. Biochar mediated control of Phytophthora blight of pepper is closely related to the improvement of the rhizosphere fungal community. Front. Microbiol. 11, 1427.
Warnock, D.D., Lehmann, J., Kuyper, T.W., Rillig, M.C., 2007. Mycorrhizal responses to biochar in soil–concepts and mechanisms. Plant Soil 300, 9–20.
Xu, H.J., Wang, X.H., Li, H., Yao, H.Y., Su, J.Q., Zhu, Y.G., 2014. Biochar impacts soil microbial community composition and nitrogen cycling in an acidic soil planted with rape. Environ. Sci. Technol. 48, 9391–9399.
Yang, Y., Awasthi, M.K., Bao, H., Bie, J., Lei, S., Lv, J., 2020. Exploring the microbial mechanisms of organic matter transformation during pig manure composting amended with bean dregs and biochar. Bioresour. Technol. 313, 123647.
Yao, Q., Liu, J., Yu, Z., Li, Y., Jin, J., Liu, X., Wang, G., 2017. Three years of biochar amendment alters soil physiochemical properties and fungal community composition in a black soil of northeast China. Soil Biol. Biochem. 110, 56–67.
Yu, O.Y., Raichle, B., Sink, S., 2013. Impact of biochar on the water holding capacity of loamy sand soil. Int. J. Energy Environ. Eng. 4, 44.
Yuan, J.H., Xu, R.K., Zhang, H., 2010. The forms of alkalis in the biochar produced from crop residues at different temperatures. Bioresour. Technol. 102, 3488–3497.
Yurkov, A.M., 2018. Yeasts of the soil–obscure but precious. Yeast 35, 369–378.
Zornoza, R., Landi, L., Nannipieri, P., Renella, G., 2009. A protocol for the assay of arylesterase activity in soil. Soil Biol. Biochem. 41, 659–662.