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Biochar significantly reduced nutrient-induced positive priming in a subtropical forest soil

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Abstract

Application of biochar to soil may stabilize soil organic carbon (SOC), concomitantly increasing nutrient retention. However, the interactive effect of biochar and nutrients on SOC and the underlying microbial mechanisms remain poorly understood, particularly in intensively managed forests where decarbonization is substantial after converting from natural forests. This 80-day incubation experiment aimed to quantify native SOC mineralization as affected by biochar (B) and nutrients [nitrogen (N) or phosphorus (P)], linking to the chemical composition of SOC, soil microbial community composition, and enzyme activities within a subtropical Moso bamboo (*Phyllostachys edulis*) forest soil. Results presented that compared to the control (nil-nutrient), nutrients (N, P, and NP) significantly destabilized native SOC [positive priming effect (PE); 20–98% increase in SOC mineralization], whereas such destabilization effect was significantly reduced by biochar (6.0–19%). The positive PE by nutrient was due to the increases in O-alkyl C, microbial biomass C, available mineral N, soil pH, β-glucosidase, and invertase activities. Meanwhile, the greater PE by N than P could be attributed to (i) decreases in diversity of bacterial and fungal communities; and (ii) increases in the relative abundances of microbial taxa such as Bacilli, Planctomycetes, and Alphaproteobacteria. Importantly, biochar's stabilization effect was because biochar not only lowered NH_4^+-N and NO_3^--N and β-glucosidase activity, but also increased the activity of C-fixing enzyme (i.e., RubisCO) and polyphenol oxidase activity. Further, biochar significantly decreased soil O-alkyl C that possibly resulted in less labile SOC mineralization, but increased aromatic C resulting in lower fungal diversity. We conclude that the biochar significantly reduces the destabilization effects of nutrients on SOC, highlighting that the biochar application is an effective approach to mitigate soil CO_2 emissions within subtropical forest.

Keywords: Decarbonization; Fertilizer management; Soil enzymes; Organic carbon fraction; Microbial diversity; Ferralsol

Introduction

Subtropical forest ecosystems have a high CO_2 uptake capacity and play a vital role in the mitigation of climate change (Yu et al. 2014; Li et al. 2021c). Yet, converting natural to intensively managed forests (such as fertilizer application, understory weed removal, and plowing) has caused a significant decline of soil organic C (SOC) storage, *e.g.*, a loss of 0.64 Mg C ha^{-1} yr^{-1} in the 0–50 cm soil layer (Cai et al. 2018; Li et al. 2018a). Biochar, as an organic material, is produced from plant residues or animal wastes by pyrolysis under oxygen-limited conditions. Application of biochar to soil can withdraw CO_2 from the atmosphere and sequestrate C in soils, therefore acting as an effective measure for climate change mitigation (Lu et al. 2019; Duan et al. 2020; Lehmann et al. 2021). Furthermore, given its characteristics (a porous structure with large surface areas), biochar has other benefits such as improving nutrient retention and soil fertility in agricultural and agroforestry systems (Song et al. 2019; Hossain et al. 2020). Yet, less is known about the effectiveness of biochar on restoring SOC in intensively managed subtropical forests (Zhou et al. 2017).

Addition of substances such as nutrients or C can increase or decrease native SOC mineralization, referred to as the positive or negative "priming effect" (Kuzyakov et al. 2000; Xiao et al. 2021). Priming effects (PEs) on SOC mineralization are greatly affected by soil nutrient availability, due to the close association between C and nutrient cycling. However, the reports remain controversial – positive, negative, and neutral PEs (Nottingham et al. 2012; Lloyd et al. 2016). Generally, positive PEs by nutrients were reported to be associated with increased microbial stoichiometric decomposition (Fang et al. 2018). On the other hand, a meta-analysis reveals that N and N plus phosphorus (NP) mostly cause negative PEs, whereas P has minimal PEs (Feng and Zhu 2021). Generally, the potential mechanism for the negative PEs by N is that the N can eliminate microbial nutrient limitations as most of soils are N-limited, resulting in changes in the microbial community composition and activity (Liu et al. 2018; Hicks et al. 2019), towards less nutrient mining on nutrient-rich native soil organic matter (SOM) (Fang et al. 2018; Zhu et al. 2022b). Nevertheless, in intensively managed subtropical forest ecosystems, it lacks understanding and quantification of the PE on native SOC mineralization during fertilizer management.

Biochar can not only sequestrate C because of its recalcitrance, but also stabilize native SOC (Yu et al. 2020; Yang et al. 2021). For example, the negative PE by biochar was found in three soil types (a Ferralsol, an Anthrosol, and a loess-soil mixture) (Fang et al. 2015; Liang et al. 2010; Kuzyakov et al. 2009). A meta-analysis study shows that about half of the short-term studies (< 6 months) reported significantly negative PEs by biochar (–8.6%) (Wang et al. 2016). However, positive PE by biochar has also been widely documented (Fang et al. 2015; Luo et al. 2017; Liu et al. 2022). The uncertainty of direction and magnitude of PE by biochar are thought to link to both labile C availability and soil nutrient status affecting microbial community composition and activities (Luo et al. 2016; Qiu et al. 2022). A positive PE can be caused by (i) "co-metabolism" due to the increased labile C input that simultaneously increases microbial activities to decompose native SOM (Luo et al. 2013; Wild et al. 2014; Meier et al. 2017); and (ii) nutrient mining due to the increased microbial nutrient demand, and consequently, investigating enzyme activities involved in C dynamics and targeting the nutrient-rich SOM (Chen et al. 2018). To better manage SOC in intensively managed subtropical forests, it is vital to understand the interactive effects of biochar and fertilizer nutrients on magnitude and direction of PE and the underlying microbial mechanisms.

The decomposition of SOC is associated with the composition, diversity, and activity of the soil microbes involved in C-cycling (Duan et al. 2021). These types of microbes and corresponding enzyme activities are affected by nutrient availability, especially N, which eventually regulates direction of PE (Fan et al. 2020). For example, long-term fertilizer application was found to destabilize SOC, possibly due to the significant decreases in the abundance of C-fixing bacteria and ribulose-1,5-bisphosphate carboxylase-oxygenase (RubisCO) activity (Zhou et al. 2019), and the increases in the C-degrading microbial abundance (Tang et al. 2021). Biochar addition was found to build SOC by stimulating the C-fixing bacterial population and RubisCO activity while decreasing the C-degrading fungal population and β-glucosidase activities (Li et al. 2018b). Hence, biochar may function as counteracting negative effect of nutrients on SOC stabilization through altering soil microbial composition, diversity, and activity. Nevertheless, few investigations have been explored the mechanisms by which microbial taxa drive SOC mineralization and PEs when the nutrient combined with biochar application in intensively managed bamboo forests.

The present study aimed to explore the impacts of nutrient and biochar application on native SOC mineralization, linking to changes in soil chemical properties, microbial community composition, and enzyme activity within a subtropical Moso bamboo (*Phyllostachys edulis*) forest soil. To achieve this, C_4 maize straw biochar was applied to the C_3 forest (Moso bamboo) soil for differentiating sources of soil CO_2. We hypothesize that:

(1) nutrients addition will cause positive PE on native SOC mineralization as continuous SOC loss over time has been observed in heavily fertilized bamboo forests, and this positive PE is linked to the changes of some keystone bacterial and fungal taxa;

(2) biochar will lead to negative PE on native SOC mineralization, and reduce the nutrient-induced positive PE on native SOC, because biochar changes microbial community and diversity, and activities of enzymes such as RubisCO and glucosidase.

This work significantly improves our understanding of the microbial mechanisms that affect the mineralization of native SOC under the nutrient and biochar amendment, through two times of microbial community and diversity determination and analysis. In addition, this study considers the enzyme activities related to both C fixation and decomposition, providing a more comprehensive representation of the impact of changes in enzyme activities on the mineralization of native SOC.

Materials and methods

Soil and biochar

The soil was sampled from a subtropical Moso bamboo forest (0–20 cm soil layer) in Sankou Township, Zhejiang Province, China (30°14′N, 119°42′E). The climate at this site is typical subtropical monsoon climate, with the annual average temperature of 15.9°C and the mean annual precipitation of 1450 mm. The soil is classified as a Ferralsol (IUSS Working Group WRB 2015). The soil has pH of 4.72, SOC of 17.6 g kg^{-1}, total N of 1.85 g kg^{-1}, available P of 8.16 mg kg^{-1} and available K of 85.2 mg kg^{-1}. The detailed methods are described below. The texture of soil is loam with clay, sand, and silt contents of 274, 354, 372 g kg^{-1} soil, respectively.

To partition CO_2 sources, C4 maize (*Zea mays* L.) straw biochar ($\delta^{13}C$ = −15.6‰) was applied to the C3 forest (Moso bamboo) soil ($\delta^{13}C$: −26.3‰). Biochar was produced via slow pyrolysis at 500°C in the condition of oxygen absence. The biochar has a specific surface area of 8.73 m^2 g^{-1}, pH of 9.33, total C of 513.7 g kg^{-1}, and total N of 10.1 g kg^{-1}, respectively.

Incubation experiment

The incubation study included eight treatments: (1) soil without biochar and nutrients (CT); (2) soil + N (N); (3) soil + P (P); (4) soil + N and P (NP); (5) soil + biochar (B); (6) soil + biochar and N (BN); (7) soil + biochar and P (BP); (8) soil + biochar, N and P (BNP). There were three replicates for each treatment. To mimic the regional farmer practices (Song et al. 2019), N and P fertilizer were added at a dose of 0.2 g $CO(NH_2)_2$-N kg^{-1} soil and 0.05 g NaH_2PO_4-P kg^{-1} soil. Biochar (< 2 mm) was amended at a rate of 10 g C kg^{-1} soil.

The mixture of the fresh soil (equivalent to 300 g oven-dry weight) with nutrients and/or biochar was adjusted to 60% of soil water holding capacity (WHC). For each control or treatment, the soils were divided into two proportions – the 150 g soil for CO_2 measurement and another 150 g soil for the destructive sampling, and packed into two 500-mL glass containers with bulk density of 1.2 g cm^{-3}.

The emitted CO_2 from the aforementioned mixture soil was trapped in an alkali solution and measured by the titration method. Briefly, a soil container, and a jar containing 20 mL of 0.5 mol L^{-1} NaOH were placed in a 500 mL Mason jar. The Mason jars were sealed and incubated at 25°C for 80 days. The soil containers were weighed, and the water was added when changing NaOH traps to maintain soil moisture at 60% of the WHC. To account for the headspace CO_2 in the Mason jar, three blank 500 mL jars were set up in the manner as the treatments. The NaOH traps were replaced and sampled on days 2, 7, 14, 28, 42, 56, and 80 for CO_2 and $^{13}CO_2$ (‰) measurements. The emitted CO_2 from the soil during the incubation was qualified by trapping CO_2 with a NaOH solution (1 mol L^{-1}, 20 mL). Then, 10 mL of aforementioned NaOH solution was used for the determination of the amount of CO_2 absorbed by the NaOH solution through titrating with a HCl solution (0.1 mol L^{-1}).

Additionally, another 8 mL of aforementioned NaOH solution was precipitated as $BaCO_3$ by a $BaCl_2$ solution (1 mol L^{-1}, 8 mL). Then, the $BaCO_3$ was carefully washed and freeze-dried, for the $\delta^{13}C$ analysis by the MAT-253 isotope mass spectrometer (Thermo Electron, Bremen, Germany). During the experiment period, destructive sampling was carried out on days 14 and 56 of incubation to determine the physicochemical properties, SOC chemical composition, enzyme activity, and microbial community characteristics in soils.

Partitioning soil CO_2 sources

The soil CO_2 emission was partitioned based on the Eq. 1 (Balesdent and Balabane 1992):

$$CO_{2SOC} = \left(\frac{\delta_{total} - \delta_{biochar}}{\delta_{soil} - \delta_{biochar}}\right) \times CO_{2total} \qquad (1)$$

Where CO_{2SOC} is the CO_2 derived from native SOC in the biochar-amended soil; δ_{total} and δ_{soil} are the $\delta^{13}C$ value of CO_2 released from biochar-amended and the control soil (without biochar and nutrients), respectively; and $\delta_{biochar}$ is the $\delta^{13}C$ value of the biochar; CO_{2total} is total CO_2 released from biochar-treated soil.

The priming effect on native SOC was calculated by the Eq. 2:

$$PE = CO_{2,SOC} - CO_{2,control} \qquad (2)$$

Where PE is the priming effect on native SOC; CO_{2SOC} is the CO_2 derived from native SOC in the biochar and/or nutrients-amended soil; $CO_{2,control}$ is the CO_2 released from the control soil.

Soil and biochar physicochemical properties

Soil pH was determined based on a soil:water ratio of 1:2.5 (w/v). Available P was quantified in the HCl-NH_4F extracts. Soil NH_4^+-N and NO_3^--N were extracted with 2 mol L^{-1} KCl based on the approach of Keeney and Nelson (1982). Soil water-soluble organic carbon (WSOC) were extracted with deionized water (1:5 w/v) and determined using a TOC-TN analyzer (TOC-V_{CPH}; Shimadzu, Kyoto, Japan). Microbial biomass C (MBC) was analyzed following the method described in Song et al. (2019). A ^{13}C NMR spectrometer was used to quantify the chemical composition of SOC (Li et al. 2017). The spectra were divided into four regions: 0–46 ppm, 46–114 ppm, 114–164 ppm, and 164–220 ppm, which represented the alkyl C, O-alkyl C, aromatic C, and carbonyl C, respectively.

The specific surface areas of biochar were characterized by a surface analyzer. The C and N contents of biochar contents were determined using a elemental analyzer (Zhang et al. 2021b). The pH was measured based on a biochar:water ratio of 1:5 (w/v) (Yu et al. 2018).

Soil enzyme activities

Soil β-glucosidase activity was quantified following the method from Alef and Nannipieri (1995). Soil invertase activity was measured according to Frankenberger and Johanson (1983). Ribulose-1,5-bisphosphate carboxylase oxygenase (RubisCO) enzyme activity was quantified following Guo et al. (2015) after minor modifications. Soil polyphenol oxidase activity was analyzed according to German et al. (2011).

Microbial community analysis

Soil DNA was extracted from 0.5 g of fresh soils (n = 3) using the Fast DNA SPIN Kit (MP Biomedicals, Santa Ana, CA). Bacterial 16S ribosomal RNA (rRNA) and fungal internal transcribed spacer (ITS) genes were amplified with 515F/806R (Fromentin et al. 2022) and

ITS3F/ITS4R (Nilsson et al. 2019) primer sets respectively. TransStart Fastpfu DNA Polymerase (TransGen Biotech, Beijing China) was adopted for the PCR amplifications, following the manufacturer's protocol. The PCR cycle included initial denaturation for 5 min at 95 °C, and the following procedures of 35 (ITS)/ 27 (16S) cycles of 94°C (ITS)/ 95°C (16S) for 30 s, 55°C for 30 s, and 72 °C for 90 s (ITS)/ 45 s (16S).

The PCR products (bacterial 16S and fungal ITS gene amplicons) were used for amplicon metagenomic sequencing with Illumina MiSeq platform (Illumina, San Diego, CA, USA). Amplicon sequencing and subsequent bioinformatic data processing were explored by Shanghai Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China), according to their standard operating procedure. Briefly, raw sequencing data were demultiplexed and quality-filtered using QIIME (Caporaso et al. 2010), followed by OTU clustering (with 97% sequence similarity) with UPARSE and chimeric sequence removal with UCHIME (Edgar et al. 2011; Edgar 2013). Representative sequences for each OTU were screened for annotation, and taxonomy was assigned to OTUs using RDP Classifier (SILVA SSU ver 138 database for 16S and UNITE ver 8.2 database for ITS) based on a confidence threshold of 70%. From each sequencing dataset, an equal number of valid sequences (minimum library size) were rarefied, and the OTU tables were used for subsequent statistical analyses. The raw sequences were deposited within the NCBI Sequence Read Archive (SRA) with ID SRP354374.

Statistical analyses

Repeated measures analysis of variance (ANOVA) was adopted to explore the main effect of treatment and sampling time, and their interactive effects on SOC mineralization, cumulative PE, chemical properties, SOC chemical composition, enzyme activities, as well as the richness and diversity of microbial community. For these variables, interactions of the treatment with time were significant, therefore the treatment comparisons were examined at each time separately using the three-way (N, P, and biochar) ANOVA. Before the ANOVA, the normality of the distribution and homogeneity of variance of the data were assessed, and data were log-transformed if necessary. When the *F*-statistic showed significant, the means were compared based on the Tukey's test. In addition, correlations between cumulative native SOC mineralized and soil properties (such as physicochemical properties, enzyme activities, as well as richness, diversity, and relative abundances of soil microbial community) were examined using a linear regression equation based on Spearman correlation analysis. These statistical analyses were performed using IBM SPSS statistics 19.0.

The statistical analyses on microbial community were performed using R ver 4.1.2 (R Core Team 2021). Alpha diversity indices, i.e. number of observed OTUs, predicted OTUs (Chao1) and Shannon diversity indices, were calculated with *phyloseq* package (McMurdie and Holmes 2013). Hellinger transformation was applied to the OTU tables (Legendre and Gallagher 2001) and the differences in the microbial community composition between the sample groups were tested with permutational multivariate analysis of variance (PERMANOVA, adonis function in *vegan*) with Euclidean distance and visualized with principal component analysis (PCA) using *vegan* package (Oksanen et al. 2020). Heatmaps were generated by grouping the taxonomy at the Class level using STAMP software (Parks et al. 2014) and plotted using *ComplexHeatmap* package (Gu et al. 2016).

Results

Total C, native SOC, and biochar C mineralization

The cumulative total C mineralized over time was higher ($P < 0.01$) in the amended soils than the control soil (Fig. 1a; Table 3). On day 80, compared to the control, the cumulative

C mineralized was enhanced (P < 0.001) by biochar alone (9.5%), nutrients only (N, P, and NP; 70% on average), and their combined application (94% on average). In the biochar-amended soils, the cumulative total C mineralized was in the order of BNP > BN > BP > biochar (B); and in the nil-biochar treatments soils, it followed the order of NP > N > P > control (CT) (Fig. 1a; Table 3).

The cumulative native SOC mineralized over time was significantly increased by nutrient (N and NP) addition and their combined with biochar applications (Fig 1b; Table 3). On day 80, compared to the control, the cumulative SOC mineralized was decreased by 15% by biochar (on average across treatments). On day 80, in the nil-biochar amended soils, native SOC mineralization was increased by nutrient addition (cf. CT), i.e., 91%, 20%, 98% in the N, P, and NP treatments, respectively (Fig. 1b; Table 3). Yet, native SOC mineralization was 6.0–19% lower when biochar and nutrients (N, P, and NP) were co-applied, compared to N, P, and NP alone.

The cumulative biochar C mineralization over time was increased by nutrients (N, P, and NP) (Table 3; Fig 1c). On day 80, cumulative biochar C mineralized was 767 mg kg^{-1} soil in biochar alone treatment, which was increased by 72% by the co-applied N, 19% by the co-applied P, and 51% by the co-applied NP, respectively (Fig 1c; Table 3).

Priming effects on SOC mineralization

Nutrient (N, P, and NP) application caused the positive PEs over time (Fig. 2). The cumulative PEs by nutrients was higher when NP and N addition than P addition treatment (Fig. 2; Table 3). Conversely, biochar resulted in a negative PE on day 80 (–387 mg kg^{-1}). Importantly, the co-applied biochar significantly reduced the magnitude of positive PE induced by N, P, and NP addition (Fig. 2). That is, on day 80, the PEs were 23% lower in the BN than N-amended soil, 86% lower in the BP than P-amended soil, and 6% lower in the BNP than NP-amended soil.

Soil chemical properties and composition of SOC

Soil pH, WSOC, MBC, NH_4^+-N, NO_3^--N, and O-alkyl C were affected ($P < 0.001$) by the treatments, time, and treatment × time (Table S1). On day 14, soil pH was significantly increased (by 0.75–2.1 units) by the addition of nutrients (N, P, and NP) or biochar alone, compared to the control soil (pH 4.60). Soil pH was further significantly increased (by 0.34–0.82 units) when biochar and nutrients (N and P) were co-applied, compared to N and P alone. Such treatment effects remained on day 56, although there was a significant decrease trend (Table 1).

The addition of nutrients (i.e., N and NP) and biochar alone significantly increased (by 1–1.5 times) soil WSOC concentration, and there was an additive effect of nutrients (N, P, and NP) and biochar on soil WSOC (by 1.1–1.9 times) (Table 1). Likewise, soil MBC was considerably enhanced (by 22–75%) by the amendment of nutrients (N, P, and NP) and biochar alone, compared to the CT (250 mg kg^{-1}), and there was an additive effect of nutrients (N, P, and NP) and biochar on soil MBC (Table 1). The MBC decreased over time (Table 1), although the differences between biochar (B, BN, BP, and BNP) and nil-biochar (CT, N, P, and NP) treatments maintained.

In comparison with the control, soil O-alkyl C was enhanced by biochar (5%), nutrients (N, P, and NP; 20% on average), and their combined application (6% on average) ($P <$ 0.001). In contrast, soil aromatic C content was increased ($P < 0.001$) by biochar (23%), and the co-applied biochar and nutrients (N and P) (23% on average), but soil aromatic C content was decreased by nutrients application (N, P, and NP; 10% on average), and their combined application. Such treatment effects remained on day 56 (Table 2).

On day 14, compared to the control, soil NH_4^+-N and NO_3^--N concentrations were enhanced by addition of nutrients (i.e., N and NP); conversely, they were decreased by biochar irrespective of nutrients (N, P, and NP) application (Table 1). In addition, the NH_4^+-N concentration decreased over time (Table S1), but NO_3^--N concentration increased over time (Table S1). However, mineral N concentrations remained lower in biochar amended soils irrespective nutrient application (B, BN, BP, and BNP) than nil-biochar (CT, N, P, and NP) treatments.

AP concentration was influenced ($P < 0.001$) by the treatment and treatment × time (Table S1). On day 14, the concentration of available P increased (by 1.3–1.8 times) by the addition of nutrients (P, and NP) or biochar alone, compared to the CT (5.8 mg kg^{-1}). The concentration of available P in the soil was further increased by 40-60% when biochar and nutrients (N, P, and NP) were co-applied, compared to N, P, and NP alone. Such treatment effects remained at day 56 (Table 1).

Soil enzyme activity

The β-glucosidase, polyphenol oxidase and RubisCO activities were influenced ($P < 0.05$) by the treatment, time, and treatment × time (Table S1). On day 14, β-glucosidase activity increased (9% on average) by the addition of nutrients (N, P, and NP) alone, and that decreased (by 24%) by the addition biochar alone, compared to the CT. Furthermore, β-glucosidase activity was 17–21% lower when biochar and nutrients (N, P, and NP) were co-applied, compared to nutrients (N, P and NP) alone (Fig. 3). In addition, polyphenol oxidase activity decreased (15–38%) by the addition of nutrients (N, P, and NP) alone, and that increased (by 85%) by the addition of biochar alone, compared to the CT. However, polyphenol oxidase activity increased (by 12–30%) when biochar and nutrients (N, P, and NP) were co-applied, compared to nutrients alone (Fig. 3). Similar to polyphenol oxidase, RubisCO activity decreased (8–41%) by the addition of nutrients (N and NP) alone, and that increased (by 20%) by the addition of biochar alone, compared to the CT. RubisCO activity was 13–83% higher when biochar and nutrients (N, P and NP) were co-applied, compared to N, P, and NP alone. The aforementioned treatment effects observed at day 14 remained at day 56 (Fig. 3).

Soil bacterial and fungal community composition

PERMANOVA shows that at both days 14 and 56, the addition of N and biochar significantly influenced the bacterial and fungal composition ($P < 0.05$), while addition of P had no significant effect on the community composition ($P > 0.05$) (Fig. 5, Table 4). PCA (Fig. 5) demonstrated bacterial communities were influenced more by the addition of N than B, which can be seen from the separation of ±N communities along the PC1 axis (contributing for 30% and 42% of the total variation, at day 14 and 56 respectively) and ±B communities along the PC2 axis (contributing for 15% and 10% of the total variation at days 14 and 56, respectively) (Fig. 5a and b). On the other hand, fungal communities were influenced more by the addition B than N, as the PCA showed the opposite trend to bacteria, i.e. separation of ±B communities along the PC1 axis and ±N communities along the PC2 axis) (Fig. 5c and d).

In line with the above results, addition of N (N, BN, NP, and BNP) decreased bacterial diversity (Shannon diversity index) compared to the control (Fig. 4). This reduction in the bacterial diversity was significant for N, BN, BP, and BNP treatments at day 14 and day 56 ($P < 0.05$) (Fig. 4; Table S2). Meanwhile, biochar treatments (B, BN, BP, and BNP) strongly reduced the fungal population diversity (Shannon diversity index) (cf. CT), and this effect was considerable for B, BN, BP, and BNP treatments at day 14 and day 56 ($P < 0.05$) (Fig. 4; Table S4). A similar trend was also observed for the other diversity indices, i.e. observed OTUs and the Chao1 index (Fig. S1; Table S2 and S3).

Several microbial taxa were affected by the nutrients (especially N) or biochar treatments. For bacterial population, addition of N (N, BN, NP, and BNP treatments) resulted in the reduction of classes Acidobacteriae, Vicinamibacteria (day 56), Acidimicrobiia, Thermoleophilia, AD3 (day 56), Ktedonobacteria (day 56), Gemmatimonadetes (day 56), Phycisphaerae (day 56) and Gammaproteobacteria (day 56), and the enrichment of classes Bacteroidia, Bacilli, S0134 terrestrial group (day 56) and Alphaproteobacteria (day 56) (Fig. 6a). Furthermore, addition of biochar (B, BN, BP, and BNP) enriched the population of Actinobacteria over time, compared to other treatments (Fig. 6a).

The fungal community composition was strongly influenced by biochar (B, BN, BP, and BNP treatments), which is in line with the PCA result (Fig. 5c and d). Reduction of classes Dothideomycetes, Leotiomycetes, unclassified Ascomycota and Mucoromycotina Incertae sedis, and enrichment of Eurotiomycetes and Sordariomycetes were observed in the biochar treatments (B, BN, BP, and BNP) (Fig. 6b).

Effects of soil properties, microbial community compositions, and soil cumulative PE on native SOC mineralization

Spearman's correlations show that the cumulative PEs across treatments (at days 14 and 56) increased (P < 0.05) with increasing pH, MBC, WSOC, NH_4^+-N, NO_3^--N, and O-alkyl C, β-glucosidase and invertase activities (Table 5). Conversely, PEs decreased (P < 0.05) with increasing bacterial Shannon index and Chao1 index, fungal Chao1 index, and polyphenol oxidase and RubisCO activities (Table 5).

In addition, CCA shows that bacterial and fungal compositions were considerably different among three clusters − nutrients (N, P, and NP) alone, biochar alone and their combination (Fig. 7). The composition of bacterial communities was related to (i) the pH, MBC, and O-alkyl ($P < 0.05$) on day 14, and (ii) MBC and O-alkyl ($P < 0.05$) on day 56 (Fig. 7a and c). In addition, the composition of fungal communities was related to (i) the pH, and O-alkyl ($P < 0.05$) on day 14, and (ii) carbonyl C and O-alkyl ($P < 0.05$) (Fig. 7b and d).

Moreover, on days 14 and 56, the cumulative PE on native SOC mineralization were positively related to ($P < 0.05$) the relative abundances of Bacilli, Leotiomycetes, Geminibasidiomycetes, and Mucoromycotina_cls_Incertae_sedis; but it negatively related to ($P < 0.01$) the relative abundances of Acidobacteriae, Acidimicrobiia, Thermoleophilia, Gammaproteobacteria, and Mortierellomycetes. In addition, on day 56, the cumulative PE on native SOC mineralization enhanced ($P < 0.05$) with the increasing relative abundances of Planctomycetes and Alphaproteobacteria; but it reduced ($P < 0.01$) with increasing the relative abundances of Ktedonobacteria, Dothideomycetes, and Tremellomycetes (Table 6).

Discussion

Nutrients increased decarbonization and the underlying mechanisms

Our study provides direct evidence of the role of fertilizer application on SOC destabilization in an intensively managed forest Ferralsol, and the associated microbial mechanisms. Our results show that addition of N, P, and NP all caused a positive PE on SOC mineralization within a subtropical forest soil (Fig. 2), which aligns with previous findings in Oxisol soil and woodland topsoil (Zhang et al. 2021a; Zheng et al. 2022). Importantly, compared to N addition, P addition resulted in a lower positive PE (Figs. 1 and 2), likely due to the decreased microbial diversity and changes in some keystone microbial taxa.

Several potential mechanisms could be involved in the increased native SOC mineralization (positive PE) by nutrients. Firstly, the addition of nutrients increased O-alkyl C (Table 2) and WSOC (Table 1) resulting in increased PE (Table 5). That is, nutrients accelerated the decomposition of recalcitrant SOC and the transformation of recalcitrant SOC pool to labile SOC pool (Luo et al. 2020; Li et al. 2021b), which could have increased soil carbon availability to microbes. Secondly, PEs were directly affected by NH_4^+-N, NO_3^--N (Table 5). The addition of N enhanced the pool size of NH_4^+-N and NO_3^--N that promoted microbial growth as evidenced by the increased MBC (Table 1), causing a positive PE, corroborating with the previous observations (Fang et al. 2020; Li et al. 2021a). Thirdly, nutrients amendment increased enzyme activities regarding C degradation (Fan et al. 2020) (i.e., β-glucosidase; Fig. 3) but decreased enzyme activities regarding C fixing (Lu et al. 2021) (RubisCO; Fig. 3), thereby increasing the native SOC decomposition (Table 5). Fourthly, PE was related to soil pH as suggested by Spearman's correlation analyses (Table 5). This is because the increased soil pH by N (urea) (Table 1) could have increased the solubility of previously locked soil P (Saentho et al. 2022), resulting in greater "microbial stoichiometric decomposition" (Hessen et al. 2004) and positive PEs (Wild et al. 2014; Li et al. 2021a). Moreover, negative associations among different species of bacteria increased with nutrients (N, P, and NP alone) (Figs. S1 and S2; Table S2), which may have increased microbial resource competition, thereby resulting in the positive PE (Wei et al. 2020; Yan et al. 2021).

More importantly, N rather than P application caused more SOC destabilization (Fig. 1). This can be partly explained by the factor that N decreased more bacterial and fungal diversity (Fig. 4; Table 5). In addition, N rather than P application significantly reduced the diversity (Fig. 4), resulting in greater responses in PEs. This is supported by the previous findings that the N addition significantly changed the microbial diversity and composition, therefore increasing SOC decomposition (positive PEs) (Luo et al. 2019). Moreover, the application of N (i.e., N and NP treatments) increased the relative abundances of Bacilli, directly relating to increased PE, i.e., days 14 and 56 (Fig. 6; Table 6). Similarly, Yin et al.

(2019) reported that relative abundance of Bacillales were closely related to the metabolism of carbohydrates and amino acids. Both N and NP treatments also increased Planctomycetes, Alphaproteobacteria and Leotiomycetes relative abundances, related to positive PE (i.e., on days 14 and 56; Table 6), which are in agreement with previous findings (Lipson et al. 2014; Ivanova et al. 2020), and N treatments (i.e., N and NP) decreased the relative abundances of Acidobacteriae, leading to increased PE, i.e., days 14 and 56 (Fig. 6). Likewise, Zhu et al. (2019) found that the Acidobacteriae was critical bacterial populations controlling CO_2 emissions. In addition, both N and NP treatments decreased the relative abundances of Acidimicrobiia and Thermoleophilia, resulting in increased PE, i.e., days 14 and 56 (Fig. 6, Table 6). This is because the relative abundances Acidimicrobiia and Thermoleophilia can affect the activities of enzymes related to CO_2 fixation (Bay et al. 2021). Furthermore, nutrients (N and NP) addition decreased the relative abundances Dothideomycetes and Mortierellomycetes (Fig. 6), which are related to metabolism of Carbohydrate C (Fan et al. 2021; Zhu et al. 2022a), resulting in increased PE (i.e., on day14 and 56; Table 6).

Compared to N addition, P addition resulted in a lower positive PE (Fig. 2). This can be explained by three mechanisms. First, although P amendment increased the soil MBC, lower MBC under the P addition was observed relative to N addition, which led to a lower positive PE on native SOC mineralization (Zhang et al. 2021b). Second, P addition increased soil P availability (Table 1), but no significant relationship between soil P availability and PE was observed (Table 5). Third, P addition did not significantly change the microbial diversity and richness (Shannon and Chao1) (Figs. 4 and S1; Tables S2 and S3), which might be associated with a lower positive PE than N addition (Li et al. 2019). The aforementioned findings indicate that the Ferralsol of the present study may be more N-limited rather than P-limited for microbial proliferation.

Biochar reduced decarbonization regardless of soil nutrient status

Our study reveals that addition of biochar caused a negative PE on SOC mineralization within a subtropical forest Ferralsol (Fig. 2). Likewise, a meta-analysis study shows that the significant negative PE was observed with the treatments of crop-derived biochar (–20.3%) (Wang et al. 2016), and this also aligned with the finding of Weng et al. (2022) that the increase in SOC stocks by biochar addition was due to the decreased net cumulative SOC mineralization in subtropical pastures. In addition, our study demonstrates that compared to nutrients addition alone (N, P, and NP), co-application of nutrients and biochar (BN, BP, and BNP) considerably decreased native SOC mineralization (by 6–19%). This finding is comparable with the observation by Lu et al. (2014) that compared to N fertilizer alone, the co-application of N and biochar markedly reduced the CO_2 emission from the native SOC by 74% in a sandy loam soil. Similarly, Oladele et al. (2021) also revealed that compared to N fertilizer alone, N fertilizer plus biochar significantly reduced the CO_2 emission by 12–40% in an agricultural cropland. Taken together, biochar has shown to be an effective approach to stabilize SOC in heavily fertilized forest.

Biochar reduced native SOC mineralization regardless soil nutrient status, which could be due to the following reasons. Firstly, the biochar having great surface area absorbed soil NH_4^+-N and NO_3^--N ions to reduce N availability in the BN, BP, and BNP treatments (Table 1), consequently leading to a negative PE on native SOC mineralization (Wild et al. 2014; Zhu et al. 2018). Secondly, biochar addition enhanced aromatic C and reduced O-alkyl C (Table 2). The reduced labile C availability in microbial hotspots consequently caused a negative PE on the native SOC mineralization (Li et al. 2018b). Thirdly, biochar addition decreased the activities of enzymes regarding C degradation (β-glucosidase; Fig. 3), but enhanced the activities of enzymes regarding C fixing (such as polyphenol oxidase and

RubisCO; Fig. 3) (Li et al. 2018b; Zhou et al. 2021). This could have thereby caused a negative PE on SOC mineralization. In addition, our results show that despite increased soil P availability (Table 1), biochar addition increase SOC stabilization (Fig. 2), This further confirms that this Ferralsol was likely more N-limited, rather than P-limited for microbial proliferation.

Biochar shifted microbial community composition linking to SOC stabilization

The negative PE by biochar was not only linked to the changes in the bacterial diversity and the relative abundances of some bacterial taxa (Figs. 4), but was more driven by the fungal community (Fig. 5 and 6). More interestingly, the bacterial and fungal richness and diversity markedly decreased with biochar addition alone (Table. S3), which aligns with the previous findings that biochar addition in a loamy clay soil induced the rhizosphere communities towards having lower richness and evenness, most likely due to enhancing the recalcitrant organic C content (Fu et al. 2022), which suggested that the biochar addition decreased the resource competition of soil fungal community, and consequently its decomposition ability of SOC (Bamminger et al. 2014). Biochar changed the relative abundances of several bacterial and fungal taxa (Fig. 6), and such changes could have resulted in the negative PE. Affected taxa such as Acidimicrobiia, Thermoleophilia, Acidobacteriae, Ktedonobacteria, and Tremellomycetes relative abundances decreased native SOC mineralization, and vice-versa for Bacilli, Planctomycetes, Alphaproteobacteria, Geminibasidiomycetes, and Mucoromycotina_cls_Incertae_sedis relative abundances (Table 6). Such correlations indicate that these microbial taxa are critical for regulating soil PEs, likely through affecting enzyme activities (Liu et al. 2020). Biochar alone considerably enhanced the relative abundance of Acidimicrobiia, which are the *K*-strategist with the potential to degrade recalcitrant compounds (Liu et al. 2020). Generally, the relative abundance of

Acidimicrobiia increased as the soil aromatic C content increased (Liu et al. 2020).

More importantly, compared to nutrient addition alone, the co-application of nutrients and biochar considerably altered the relative abundances of some keystone microbial taxa, resulting in a lower positive PE on native SOC mineralization. In our study, co-application of nutrients and biochar markedly reduced the relative abundance of Alphaproteobacteria (r-strategists) (on day 56) that are related to SOC mineralization (Bian et al. 2022), compared to nutrient addition alone (Fig. 6). In addition, we found that the relative abundances of Leotiomycetes and Geminibasidiomycetes were lower in the co-application of nutrients and biochar (BN and BNP) than nutrient addition alone (N and NP) (Fig. 6). Likewise, observations of Lipson et al. (2014) and Zhu et al. (2022) demonstrated that Leotiomycetes and Geminibasidiomycetes were the dominant class of resident fungal populations that affect soil C turnover and SOC priming.

Implications for building SOC in intensively managed forests

Intensive management measures of the subtropical forest have resulted in a significant decline in SOC stock (Li et al. 2018a). To achieve zero emissions target, it is important to improve the C sink function of the Moso bamboo forest ecosystem. Generally, the addition of nutrients (N, P and NP) enhances plant growth and consequently increases plant C stock (Carrara et al. 2018; Garret et al. 2021), but this positive effect was weakened by the nutrient-induced positive PE on SOC mineralization (Fig. 2). Our results suggest that the application of biochar can be an effective approach to reduce the PE on SOC mineralization through altering soil nutrient availability as well as microbial composition and activity in subtropical forest ecosystems. Therefore, co-application of nutrients (N, P, and NP) and biochar should be recommended as soil management practice to enhance the growth of forest plants while reducing the SOC loss induced by nutrient addition in subtropical forest ecosystems. In addition, understanding the microbial mechanisms of responses of SOC to nutrient and biochar management will help future adaption of such practices to reduce CO_2 emissions.

Conclusions

In this study, we explored the linkages between SOC mineralization and changes in microbial activity and community composition, responding to nutrient (N and P) management in a subtropical forest soil. Our study gives new insights into a promising solution – through biochar application – to restore and retain SOC in cash-crop forests that are often having lower SOC compared to natural forest (before land-use conversion). SOC was significantly destabilized by nutrient additions (N, P, and their combination), i.e., 4.70–7.74% SOC loss, compared to the control (3.90% SOC loss). Such destabilization was linked to the increases in O-alky C, N availability, the relative abundances of Bacilli, Planctomycetes, Alphaproteobacteria, Leotiomycetes and Geminibasidiomycetes, and decreases in Acidobacteriae and Acidimicrobiia, Thermoleophilia, Ktedonobacteria, Dothideomycetes and Mortierellomycetes. Importantly, biochar stabilized SOC due to the decreases in soil O-alky C, N availability and β-glucosidase activity, as well as the increases in aromatic C, polyphenol oxidase and RubisCO. Furthermore, the SOC stabilization by biochar in the nutrient-rich soils were also attributed to the decreased the relative abundances of Alphaproteobacteria, Leotiomycetes and Geminibasidiomycetes. Our study highlights that biochar application can be an effective and economic approach to reduce soil CO_2 emissions within subtropical cash-crop forests achieving net-zero carbon emissions in agriculture. In addition, feedstock types and pyrolysis conditions affect the physicochemical properties of biochar. Thus, the impact of nutrient combined with the different biochar types on native soil-derived CO_2 in bamboo forest ecosystems needs to be addressed in the future.

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Declarations

Competing interests The authors declare no competing interests.

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Supporting Information

Table S1 Statistical significance (*P* values) of the repeated measures ANOVA on the dependent variables tested.

Table S2 Effects of nutrients and biochar application on the richness and diversity of the bacterial community in a Ferralsol from Moso bamboo forest. The numbers indicate mean ± standard deviations (n = 3).

Table S3 Effects of nutrients and biochar application on the richness and diversity of the fungal community in a Ferralsol from Moso bamboo forest. The numbers indicate mean ± standard deviations (n = 3).

Fig. S1 Biochar and the nutrient treatments affected the microbial diversity in the Moso bamboo forest soil microbiome. Box plots show the (a) observed number of OTUs and (b) Chao 1 indices of bacterial and fungal communities in the soils incubated for 14 and 56 days, with the respective biochar and nutrient treatments (n = 3).

Table 1 Effects of nutrients and biochar on soil physicochemical properties after 14 and 56 days of incubation in a Ferralsol from Moso bamboo forest. The mean values after "±" are the standard deviation.

Treatment	Day 14						Day 56					
	pH	WSOC[†] ($mg\ kg^{-1}$)	MBC ($mg\ kg^{-1}$)	NH_4^+-N ($mg\ kg^{-1}$)	NO_3^--N ($mg\ kg^{-1}$)	AP ($mg\ kg^{-1}$)	pH	WSOC ($mg\ kg^{-1}$)	MBC ($mg\ kg^{-1}$)	NH_4^+-N ($mg\ kg^{-1}$)	NO_3^--N ($mg\ kg^{-1}$)	AP ($mg\ kg^{-1}$)
CT[‡]	4.60 ± 0.2	54.5 ± 3.7	249.9 ± 8.8	80.2 ± 4.4	19.9 ± 1.4	5.8 ± 0.5	4.43 ± 0.1	23.9 ± 1.6	136.0 ± 5.0	35.5 ± 2.1	42.3 ± 1.7	4.7 ± 0.1
N	6.69 ± 0.2	137.8 ± 7.3	435.8 ± 25.6	115.2 ± 3.7	25.6 ± 3.6	5.9 ± 0.6	6.65 ± 0.3	55.5 ± 4.0	234.5 ± 6.4	50.3 ± 3.4	78.8 ± 2.5	4.7 ± 0.2
P	5.32 ± 0.1	58.3 ± 3.1	305.0 ± 17.0	63.2 ± 2.9	17.7 ± 1.3	13.1 ± 0.8	4.95 ± 0.2	24.1 ± 1.5	212.3 ± 6.1	26.2 ± 0.9	38.0 ± 1.9	18.6 ± 0.5
NP	6.81 ± 0.2	134.4 ± 3.5	447.7 ± 20.4	112.7 ± 2.5	22.5 ± 2.1	16.6 ± 1.5	6.76 ± 0.2	57.6 ± 3.3	241.5 ± 5.9	50.3 ± 1.8	67.0 ± 5.8	17.7 ± 0.9
B	6.25 ± 0.2	111.1 ± 6.4	305.2 ± 10.2	45.9 ± 1.8	15.3 ± 0.9	9.5 ± 0.4	5.21 ± 0.1	44.9 ± 2.1	362.4 ± 15.4	27.0 ± 1.5	33.1 ± 0.8	7.8 ± 0.4
BN	7.03 ± 0.2	154.0 ± 7.5	491.7 ± 15.7	51.6 ± 2.3	18.5 ± 1.5	9.3 ± 0.6	6.64 ± 0.2	64.9 ± 2.8	391.9 ± 14.4	34.2 ± 1.8	45.2 ± 1.1	7.7 ± 0.2
BP	6.14 ± 0.1	112.5 ± 5.6	353.1 ± 17.3	42.5 ± 2.3	11.7 ± 0.7	19.6 ± 1.2	6.10 ± 0.2	47.6 ± 2.1	351.2 ± 21.5	21.9 ± 1.4	32.3 ± 1.3	21.3 ± 1.0
BNP	6.97 ± 0.2	157.1 ± 8.4	529.1 ± 28.2	50.6 ± 2.2	17.5 ± 1.1	23.0 ± 1.1	6.73 ± 0.2	65.8 ± 3.7	417.5 ± 13.3	32.0 ± 1.3	37.5 ± 1.0	22.4 ± 0.6
Statistical significance												
N	***[§]	***	***	***	***	***	***	***	***	***	***	NS
P	*	NS	***	***	***	***	***	NS	***	***	***	***
B	***	***	***	***	***	***	***	***	***	***	***	***
N × P	NS[‡]	NS	NS	**	NS	***	**	NS	NS	**	**	NS
N × B	***	***	NS	***	NS	NS	***	***	NS	***	***	NS
P × B	**	NS	NS	**	NS	**	NS	NS	**	NS	NS	NS
N × P × B	*	NS	NS	*	NS	NS	NS	NS	***	NS	NS	*

[†]WSOC = water-soluble organic C, MBC = microbial biomass C, AP = available P

[‡] CT = control soil without biochar and nutrient, N = N nutrient treatment, P = P nutrient treatment, NP = N + P treatment, B = biochar treatment, BN = biochar + N nutrient treatment, BP = biochar + P nutrient treatment, BNP = biochar + N + P treatment

[§] Note that "NS", *, ** and *** represent non-significant, $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.

Table 2 Effects of nutrients and biochar on SOC chemical composition after 14 and 56 days of incubation in a Ferralsol from Moso bamboo forest. The mean values after "±" are the standard deviation.

	Day 14				**Day 56**			
Treatment	**alkyl C** (%)	**O-alkyl C** (%)	**Aromatic C** (%)	**Carbonyl C** (%)	**alkyl C** (%)	**O-alkyl C** (%)	**Aromatic C** (%)	**Carbonyl C** (%)
CT†	32.3 ± 1.8	20.0 ± 1.5	22.8 ± 0.5	24.9 ± 1.0	31.4 ± 0.9	17.3 ± 0.9	17.0 ± 1.3	34.3 ± 1.3
N	28.1 ± 1.7	40.1 ± 1.7	14.1 ± 0.6	17.7 ± 1.1	27.2 ± 1.3	41.9 ± 1.1	14.1 ± 0.5	16.8 ± 1.3
P	30.6 ± 0.9	38.4 ± 1.6	11.8 ± 1.1	19.3 ± 2.1	27.2 ± 0.6	29.3 ± 0.8	14.2 ± 0.5	29.4 ± 1.7
NP	32.0 ± 1.1	43.5 ± 1.8	14.0 ± 0.4	10.5 ± 0.5	28.2 ± 1.5	41.3 ± 1.1	14.1 ± 0.8	16.3 ± 0.9
B	17.2 ± 0.5	25.3 ± 0.9	45.6 ± 2.3	11.9 ± 1.4	11.5 ± 0.7	19.0 ± 1.5	57.3 ± 2.1	12.1 ± 1.7
BN	17.3 ± 1.4	25.2 ± 1.3	46.8 ± 1.8	10.6 ± 0.7	11.0 ± 2.1	26.0 ± 1.9	48.3 ± 2.0	14.7 ± 1.4
BP	15.3 ± 1.1	23.8 ± 0.7	46.7 ± 2.3	14.2 ± 1.7	13.5 ± 1.4	25.6 ± 1.2	47.8 ± 2.0	13.1 ± 0.5
BNP	16.7 ± 0.2	29.8 ± 1.1	43.0 ± 1.1	10.6 ± 0.9	16.4 ± 2.8	26.6 ± 1.6	47.2 ± 1.9	9.90 ± 1.1
Statistical significance								
N	NS‡	***	**	***	NS	***	***	***
P	NS	***	***	***	NS	***	***	***
B	***	***	***	***	***	***	***	***
N × P	**	**	*	NS	**	***	***	NS
N × B	*	***	NS	***	*	***	*	***
P × B	*	***	**	***	**	NS	**	NS
N × P × B	*	***	***	NS	NS	**	*	***

† CT = control soil without biochar and nutrient, N = N nutrient treatment, P = P nutrient treatment, NP = N + P treatment, B = biochar treatment, BN = biochar + N nutrient treatment, BP = biochar + P nutrient treatment, BNP = biochar + N + P treatment

‡ Note that "NS", *, ** and *** represent non-significant, $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.

Table 3 Three-way ANOVA of the effects of nutrient type, biochar application and their interaction on cumulative CO_2 emission, cumulative SOC-derived CO_2 emission and cumulative priming effect (PE), two-way ANOVA of the effects of nutrient type and their interaction on cumulative biochar-derived CO_2 emission.

Source of variation	Total CO_2 emission[†] (mg CO_2 kg^{-1} soil)	CO_2-C_{soil} emission (mg CO_2 kg^{-1} soil)	Priming effect (mg CO_2 kg^{-1} soil)	CO_2-$C_{biochar}$ emission (mg CO_2 kg^{-1} soil)
N[‡]	***[§]	***	***	***
P	***	***	***	NS
B	***	**	***	-
N × P	***	NS	***	*
N × B	***	NS	**	-
P × B	**	***	***	-
N × P × B	NS	NS	***	-

[†]Total CO_2 emission, CO_2-C_{soil} emission, Priming effect and CO_2-$C_{biochar}$ emission represent the cumulative CO_2 emission, cumulative SOC-derived CO_2 emission, cumulative priming effect and cumulative biochar-derived CO_2 emission on day 80, respectively.

[‡] N = N nutrient treatment, P = P nutrient treatment, NP = N + P treatment, B = biochar treatment, BN = biochar + N nutrient treatment, BP = biochar + P nutrient treatment, BNP = biochar + N + P treatment

[§]Note that "NS", *, ** and *** represent non-significant, $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.

Table 4 Effects of nutrients and biochar on bacterial and fungal community composition tested by PERMANOVA based on the weighted UniFrac distance matrices

		Day 14				Day 56			
		Mean squares	F	R^2	P	Mean squares	F	R^2	P
Bacteria	Biochar	0.676	3.981	0.153	0.002	0.557	2.336	0.096	0.036
	Phosphorus	0.194	1.013	0.044	0.390	0.217	0.855	0.037	0.444
	Nitrogen	1.204	8.268	0.273	0.001	2.366	15.15	0.408	0.001
Fungi	Biochar	3.181	36.25	0.622	0.001	2.475	12.53	0.363	0.001
	Phosphorus	0.067	0.291	0.013	0.912	0.201	0.669	0.030	0.616
	Nitrogen	0.532	2.558	0.104	0.042	1.624	6.872	0.238	0.001

Table 5 Spearman's correlations (ρ) between cumulative PE on native SOC mineralization and soil properties in a Ferralsol from Moso bamboo forest (* and ** indicate $P < 0.05$ and $P < 0.01$, respectively).

	Cumulative PE	
	day 14	day 56
pH	0.724**	0.832**
Microbial biomass C (MBC)	0.846**	–0.115
Water-soluble organic C (WSOC)	0.682**	0.666**
Ammonium N (NH_4^+-N)	0.659**	0.791**
Nitrate N (NO_3^--N)	0.755**	0.704**
Available P (AP)	0.018	–0.028
Alkyl C	0.366	0.446*
O-alkyl C	0.533*	0.715**
Aromatic C	–0.345	–0.453*
Carbonyl C	–0.301	–0.137
β-glucosidase	0.502*	0.658**
Invertase	0.780**	0.921**
Polyphenol oxidase	–0.685**	–0.702**
RubisCO	–0.466*	–0.499*
Bacterial observed OTU	-0.008	0.260
Bacterial Shannon index	-0.526*	-0.719**
Bacterial Chao1 index	-0.411	-0.742**
Fungal observed OTU	0.172	0.058
Fungal Shannon index	0.378	-0.076
Fungal Chao1 index	-0.403	-0.588**

Table 6 Spearman's correlations (ρ) between dominant class of microbial taxa and cumulative priming effect (PE) on native SOC mineralization in the Ferralsol from Moso bamboo forest (* and ** indicate $P < 0.05$ and $P < 0.01$, respectively)

	Cumulative priming effect (PE) on native SOC mineralization				
Bacteria	Day 14	Day 56	Fungi	Day 14	Day 56
Acidobacteriae	-0.670**	-0.847**	Dothideomycetes	-0.193	-0.466*
Acidimicrobiia	-0.566**	-0.766**	Eurotiomycetes	0.020	0.001
Actinobacteria	-0.132	-0.246	Leotiomycetes	0.490**	0.580**
Thermoleophilia	-0.581**	-0.735**	Sordariomycetes	-0.095	0.187
Ktedonobacteria	-0.374	-0.693**	Agaricomycetes	-0.176	-0.196
Bacilli	0.828**	0.750**	Geminibasidiomycetes	0.566**	0.810**
Planctomycetes	0.210	0.417*	Tremellomycetes	-0.068	-0.456*
Alphaproteobacteria	0.230	0.697**	Mortierellomycetes	-0.523**	-0.557**
Gammaproteobacteria	-0.481*	-0.765**	Mucoromycotina_cls_Incertae_sedis	0.555**	0.521**
Verrucomicrobiae	-0.141	-0.207	Umbelopsidomycetes	0.348	-0.307

Figure captions

Fig. 1 Cumulative CO_2 emission within a Moso bamboo forest Ferralsol under the amendments of nutrients and biochar. CT = control soil without biochar and nutrient, N = N nutrient treatment, P = P nutrient treatment, NP = N and P treatment, B = biochar treatment, BN = biochar + N nutrient treatment, BP = biochar + P nutrient treatment, BNP = biochar + N and P treatment.

Fig. 2 Temporal dynamics of cumulative priming effect (PE) on mineralization of native soil organic C (SOC). CT = control soil without biochar and nutrient, N = N nutrient treatment, P = P nutrient treatment, NP = N and P treatment, B = biochar treatment, BN = biochar + N nutrient treatment, BP = biochar + P nutrient treatment, BNP = biochar + N and P treatment.

Fig. 3 Effect of nutrients and biochar application on the activities of β-glucosidase (a), invertase (b), polyphenol oxidase (c) and RubisCO (d) at day 14 and day 56 of incubation in a Ferralsol from a Moso bamboo forest. Note that *, ** and *** represent $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Fig. 4 Biochar and the nutrient treatments affected the microbial diversity in the Moso bamboo forest soil microbiome. Box plots show the Shannon diversity indices of bacterial and fungal communities within the soils incubated for 14 and 56 days, with the respective biochar and nutrient treatments ($n = 3$).

Fig. 5 Effect of biochar (B) and nitrogen (N) treatments on the Moso bamboo forest soil microbiome. PCA plots show the bacterial (a, b) and fungal (c, d) community composition in the soil incubated for 14 (a, c) and 56 (b, d) days. PERMANOVA showed the B and N treatments significantly affected the microbial community composition ($P < 0.05$), but not the P treatment (Table 4). Therefore, in these PCA plots the samples were grouped into four treatments: "-B, -N" (CT and P), "+B, -N" (B and BP), "-B, +N" (N and NP) and "+B, +N" (BN and BNP) ($n = 6$).

Fig. 6 Biochar and nutrient treatments altered the microbial composition in the Moso bamboo forest soil. Heatmaps depicting the relative abundances of (a) bacterial and (b) fungal classes in each treatment (CT, B, N, BN, P, BP, NP, and BNP). Minor classes (< 0.2% for bacteria and < 0.1% for fungi) were compiled into "Others". The color scale represents the square root of relative abundance.

Fig. 7 Canonical correspondence analysis (CCA) of soil bacterial (a, c) and fungal (b, d) community composition with soil environmental parameters within a Moso bamboo forest Ferralsol under the amendments of nutrients and biochar at days 14 (a, b) and 56 (c, d).

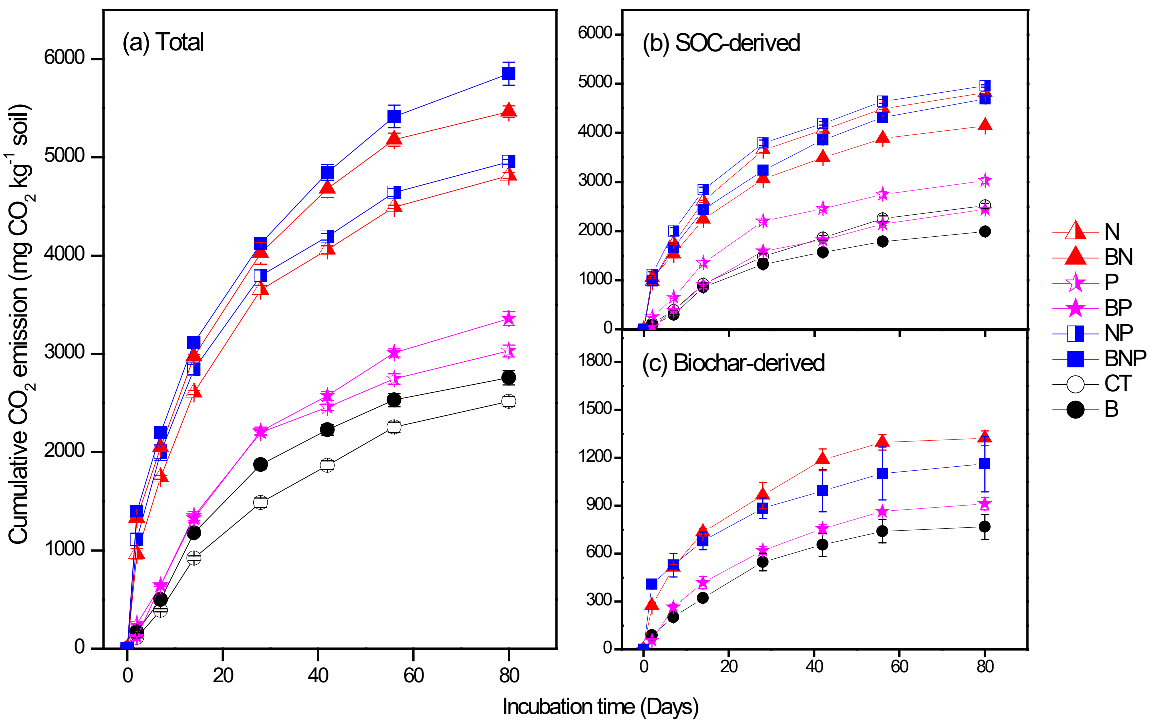

(a) Total
(b) SOC-derived
(c) Biochar-derived
Cumulative CO_2 emission (mg CO_2 kg^{-1} soil)
Incubation time (Days)
N
BN
P
BP
NP
BNP
CT
B

Fig. 1

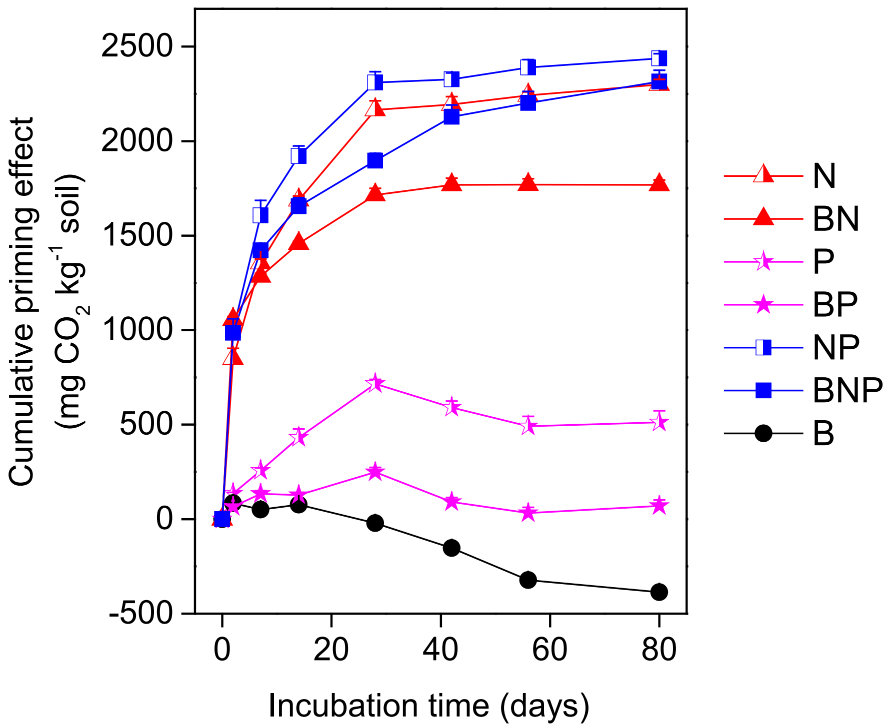
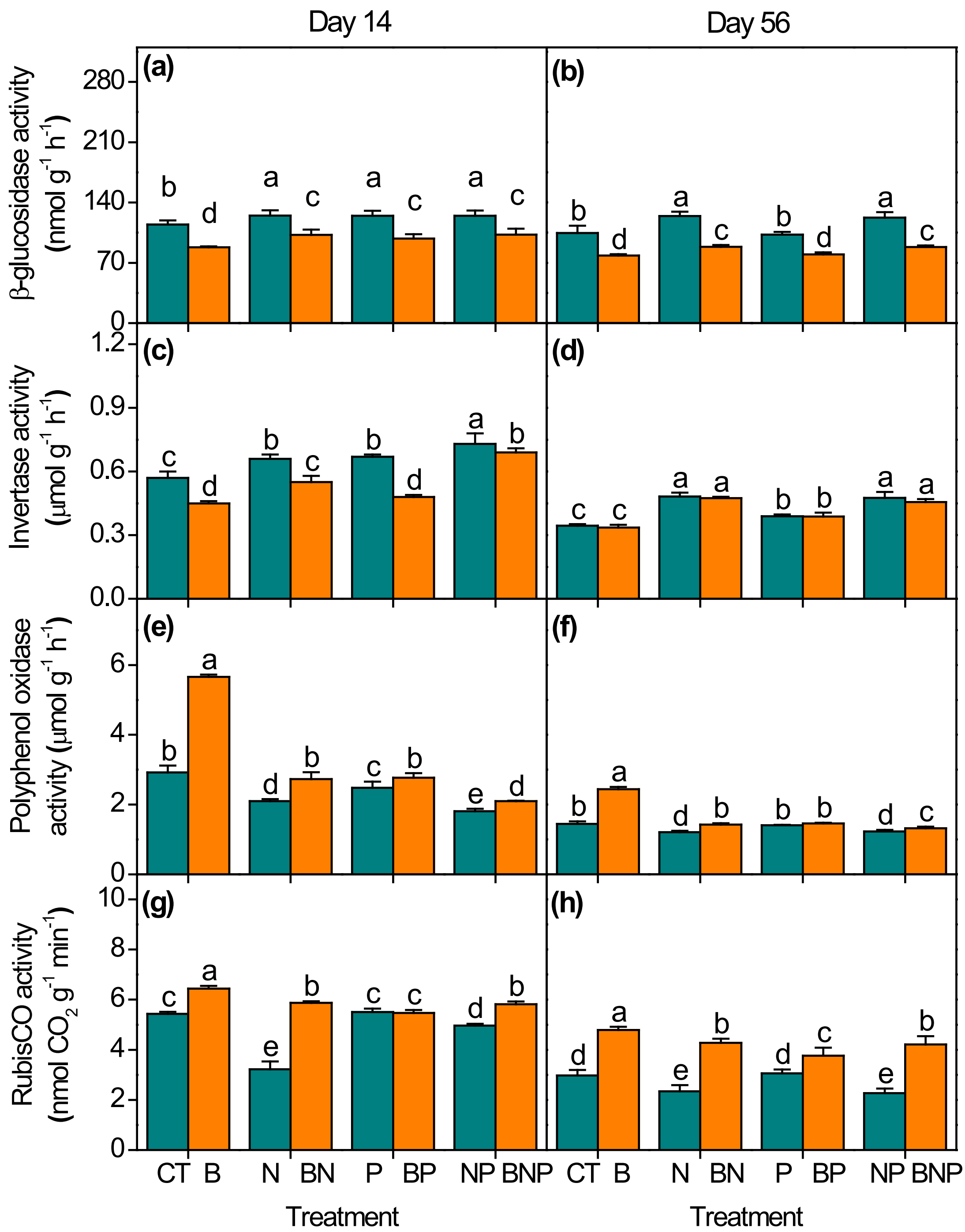

Cumulative priming effect
(mg CO_2 kg^{-1} soil)
2500
2000
1500
1000
500
0
-500
0
20
40
60
80
Incubation time (days)
N
BN
P
BP
NP
BNP
B

Fig. 2



Day 14
Day 56
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
β-glucosidase activity (nmol g-1 h-1)
Invertase activity (μmol g-1 h-1)
Polyphenol oxidase activity (μmol g-1 h-1)
RubisCO activity (nmol CO2 g-1 min-1)
CT B N BN P BP NP BNP
Treatment

Fig. 3

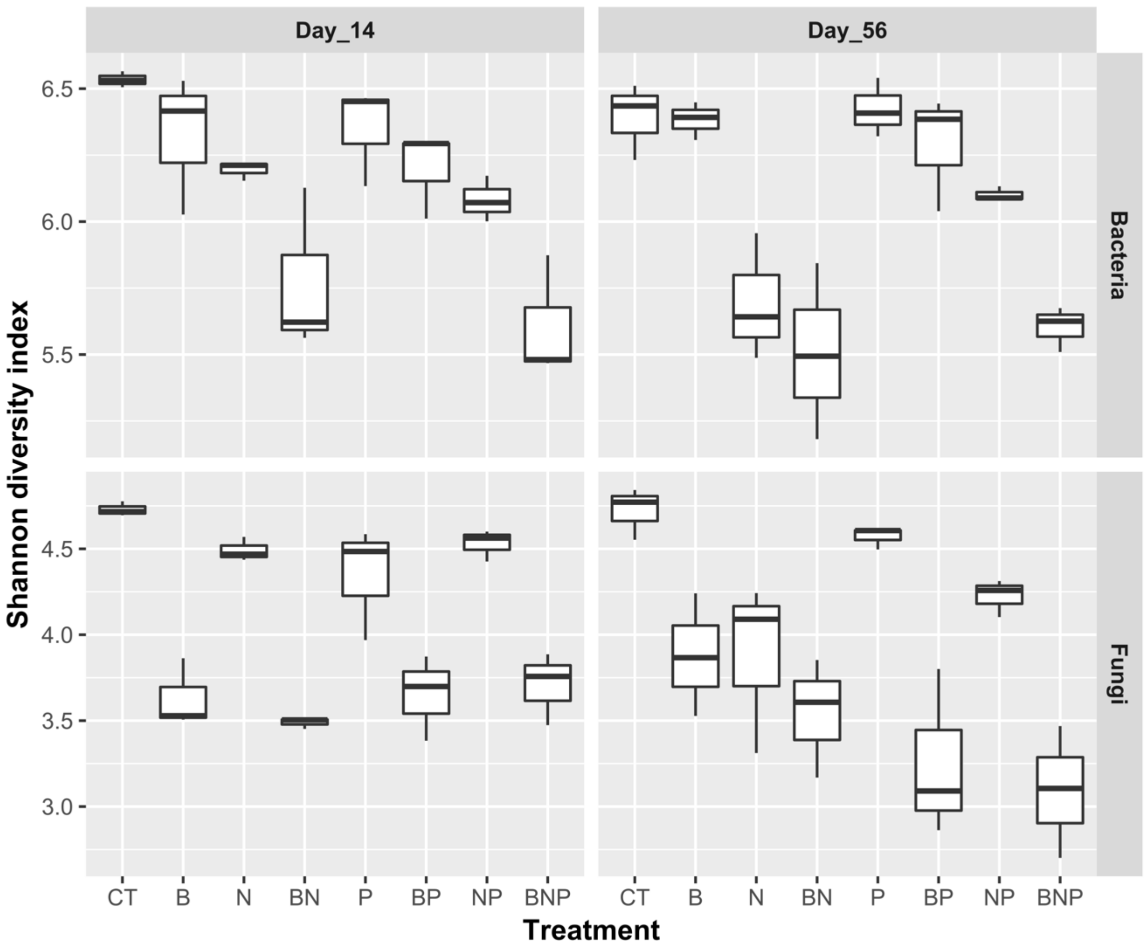

Day_14
Day_56
Shannon diversity index
6.5
6.0
5.5
4.5
4.0
3.5
3.0
Bacteria
Fungi
CT
B
N
BN
P
BP
NP
BNP
Treatment

Fig. 4

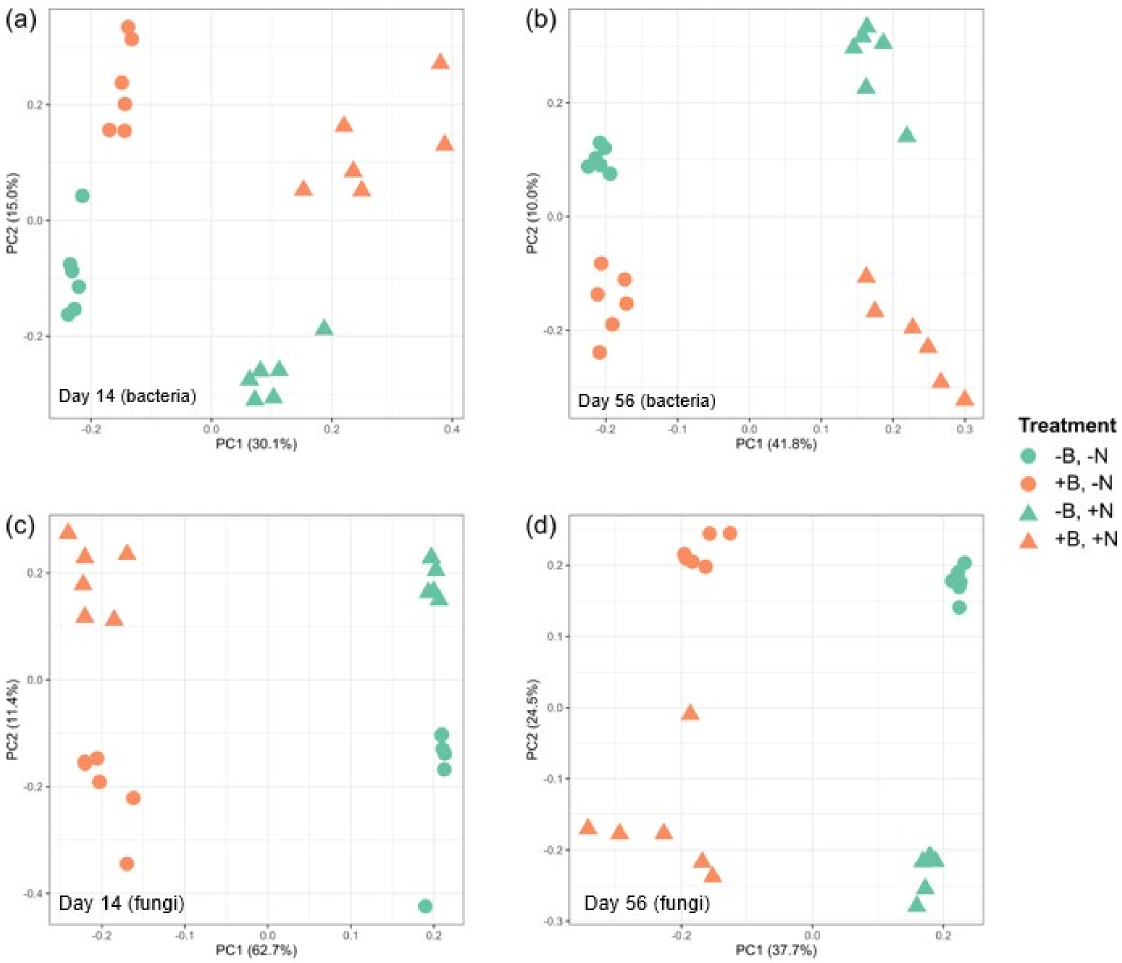

(a)
Day 14 (bacteria)
PC1 (30.1%)
PC2 (15.0%)
(b)
Day 56 (bacteria)
PC1 (41.8%)
PC2 (10.0%)
(c)
Day 14 (fungi)
PC1 (62.7%)
PC2 (11.4%)
(d)
Day 56 (fungi)
PC1 (37.7%)
PC2 (24.5%)
Treatment
-B, -N
+B, -N
-B, +N
+B, +N

Fig. 5

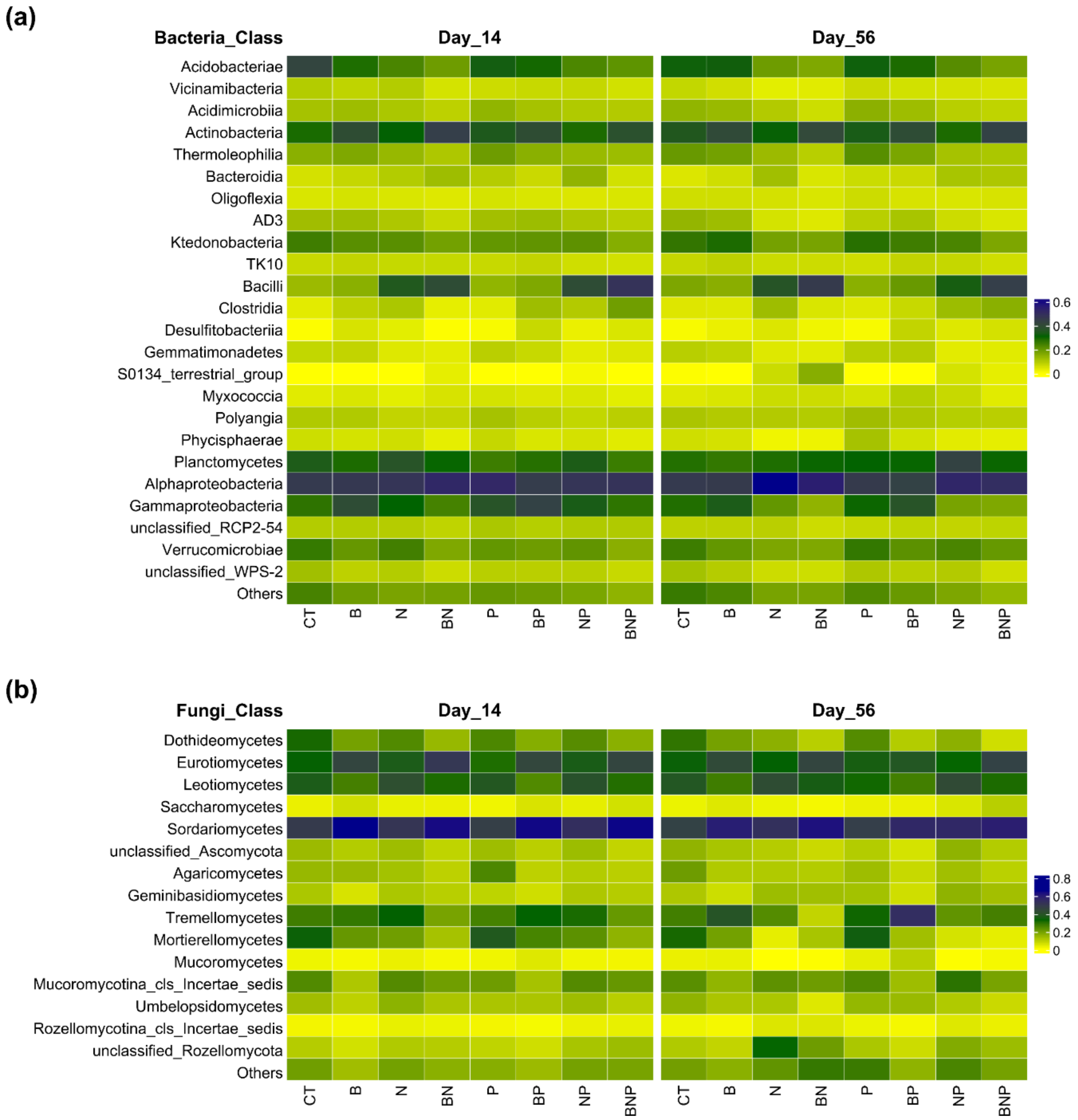
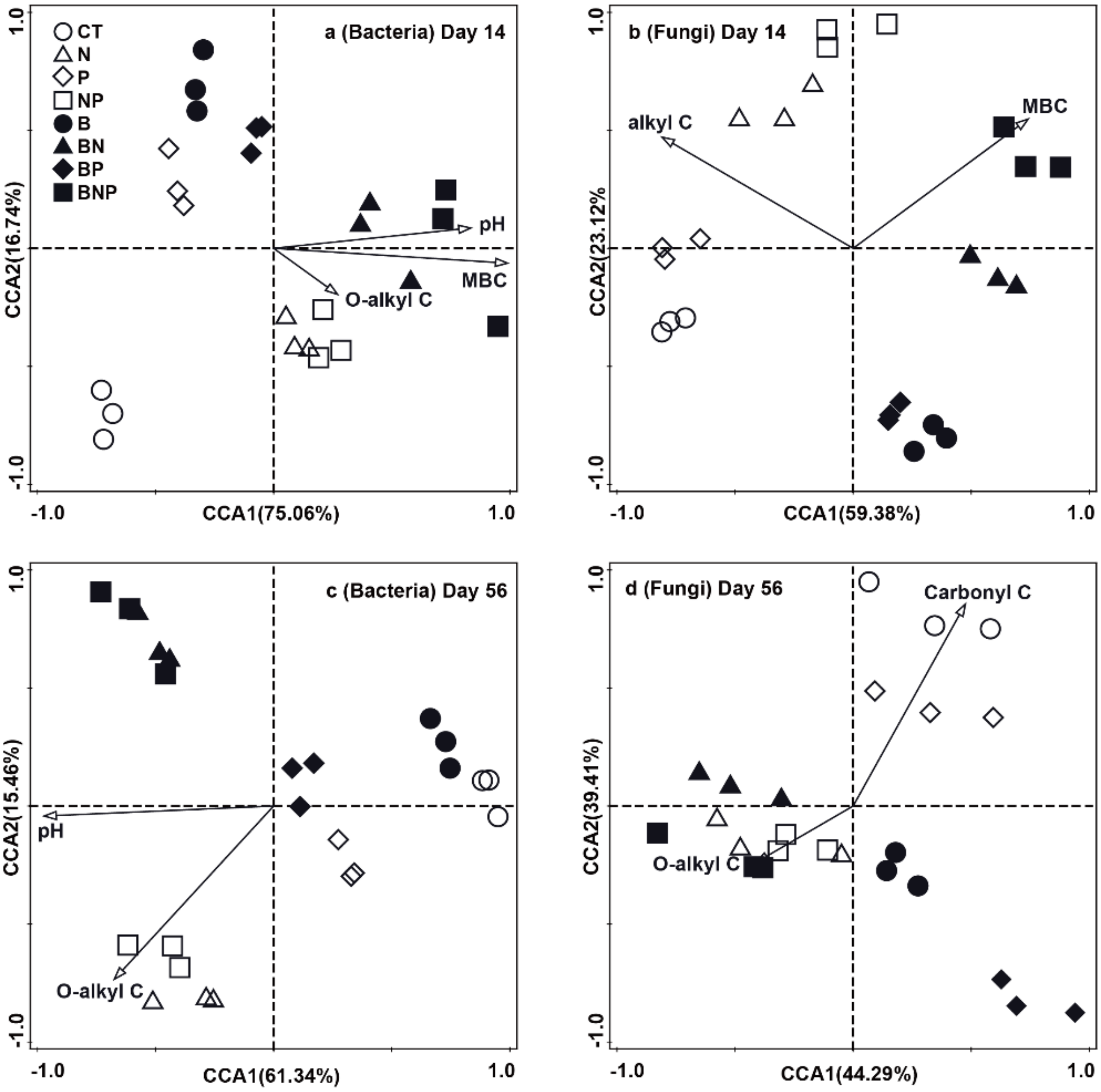

(a)
Bacteria_Class
Day_14
Day_56
Acidobacteriae
Vicinamibacteria
Acidimicrobiia
Actinobacteria
Thermoleophilia
Bacteroidia
Oligoflexia
AD3
Ktedonobacteria
TK10
Bacilli
Clostridia
Desulfitobacteriia
Gemmatimonadetes
S0134_terrestrial_group
Myxococcia
Polyangia
Phycisphaerae
Planctomycetes
Alphaproteobacteria
Gammaproteobacteria
unclassified_RCP2-54
Verrucomicrobiae
unclassified_WPS-2
Others
CT
B
N
BN
P
BP
NP
BNP
0.6
0.4
0.2
0
(b)
Fungi_Class
Day_14
Day_56
Dothideomycetes
Eurotiomycetes
Leotiomycetes
Saccharomycetes
Sordariomycetes
unclassified_Ascomycota
Agaricomycetes
Geminibasidiomycetes
Tremellomycetes
Mortierellomycetes
Mucoromycetes
Mucoromycotina_cls_Incertae_sedis
Umbelopsidomycetes
Rozellomycotina_cls_Incertae_sedis
unclassified_Rozellomycota
Others
CT
B
N
BN
P
BP
NP
BNP
0.8
0.6
0.4
0.2
0

Fig. 6



CT
N
P
NP
B
BN
BP
BNP
a (Bacteria) Day 14
pH
MBC
O-alkyl C
CCA2(16.74%)
CCA1(75.06%)
b (Fungi) Day 14
alkyl C
MBC
CCA2(23.12%
CCA1(59.38%)
c (Bacteria) Day 56
pH
O-alkyl C
CCA2(15.46%)
CCA1(61.34%)
d (Fungi) Day 56
Carbonyl C
O-alkyl C
CCA2(39.41%)
CCA1(44.29%)
1.0
-1.0

Fig. 7