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Biochar

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Short-term microbial community dynamics induced by ^{13}C-labeled maize root, its derived biochar and NPK in long-term amended soil

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Abstract

Crop residues and their derived biochar are frequently used for their potential to improve grain yield, soil fertility and carbon (C) sequestration. However, the effects of root are often overlooked, and the effects of chemical fertilizer (NPK) combined with root or its biochar on microbial community structure need further study. This study used ^{13}C-labeled maize root, its biochar and soil with different fertilization for 8 years as materials and substrates. A 112-day incubation experiment was conducted to explore the effects of microbial community on the C processing. During incubation, the root-C (54.9%) mineralized significantly more than biochar-C (12.8%), while NPK addition significantly increased the root-C mineralization. Adding biochar alone did not significantly change the microbial community. Compared to the biochar treatment (BC), the root treatment (R) notably increased the contents of total phospholipid fatty acids (PLFAs), ^{13}C-PLFA and the proportion of fungi and Gram-negative bacteria, but reduced the proportion of actinomycetes. The root mineralization was significantly correlated with the relative content of ^{13}C-Gram-positive bacteria and ^{13}C-fungi, while biochar mineralization was significantly correlated with the relative content of ^{13}C-Gram-positive bacteria and ^{13}C-actinomycetes. Notably, NPK addition significantly increased the contribution of biochar-C to PLFA-C pool, while decreasing the contribution of root-C. In summary, due to microbial adaptation to the lack of bioavailable C in biochar-amended soil, biochar can act as a buffer against the significant disturbance caused by NPK to microbial communities and native soil organic carbon (SOC), which contributes to the steady enhancement in soil C storage.

Highlights

- The addition of biochar alone for 8 consecutive years did not change the composition of the microbial community structure, but the total PLFA content increased significantly compared to the control.
- NPK addition reduced the proportion of microbial assimilation of root-C, while increasing the proportion of microbial assimilation of biochar-C.
- The effect of NPK on microbial biomass is short-lived, but the effect on microbial community structure is long-lasting.

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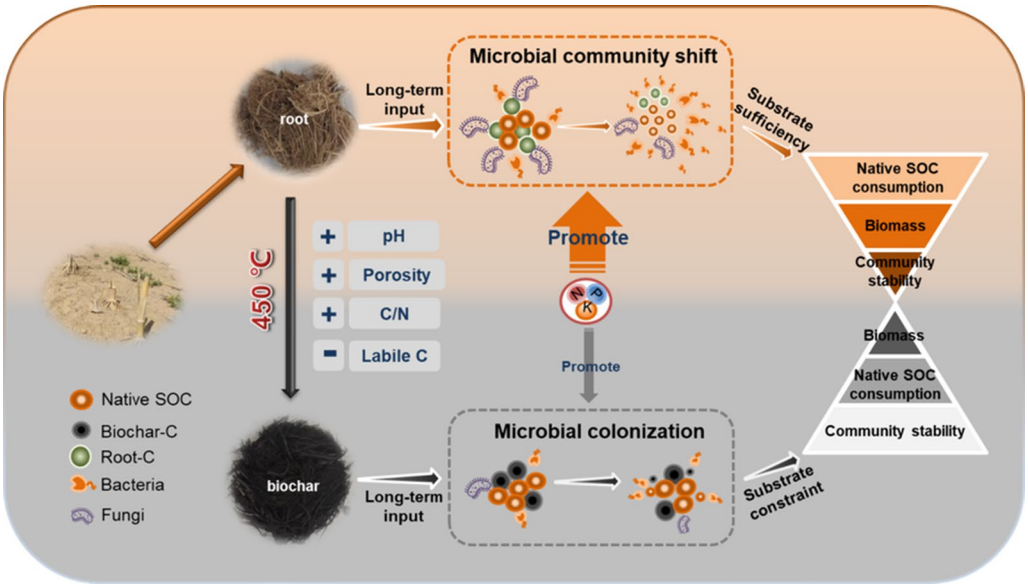
- Biochar has a stronger buffering effect on the drastic changes in microbial communities and native SOC caused by NPK.

Keywords Biochar, Maize root, ^{13}C-PLFA, Microbial community structure, NPK

Graphical Abstract



1 Introduction

Soil organic carbon (SOC), one of the Earth's most substantial terrestrial C reservoirs, is pivotal for boosting soil fertility, combating global climate change, and upholding ecosystem stability (Bossio et al. 2020; Liang et al. 2017). Historically, plant residues were incorporated into agricultural soils not only to maintain soil structure, organic content, and moisture retention but to act as a vital energy substrate for microbial activities (Pan et al. 2016a; Powlson et al. 2011; Schmatz et al. 2016). However, the effects of roots routinely left in the soil after harvest are often overlooked despite their relevance. Biochar, originating from crop residues, is more resistant to chemical decomposition than its precursor and shows immense promise in boosting SOC accumulation, mitigating greenhouse gas emissions, and curbing both organic and inorganic pollutant bioavailability and accumulation (Khan et al. 2015; Lehmann et al. 2011; Stewart et al. 2013; Waqas et al. 2014). Discussions persist about the degradation pathways of crop roots and biochar in the soil, especially concerning their influence on microbial community configurations. Filling the knowledge void about this process, particularly under diverse fertility conditions, is crucial.

The dynamics of microbial communities are responsible for the metabolism of soil organic matter (SOM) (Waldrop and Firestone 2004) and regulate the formation and mineralization of SOM. Varied soil microorganisms, each with distinct life cycles and physiological traits, demonstrate differential utilization of substrates and metabolism of crop residues(Andresen et al. 2014; Fierer et al. 2007). Recalcitrant C is mainly tapped by K-strategists like certain fungi, attributed to their adaptability in nutrient-depleted environments and prowess in mineralizing resistant C (Terrer et al. 2021). Labile C can boost microbial communities dominated by r-strategists such as Gram-negative bacteria (Fontaine et al. 2011; Paterson and Sim 2013), which results in CO_2 emissions and diminishes the fraction of residual C incorporated into SOM (Arcand et al. 2016; Li et al. 2019a; Liang et al. 2017). However, a recent study by Kramer et al. (2016) posited that bacterial utilization of labile C and fungal utilization of recalcitrant C sources were not distinctly separated during a decomposition experiment employing diverse plant C resource. For example, it has

been reported that fungi are strong utilizers of labile C sources (Müller et al. 2017).Such finding underscores the considerable variability in microbial functions which has spurred continued discussions regarding the roles of specific microbial groups at varied decomposition stages (Strickland and Rousk 2010). Therefore, understanding the influence of the complexity of microbial function on residue decomposition is important for predicting SOM turnover.

The quality of organic matter is the most common limiting factor for microbial activity in the soil (Kuzyakov 2010; Potthast et al. 2010). Maize roots are composed of biopolymers with varying degrees of persistence, resulting in different microbial community structures adapted to the metabolism of various substrates. In contrast, biochar after pyrolysis is difficult to be used by microorganisms due to its inertness (Lehmann et al. 2011), and thus it has been reported that biochar's addition has minimal (Pan et al. 2016b) or even no effects (Tian et al. 2016) on microbial community composition. However, biochar's unique porous structure and high surface aromatization can modulate soil microbial communities (Atkinson et al. 2010; Chen et al. 2019). There have been some different reports stating that biochar notably impacts microbial community composition in different ways (Yang et al. 2022; Zhang et al. 2021). For example, biochar promoted fungal growth (Azeem et al. 2020) and greatly reduced bacterial abundance and total PLFA content (Wang et al. 2015b). In contrast, Jones et al. (2012) reported that biochar led to a shift from microbial to bacterial communities, but the dominance of Gram-positive (Mitchell et al. 2015) or Gram-negative bacteria (Gomez et al. 2014) was inconsistent. Such inconsistencies may arise from variations in biochar types, application quantity, soil types and plant system responses (Chen et al. 2017; Gomez et al. 2014; Gul et al. 2015). The inconsistency in the extent and direction of biochar's effects on microbial communities creates barriers to exploring the functionality of biochar. Additionally, the microbial community regulates the decomposition of exogenous C and also receives its influence, and clarifying the relationship between them is important for understanding the C process.

Fertilization is a necessary measure to ensure crop yield, while also significantly affecting the biochemical processes of soil microorganisms. There have been studies highlighting that prolonged fertilizer application significantly modifies microbial community composition in agricultural soils (Carrara et al. 2021; Yuan et al. 2023; Zheng et al. 2016), thus compromising their stability (Ibrahim et al. 2020; Li et al. 2020; Pang et al. 2022). For instance, chemical fertilizer (NPK) addition significantly increased and decreased the diversity of bacteria and fungi in the top soil layer, affecting microbial community composition (Yuan et al. 2023). Compared to NPK applied alone, the combination of NPK and organic matters creates a different microbial community by providing a source of available nutrients and C source for a more active microbiota (Li et al. 2017). In a 6-year field trial, Wang et al. (2018) showed that the synergistic effect of biochar and NPK increased total microbial biomass and bacterial biomass. However, it has also been shown that microbial response to NPK may be weaker in biochar-amended soils (Watzinger et al. 2013) and the combination of biochar and NPK does not affect fungal populations (Dangi et al. 2020). This may be because biochar can help to maintain the stability of microbial community structure (Song et al. 2020). In addition, we speculate that the pattern of microbial community response to newly added exogenous C and NPK may be altered in soil amended with different fertilization practices over time due to adaptation to long-term substrate supply (Blagodatskaya and Kuzyakov 2013; Schimel et al. 2007). It's essential to further explore the effects of NPK on the decomposition and transformation of diverse organic substrates in soil amended with different ways over time.

In this study, ^{13}C-labeled maize root and its biochar were applied to brown soil with different fertilization regimes for up to 8 years for a 112-day incubation experiment. To study the fate of maize root and its derived biochar, the abundance of ^{13}C in SOC and PLFAs was analyzed using a combination of biomarker and isotopic technology, and the response of soil microbial community structure and function to the addition of maize root, biochar and NPK was investigated. Our hypotheses are as follows. (1) The structure of the microbial community changes significantly during root decomposition, while the response to biochar addition may not be significant due to the difficulty of microbial utilization. (2) Microorganisms can assimilate more exogenous C in the case of NPK because of higher biomass and greater activity. (3) Irrespective of NPK application and exogenous materials' quality, exogenous C would contribute more to fungal PLFA compared to other microbial communities due to the higher capacity of fungi to decompose recalcitrant C.

2 Materials and methods

2.1 Site description and biochar production

The soil was collected from the brown soil long-term test base (40°48′N and 123°33′E) in Shenyang Agricultural University, Liaoning Province, northeastern China (2013–present), where the experimental soil is a brown soil of loess origin with a clay loam soil texture (48% sand, 29% silt and 23% clay). The climate is temperate, characterized by humid-semi-humid monsoons. The average annual precipitation ranges from

574 to 684 mm, with an average annual temperature of 7.0–8.1 °C. The frost-free period lasts 147–164 days annually. To investigate the decomposition status of roots and their derived biochar, as well as their impact on microbial communities under varying fertility levels, we conducted indoor incubation experiments using six field soils (0–20 cm) with different treatments spanning up to eight years. The treatments included the treatments without chemical fertilizer: no fertilizer (CK), biochar (C), straw (S), and the treatments with chemical fertilizer: NPK-only (NPK), biochar and NPK (C + NPK) and straw and NPK (S + NPK). Maize straw and its derived biochar were utilized as organic materials. The soil was fertilized annually prior to planting starting in 2013. The application rate was 9 $t{\cdot}ha^{-1}$ for straw and 3 $t{\cdot}ha^{-1}$ for biochar. Before planting, urea, calcium superphosphate and potassium chloride were applied once, with application rates of 195 $kg{\cdot}ha^{-1}$ N, 90 $kg{\cdot}ha^{-1}$ P_2O_5 and 75 $kg{\cdot}ha^{-1}$ K_2O. Subsequently, the tractor pulled the ridge (20 cm high, 60 cm wide), and the maize seeds were sown on the ridge at a spacing of 30 cm. The maize grew naturally throughout the season, without any human intervention. The crop was artificially harvested in early October and the maize remnants were removed from the field.

The ^{13}C-labeled root was prepared with reference to Bei et al. (2013). The labeled root was washed with deionized water, dried at 105 °C for 2h and 75 °C for 24 h, and then cut into pieces of about 2 mm and set aside. Biochar was produced by slow pyrolysis of maize root under anaerobic conditions for more complete carbonization. After N_2 purge, the ^{13}C-labled root was placed in a muffle furnace, pyrolyzed at 450 °C for 20 min at an elevated rate of 22.5 $°C{\cdot}min^{-1}$, and then cooled by N_2 purging and stored in a dryer for later use. Total ash content of the biochar was determined to be 40.1%. The basic physical and chemical properties of field treated soil and ^{13}C-labeled root and biochar are shown in Table 1.

2.2 Experimental design

In 2021, six topsoil samples (0–20 cm) that had been treated in the field were chosen for a laboratory incubation experiment. The soil was air-dried and screened (< 2 mm), with fine roots, crop debris, and small stones carefully removed for backup. Before adding ^{13}C-labeled root, its derived biochar and NPK, the soil equivalent to 60 g oven-dried soil weight was adjusted to 60% of the field maximum water holding capacity in a culture tank, and pre-incubated in a dark space at 25 °C for 14 days to stabilize the soil microbial community. After pre-incubation, labeled root, its derived biochar and/or fertilizer were mixed evenly into the soil. New treatments were formed: control (CK), maize root biochar (BC), maize root (R), NPK-only (NPK), maize root biochar with NPK (BC + NPK), and maize root with NPK (R + NPK). Each treatment was repeated three times. The cells were cultured at 25 °C in darkness for 112 days. During the incubation, the soil water content was maintained by weighing method with deionized water every 4–5 days and all the bottles were sealed with a preservative film with pinholes to maintain aerobic conditions. Details of the treatments are shown in Table 2.

2.3 Soil sampling

During the incubation period, destructive sampling was performed on each treatment sample on days 0, 7, 14, 28, 56 and 112. Part of the sample was stored in a refrigerator at − 80 °C for extraction of soil PLFAs content and its ^{13}C abundance value to study microbial community changes, tracking the transfer of organic residue C to different microbial communities, and the remaining soil was air-dried for determination of soil pH, total C, N content and ^{13}C abundance.

Table 1 Chemical properties of soil samples and ^{13}C-labeled organic materials

		pH	Total N ($g\ kg^{-1}$)	Total C ($g\ kg^{-1}$)	C:N ratio	$\delta^{13}C$ value (‰)	Available P ($mg\ kg^{-1}$)	Available K ($mg\ kg^{-1}$)
Soil	CK	5.41	0.89	7.34	8.29	–	7.61	75.94
	NPK	5.33	1.26	11.63	9.21	–	25.25	88.67
	C	5.89	1.28	16.46	12.84	− 17.82	15.63	102.64
	S	5.57	1.00	13.11	13.07	− 18.78	16.70	89.20
	C + NPK	5.45	1.47	19.06	12.96	− 18.93	25.37	111.46
	S + NPK	5.32	1.27	14.92	11.75	− 17.76	25.85	93.04
Organic material	Maize root	–	18.05	353.20	19.57	2835.52	–	–
	Biochar	8.75	19.45	483.85	24.88	2969.44	–	–

Table 2 Details of the formation of incubation experiment treatments

Treatment	Incubation substrate from field treatment	Amendment material				
		N (g kg^{-1})	P_2O_5 (g kg^{-1})	K_2O (g kg^{-1})	Root (g kg^{-1})	Biochar (g kg^{-1})
CK	CK	–	–	–	–	–
BC	C	–	–	–	–	0.89
R	S	–	–	–	2.67	–
NPK	NPK	0.17	0.08	0.06	–	–
BC + NPK	C + NPK	0.17	0.08	0.06	–	0.89
R + NPK	S + NPK	0.17	0.08	0.06	2.67	–

2.4 Chemical analysis

Soil moisture was measured by drying at 105 °C until a constant weight was achieved. The soil, maize root, and biochar were subsequently air-dried and ground. Total C, N content and ^{13}C composition of soil and organic residues were analyzed using the EA-IRMS (Elementar Vario PYRO cube coupled with IsoPrime 100 Isotope Ratio Mass Spectrometer) instrument from Germany. Soil pH was measured with a pH ion meter, using soil: water at a 1:5 ratio. The $\delta^{13}C$ values for each index were determined using an EA-IRMS (Elementar Vario PYRO cube coupled to IsoPrime 100 Isotope Ratio Mass Spectrometer, Germany).

2.5 Soil PLFA analysis

The extraction and quantification of phospholipid fatty acids were carried out according to the method proposed by Zelles (1999) as modified by Hamer et al. (2007). Briefly, lipids were extracted from 4 g freeze-dried soil with phosphate buffer solution, chloroform and methanol (0.8:1:2, v:v:v), transferred to the organic phase and concentrated under N_2. After that, phospholipids were eluted with chloroform, acetone and methanol, and purified from neutral lipids and glycolipids by solid phase extraction column, and concentrated under N_2. The purified phospholipids were methylated by methanol-toluene (1:1), 0.2 M KOH–methanol and n-hexane-chloroform (1:1) to derive their respective fatty acid methyl esters (FAMES). The solution was prepared by dissolving the sample in n-hexane and adding methyl nonadecanoate (19:0, Sigma) as an internal standard. The sample was then analyzed and quantified using Agilent 7890A gas chromatography equipped with MIDI peak recognition software (Version 4.5; MIDI Inc., Newark, DE, USA). The $\delta^{13}C$ values of PLFA individuals were determined by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) using Thermo Scientific Trace GC Ultra attached to Finnigan MAT 253 IRMS (CuO / Pt Finnigan MAT Mark I combustion interface maintained at 850 °C).

The 23 PLFA individuals were grouped as follows: 14:0, 15:0, 16:0, 17:0 and 18:0 were characterized as non-specific (general) PLFA; i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0 were characterized as Gram-positive bacteria; 16:1ω7c, 17:1ω8c, cy17:0ω7c, 18:1ω7c and cy19:0ω7c were characterized as Gram-negative bacteria; 16:1ω5c, 18:2ω6c and 18:1ω9c were characterized as fungi; 10Me 16:0, 10Me 17:0, 10Me 17:1ω7c, 10Me 18:0 were characterized as actinomycetes (Dungait et al. 2010; Olsson 1999; Pan et al. 2016a, 2016b; Potthast et al. 2010). The sum of all microbial PLFAs was characterized as total microbial biomass.

2.6 Calculations

For $\delta^{13}C$ of each PLFA molecule, the mass balance equation is used to correct an additional C atom introduced during the methylation process:

$$n_{cd}\delta^{13}C_{cd} = n_c\delta^{13}C_c + n_d\delta^{13}C_d$$

where, n is number of C atoms, n_c is the number of C atoms of the underived compound, n_d is the number of C atoms of the derivatization reagent (methanol, $n_d = 1$), and n_{cd} is the number of C atoms of the corresponding derivatized compounds. The $\delta^{13}C_c$ is the ^{13}C abundance of the underivatized compound, the $\delta^{13}C_d$ is the ^{13}C abundance of the derivatization reagent (the $\delta^{13}C$ value of methanol measured by GC / IRMS is − 29.33‰), and the $\delta^{13}C_{cd}$ is the ^{13}C abundance of the corresponding derivative compound.

The proportion of exogenous C in SOC and PLFAs (F_e) was calculated by the following formula:

$$F_e = \frac{\delta^{13}C_t - \delta^{13}C_0}{\delta^{13}C_{biochar\ or\ root} - \delta^{13}C_0} \times 100$$

where, $\delta^{13}C_t$ and $\delta^{13}C_0$ represent the $\delta^{13}C$ value of the C pool of the root or biochar addition treatment

and control soil (no organic materials addition), and $\delta^{13}C_{biochar\ or\ root}$ represents the $\delta^{13}C$ value of the corresponding biochar or root.

The amount of root- and biochar-derived labeled C in each PLFA was calculated by formula:

$$A_e = C_{PLFA} \times F_e/100$$

where, C_{PLFA} is the amount of C in each PLFA.

The ratio of exogenous C mineralization (M_e) was calculated by the following formula:

$$M_e = \left(1 - \frac{W_{soil} \times F_e \times C}{A_{biochar\ or\ root}}\right) \times 100$$

where, W_{soil} is the weight of soil in each treatment (60 g dry soil), and C (g kg^{-1}) represents the content of SOC of each treatment. $A_{biochar\ or\ root}$ (g) is the amount of initial C of the biochar or root.

2.7 Statistical analysis

Statistical analysis was performed using SPSS 19.0 (SPSS Inc., Chicago, IL). The results were expressed as mean and standard deviation of three replicates. The ANOVA and Duncan test were used to compare the minimum significant differences between different sampling times and treatments at the 95% level. The relative abundance of individual PLFAs in total PLFA was calculated, and then normalized to unit variance using principal component analysis (PCA) to describe the soil microbial community structure under different treatments. All charts were drawn using Origin 2023b (Origin Lab Corporation, Northampton, USA).

3 Results

3.1 Mineralization rate of ^{13}C-labeled maize root and biochar

The rate of exogenous C mineralization sharply increased in the first 14 days and then gradually stabilized. The rate of exogenous C mineralization was significantly affected by the type of organic materials. On day 112 of the incubation, the root mineralization rate accounted for approximately 54.9% of the initially added root, whereas the biochar mineralization rate was significantly lower, at about 12.8% of the initially added biochar ($p < 0.05$, Fig. 1). NPK addition significantly increased the mineralization rate of root-C by 29.7%, but had no obvious effect on biochar-C mineralization ($p > 0.05$).

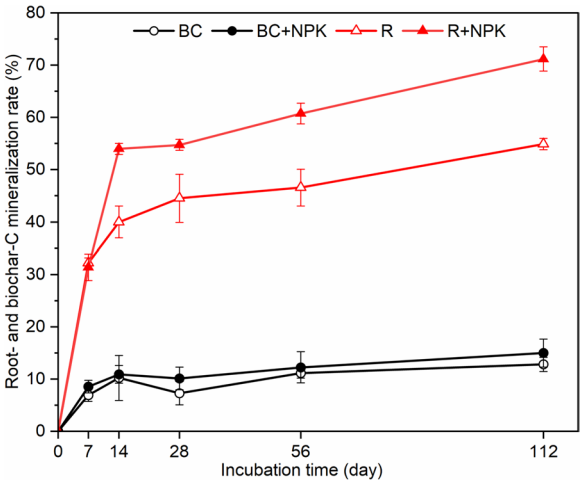


Fig. 1 Proportion of root- and biochar-C mineralization relative to initially added C (%) over 112 d incubation. NPK represents the addition of chemical fertilizer. R and BC represent root and biochar, respectively. The error bars represent the standard deviation of the mean (n = 3)

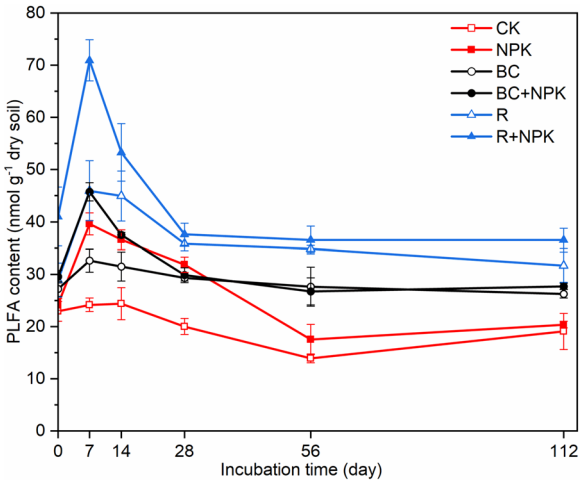


Fig. 2 Sum of PLFAs under different treatments over 112 d incubation (nmol g^{-1} dry soil). CK represents the control treatment (without organic materials and chemical fertilizer addition). NPK represents the addition of chemical fertilizer. R and BC represent root and biochar, respectively. The error bars represent the standard deviation of the mean ($n = 3$)

3.2 Dynamic changes of soil PLFA and microbial community structure

The application of organic materials and NPK notably augmented the total PLFA content in the early stage ($p < 0.05$), peaking on the seventh day and then gradually decreasing before stabilizing thereafter (Fig. 2). Throughout the incubation period, the total PLFA content in R and R + NPK treatments was significantly higher than BC and BC + NPK treatments ($p < 0.05$). Combining organic matter with NPK significantly increased the total PLFA content compared to using organic matter alone (0–28 days), but thereafter there were no significant differences and it was significantly higher compared to

the treatments without organic matter (CK and NPK, $p < 0.05$). The variation trend of PLFA content in microbial community was similar to that of total PLFA content (Table. S1). Throughout the incubation period, the microbial community composition did not change significantly ($p > 0.05$) in the BC treatment compared to the CK treatment. However, the proportion of fungi and Gram-negative bacteria increased significantly in R treatment ($p < 0.05$), while actinomycetes and positive bacteria decreased. Compared to the NPK-free treatments, adding NPK significantly enhanced the proportion of bacterial PLFAs while reducing the proportion of fungal PLFAs ($p > 0.05$). Notably, the proportion of fungal PLFAs was significantly higher in root-added treatments than in biochar-added treatments, while the scenario for actinomycete PLFAs was reversed (Fig. S1).

Principal component analysis (PCA) was created to illustrate the response of microbial communities to maize root, its derived biochar and fertilizer in different incubation periods (Fig. 3). The score plot (Fig. 3a) showed that the scores of all treatments in PC1 gradually increased with the prolongation of incubation time. The PLFA profiles of CK and BC treatments remained stable throughout the entire incubation period, whereas the community structure in other treatments underwent significant alterations during this period. The scores of biochar-amended treatments (BC and BC + NPK) on PC2 were significantly higher than those of root-amended treatments (R and R + NPK) and the scores of NPK addition treatments (NPK, BC + NPK and R + NPK) on PC1 were significantly higher than those of NPK-free treatments (CK, BC and R). The PLFAs loading plot (Fig. 3b) results identified 18:2ω6c, a15:0, and a17:0 as key explanatory variables for microbial alterations in R + NPK treatment, serving as indicators of fungi and Gram-positive bacteria. Similarly, PLFAs i15:0, 10Me 16:0 and 10Me 18:0 were pinpointed as indicators of Gram-positive bacteria and actinomycete, elucidating the observed microbial community shifts in CK and BC treatments. Cy17:0ω7c, cy19:0ω7c (indicators of Gram-negative bacteria), 17:0 10-methyl, 17:1ω7c 10-methyl (indicators of actinomycetes), i16:0 and i17:0 (indicators of Gram-positive bacteria) served to elucidate the primary microbial community alterations in NPK and BC + NPK treatments. The microbial community alterations in the R treatment were predominantly characterized by fungi (16:1ω5c, 18:1ω9c, 18:2ω6c), Gram-negative bacteria (16:1ω7c, 17:1ω8c), and Gram-positive bacteria (i14:0).

3.3 Microbial uptake of ^{13}C-labeled root and biochar

The uptake of exogenous C by microorganisms was significantly influenced by various exogenous organic materials and NPK. Root- and biochar-C were rapidly assimilated by microorganisms during the incubation period, and the amount of assimilation decreased gradually over time with significant differences ($p < 0.05$, Table S2). Microbial utilization of root-C was greater compared to biochar-C, with total ^{13}C-PLFA derived from R treatment averaging 1970 ng·g^{-1} and BC treatment averaging 14.7 ng·g^{-1}. The assimilation of exogenous C by Gram-positive bacteria was at the highest-level during incubation (Fig.S2). In contrast, the assimilation

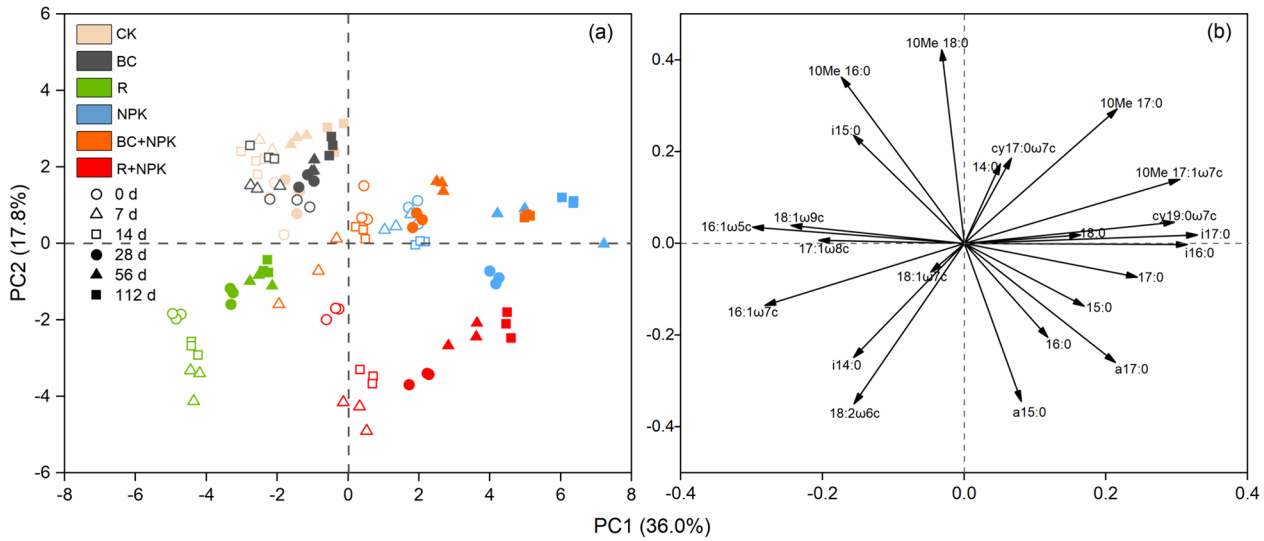


Fig. 3 Principal component analysis (PCA) based on the relative proportion of individual PLFAs for different treatments. The score plot (**a**) shows the community structure changes over time in different treatments. Different colors represent different treatments and different symbols represent the sampling times. The loadings plot (**b**) shows the shift in dominant PLFAs

of root-C by the fungi was at a high level on day 7 but then declined rapidly. The addition of NPK enhanced the microbial assimilation towards biochar-C, unlike the application of root alongside fertilizer, which only increased the microbial assimilation towards root-C on day 7 of the incubation period, but significantly reduced it for the remainder of the sampling duration.

Based on the contribution of exogenous C to the PLFAs of distinct microbial communities (Fig. 4), there were notable differences in the utilization of exogenous organic materials by microbial communities. In addition to the higher contribution of exogenous C to the PLFAs of Gram-negative bacteria in BC treatment on the 7th day, the fungal community in the other treatments was the most active microbial population using exogenous organic materials. The contribution of exogenous C to the PLFAs of fungi surpassed that of Gram-negative and Gram-positive bacteria, with actinomycete PLFAs displaying the lowest contribution. Notably, NPK addition significantly increased the proportion of exogenous C incorporated into the PLFA of the microbial community in the biochar-amended treatments, whereas the opposite trend was observed in the root-amended treatments except for the proportion of exogenous C incorporated into the fungal PLFA, which was only slightly elevated in the early stages (0–28 day).

3.4 Linking ^{13}C-PLFA partitioning dynamics in microbial communities to exogenous C decomposition

In each treatment, microorganisms showed a heterogeneous distribution of synthesized ^{13}C-PLFA among various communities and individual PLFA, reflecting varied patterns of microbial utilization of root- and biochar-C. Over the incubation duration, the average synthesis of

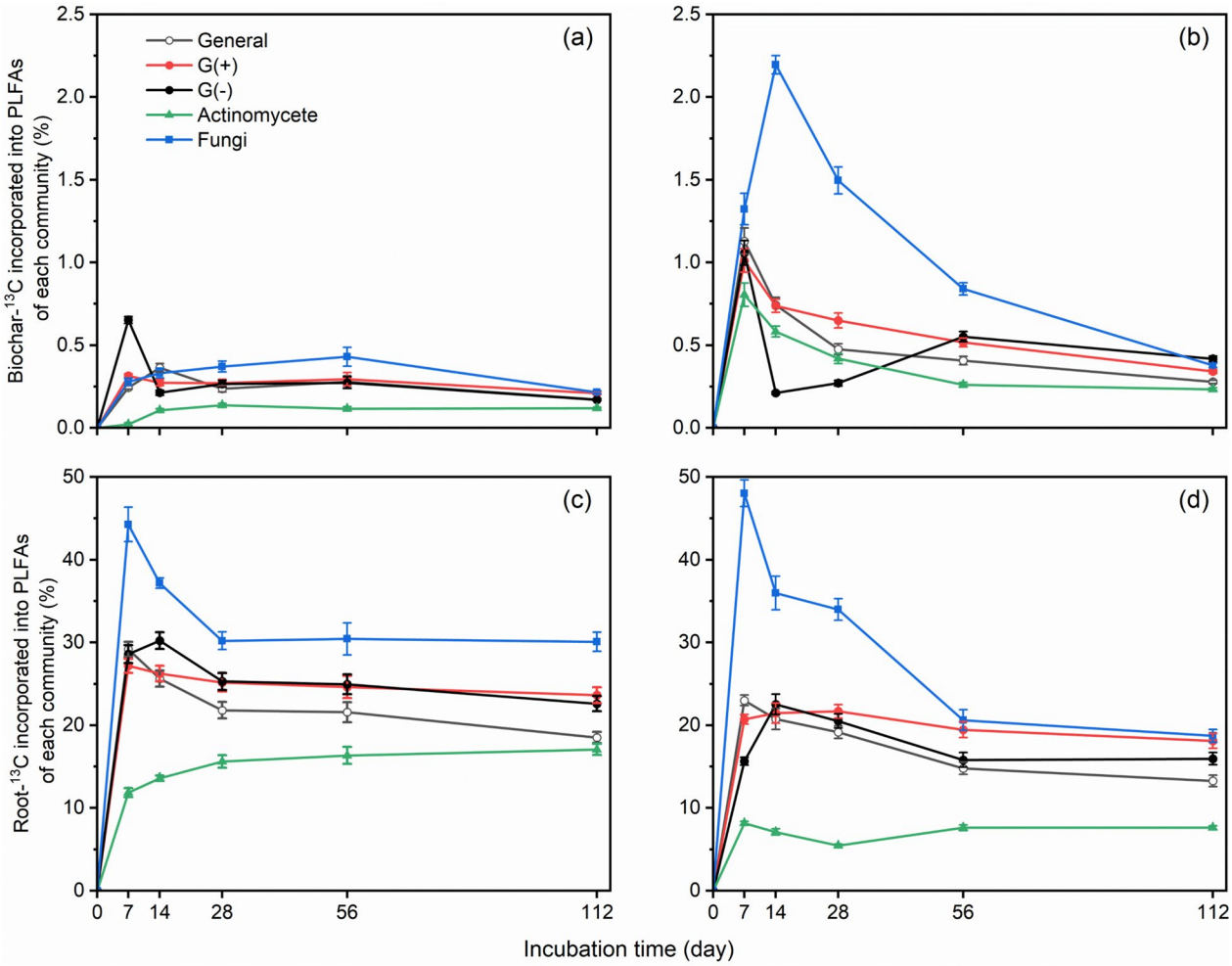


Fig. 4 Root- and biochar-C incorporation into microbial community PLFAs, expressed as a percentage of ^{13}C-PLFA synthesized by each community to the PLFAs of distinct microbial communities. **a**–**d** represent BC, BC + NPK, R and R + NPK treatments, respectively. The error bars represent the standard deviation of the mean (n = 3)

general PLFA accounts for about 18.6–20.9% of the total ^{13}C-PLFA (Table S2). The distribution ratio of ^{13}C in Gram-positive bacteria was increased in each treatment. In BC treatment, the relative content of Gram-negative bacterial ^{13}C-PLFA dropped markedly from 37% at the onset to 19% by the end of incubation. NPK application increased the distribution of ^{13}C in fungi during the early stages of incubation but significantly decreased it at the end. During the incubation period, the distribution of exogenous C across the 22 individual PLFAs showed that the partitioning of ^{13}C between the general and actinomycetes PLFA remained basically stable. The proportion of ^{13}C incorporated into PLFA 16:0 was always at a high level during incubation, while that incorporated into actinomycete PLFA was always the lowest. On the seventh day of incubation, root-C was heavily incorporated into PLFA 18:2ω6c (fungi) and 16:0 (general), followed by a15:0, i15:0, a16:0, 16:1ω7c, cy17:0ω7c and 18:1ω9c. During the incubation, the allocation of root-C decreased in fungal community and gradually increased in bacterial community while the distribution of biochar-C was more stable (mainly in Gram-positive bacterial PLFA). The results of the regression analysis indicated a significant correlation between the root mineralization rate and the dynamics of the relative content of ^{13}C-PLFA derived from root-C (^{13}C-PLFA/TPLFAs, ^{13}C-general (%), ^{13}C-Gram-positive bacteria (%) and ^{13}C-fungal (%)) ($p < 0.01$). Conversely, the biochar mineralization rate showed a significant increase with the increase in the relative content of biochar-derived ^{13}C-PLFAs [^{13}C-Gram-negative bacteria (%) and ^{13}C-actinomycetes (%)] ($p < 0.01$). There was no linear relationship between the rate of exogenous C mineralization rate and Gram-negative bacteria (Figs. 5, 6).

4 Discussion

4.1 Effects of root and its derived biochar on soil microorganisms

In this study, we analyzed the total PLFA content across different treatments (Fig. 2). Aside from CK treatment, all treatments saw their PLFA content peak on the 7th day, which is in accordance with prior research showing that the PLFA content peaks within 7 days (Bai et al. 2016). During the incubation period, the soil amended with maize root exhibited a significantly higher PLFA content than biochar-amended soil ($p < 0.05$), which was attributed to the higher level of labile C content of the root provided more substrate for microbial growth. Whereas, there was no significant effect of biochar on PLFA content except for an increase on day 7. This may be due to the rapid mineralization of a small fraction of labile compounds in biochar within a few days (Smith et al. 2010), which can provide some of the energy and nutrients to the microorganisms (Bowen 2006; Farrell et al. 2013), yet the remaining fraction (about 97% of biochar-C) is the recalcitrant C that is challenging for microbial utilization (Wang et al. 2015a). Liu et al. (2024) has reported no direct effect of biochar application on microbial abundance. Notably, throughout the entire incubation period, the BC treatment displayed a significantly higher total PLFA content than the CK treatment ($p < 0.05$). We suggest that possibly because the long-term application of biochar (for up to 8 years) elevated organic C levels and enhanced nutrient retention and microbial accessibility on particular surfaces (Lehmann et al. 2011). Additionally, multi-year application of biochar might offer increased attachment sites for microorganisms (Awad et al. 2018; Dai et al. 2021), thereby accelerating microbial colonization (Singh et al. 2022).

PCA analysis revealed that there were no discernible differences in microbial community structures between BC and CK treatments (Fig. 3), echoing the findings of Pan et al. (2016b). Helfrich et al. (2015) concluded that biochar's quality variation doesn't sufficiently incite competition in soil microbial communities attuned to diverse organic matter. Meanwhile, Yuan et al. (2023) also noted that the addition of biochar is beneficial to maintain the stability of microbial community. In contrast, root addition significantly altered soil microbial community structure, which is consistent with our hypothesis (1). To be precise, root incorporation led to an increase in the relative abundance of fungal and Gram-negative bacterial PLFAs, while decreasing the relative abundance of Gram-positive bacterial and actinomycete PLFAs relative to the CK and BC treatments throughout the incubation period (Fig. S1). This may be attributed to the fact that fungi and Gram-negative bacteria likely prefer labile C from fresh exogenous residues, while Gram-positive bacteria opt for stable C in organic residues or tap into SOM over plant residues' endogenous C (Li et al. 2019b; Liu et al. 2019; Zhang et al. 2020). The proportion of actinomycetes in the biochar-amended treatments was significantly higher than that in the root-added treatments, which may be related to the strong adaptation of actinomycetes to recalcitrant C metabolism (Yuan et al. 2023).

This study demonstrated that different microbial community structures were formed in soil amended with distinct organic materials for 8 consecutive years. The complexity of root components and the differing resistance to decomposition properties support different decomposition kinetics and longer decomposition cycles. Differences in metabolic capacity and competition for substrates lead to dynamic changes in microbial community composition in root-amended soil. Concurrently, the longer decomposition cycle ensures a continuous supply of available C for microorganisms, thereby augmenting

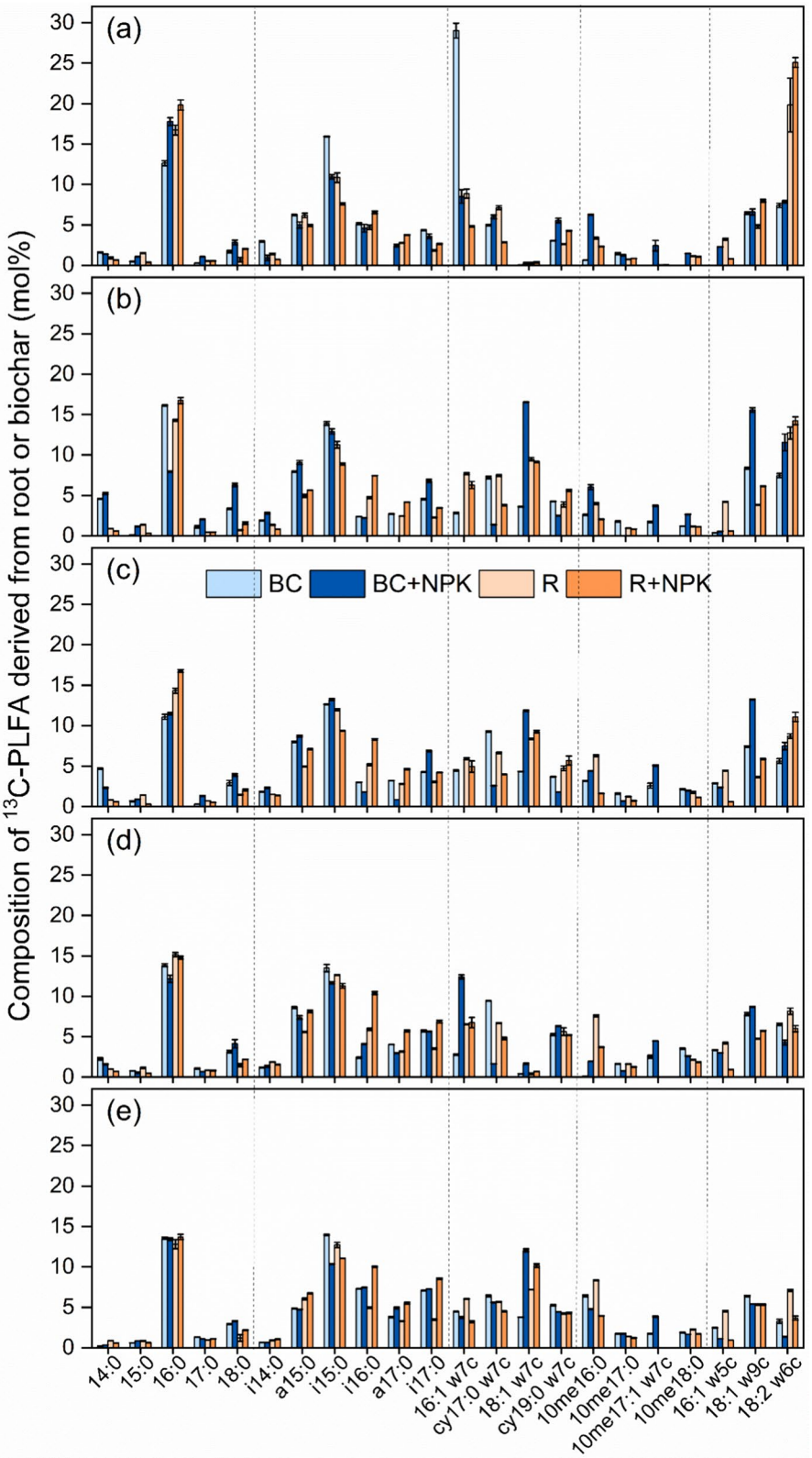


Fig. 5 Distribution of root- and biochar-C in individual PLFAs, expressed as a percentage of ^{13}C-PLFA synthesized by individual PLFAs in total ^{13}C-PLFA (mol%). **a**–**e** represent the 7th, 14th, 28th, 56th and 112th day of incubation, respectively

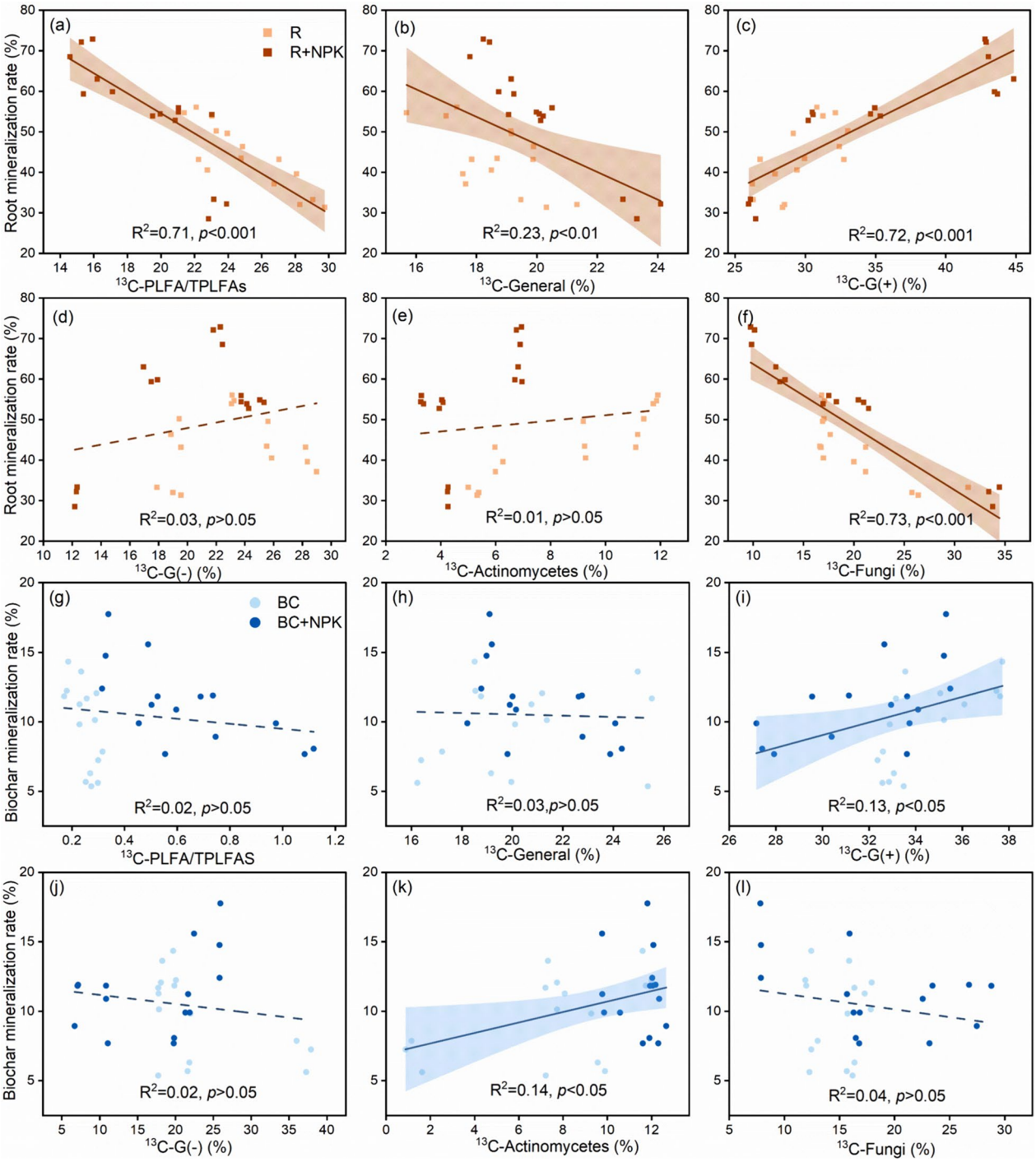


Fig. 6 Regressions between biomarkers of microbial groups and the mineralization of root (**a–f**) and biochar (**g–l**). ^{13}C-General (%), ^{13}C-G(+) (%), ^{13}C-G(−) (%), ^{13}C-Actinomycetes (%) and ^{13}C-Fungi (%) represent the proportions of distribution of total ^{13}C-PLFA across microbial communities, respectively

microbial biomass. In contrast, since the majority of biochar-C is not available to microorganisms (^{13}C-PLFA derived from biochar-C averaged only 14.7 ng·g^{-1}, Table S2), the microorganisms continue to adopt the strategy of mineralizing native SOC, which has a negligible effect on microbial abundance and community structure. Nevertheless, the porous structure and extensive surface area of the biochar facilitated the adsorption of

SOC and nutrients, while providing habitats for microorganisms, resulting in a slow yet sustained increase in microbial populations.

4.2 Microbial assimilation of exogenous organic C

The microbial uptake of root-C was observed to be 34–152 times greater than biochar-C (Table S2), which is related to the significant difference in the proportion of labile and resistant C fraction between root and biochar. Consistent with the trend of total PLFA content, ^{13}C-PLFA content peaked on the 7th day, indicating the rapid assimilation of labile C by microorganisms. As the proportion of resistant components in organic materials increases with time (Abiven et al. 2005), the microorganisms' utilization of substrates shifts from labile C to resistant C (Xu et al. 2020), and the assimilation of exogenous C gradually decreased. While some studies have suggested that microbial accessibility might control organic C turnover (Dungait et al. 2012; Han et al. 2016; Schmidt et al. 2011), our study demonstrates that the recalcitrance of organic C remains a challenge for microbial utilization, especially given the significant disparities in the physical and chemical attributes of biochar and its precursors (Abiven et al. 2005; Puget and Drinkwater 2001).

Different microbial communities exhibit varied patterns in the assimilation of organic materials. The contribution of both root- and biochar-C in fungal PLFA was higher than the other microbial communities (Fig. 4), suggesting that fungi use exogenous organic C more efficiently to build their own biomass, which is in line with our hypothesis (3). This is related to the secretion of extracellular enzymes by fungi, which give them a high capacity to degrade complex compounds (Genre et al. 2020; Yang et al. 2022). Additionally, fungi can adopt saprophytic nutritional strategies (Nilsson et al. 2018) and absorb nutrients at the soil-residue interface through hyphae (Acosta-Martínez et al. 2008), bolstering their competition for exogenous organic C.

The assimilation of root-C was initially dominated by fungi and bacteria, but the dominant position of fungi gradually decreased with the consumption of liable C and root-C was gradually enriched in bacterial PLFAs due to the higher biomass (Table S2 and Fig. 5). In contrast, no fungal dominance of biochar-C assimilation was observed in the early stages, which may be due to the fungal preference for lignin C over pyrolytic C (Santos et al. 2012). This finding also aligns with the results of Ippolito et al. (2014), indicating that the labile C substrate provided by biochar may be more conducive to the growth of fast-growing bacteria than fungi. Notably, the assimilation of exogenous C by Gram-positive bacteria was basically at the highest level in each treatment due to its ability to decompose recalcitrant C (Kramer and Gleixner 2008; Santos et al. 2012). The rapid decrease in the distribution of ^{13}C in Gram-negative bacterial PLFAs (especially in BC treatment) may highlight the sensitivity of Gram-negative bacteria to rapid C sources. The assimilation of exogenous C by actinomycetes was always the lowest, which may be related to the lower biomass and the physiological adaptability of actinomycetes to the degradation of C-rich resistant substances (O'Neill et al. 2009). Our study suggests that fungi effectively assimilate exogenous C, credited to their proficient degradation capability, whereas Gram-positive bacteria become the main actor of exogenous C assimilation due to their high biomass and the ability to decompose recalcitrant C.

4.3 The effect of NPK

In this study, we observed a swift increase in microbial biomass shortly after NPK addition, but this promotive effect diminished as time progressed (Fig. 2). Furthermore, the PLFA content of the treatments with the same organic material had no significant difference regardless of NPK addition, suggesting that NPK application may only have a short-term positive effect on microbial biomass. This was further supported by the fact that there was no significant difference in PLFA content between NPK and CK treatments in the incubation's later stage. Moreover, NPK addition significantly altered the microbial community composition during incubation (Fig. S1). Research has shown that applying N influences bacterial and fungal diversity, resulting in instability of the microbial community and pronounced effects on the composition of soil microbes (Carrara et al. 2021; Ibrahim et al. 2020; Li et al. 2020; Pang et al. 2022). In the NPK-applied groups, fungal PLFAs decreased notably, while bacterial PLFAs saw a significant rise compared to the NPK-free groups, reflecting differential responses of bacteria and fungi to inorganic fertilizer (Ge et al. 2016). In the same vein, Strickland and Rousk (2010) have shown that introducing N shifts communities from fungal-dominated to bacterial-dominated. Totally, the enhancement of microbial biomass by NPK is short-term, whereas the effect on microbial community structure is long-lasting.

The results demonstrated that in the case of NPK addition, the microbial community had different modes of utilization of two exogenous C (Table.S2 and Fig.S2). Microbial uptake of biochar-C significantly increased after fertilization, while the uptake of root-C only increased on the 7th day of incubation, which is not in accordance with our hypothesis (2). Several factors could explain this observation. Firstly, we suggest that NPK addition provided the microorganisms with generous inorganic nutrients for growth and development, thereby simultaneously enhancing their uptake capability for

both root-C and native SOC. Yet, as the labile C in root is consumed, the fraction of resistant C rises, which makes microorganisms change the strategy of using root-C to the utilization of SOC. It has been reported that microorganisms prefer to use SOC with high C content to maintain their internal element balance after N application (Chen et al. 2020). The microbial uptake of native SOC amplifies, consequently diminishing the assimilation ratio of root-C (Moore-Kucera and Dick 2008). Secondly, there is no doubt that the soil perennially amended by maize residues contains more incompletely decomposed plant residue-derived compounds, which provide more alternative C sources for microorganisms. In contrast, with perennial application of biochar, the depletion of labile C in soil and fewer C sources for microorganisms to choose from make the microbial community adaptive to the utilization of recalcitrant C. The NPK addition provides energy for the microbial community to decompose recalcitrant C and thus increases the absorption of biochar-C.

4.4 Linking C process to microbial community dynamics and NPK

Due to the significant differences in the labile C fractions, the mineralization rate of root was considerably higher than that of biochar. As the labile C content decreases, microbial utilization of exogenous C becomes more difficult, so we observed a gradual slowing down of mineralization and a subsequent decrease in the content of total PLFA and ^{13}C-PLFA. NPK addition significantly increased the mineralization rate of root but not that of biochar, since NPK can provide nutrients and power for microorganisms to support further decomposition of root composed of biopolymers with varying degrees of persistence, but cannot overcome the extreme recalcitrance of biochar. Unexpectedly, the mineralization rate of biochar in this study was 12%, which is somewhat high. Wang et al. (2015a) estimated that the labile C pool was about 3% of biochar. It may be because the biochar used in this study was prepared by slow pyrolysis and short residence time, so the proportion of liable C in biochar was higher. Studies have shown that slow pyrolysis leads to a more complete carbonization process favoring the formation of carbonaceous biochar (Huang et al. 2013; Keiluweit et al. 2010), while shorter residence time usually results in higher content of labile C (Zornoza et al. 2016).

There is a significant opposite correlation between the root mineralization rate and the allocation of root-C in fungal and Gram-positive bacterial communities (Fig. 6), representing the shift in the contribution of microbial communities during root decomposition. Since maize root contains a large amount of cellulose and hemicellulose and fungi are increased as the main participants in cellulose decomposition, there was a large allocation of root-C to the saprophytic fungal PLFA in the initial stage of incubation (particularly in 18:2ω6c, Fig. 5). Some studies have shown that saprophytic fungi dominate the decomposition of newly added residues (De Deyn et al. 2011; Theuerl and Buscot 2010) and then the filamentous hyphae of fungi promote subsequent bacterial growth by breaking down complex substrates into readily available compounds (Bai et al. 2016). Due to the preference of Gram-positive bacteria for recalcitrant C, they become the main decomposer after fungi. In contrast, the mineralization rate of biochar increased significantly with higher allocation of biochar-C to Gram-positive bacteria and actinomycetes. Farrell et al. (2013) reported that Gram-positive bacteria can utilize the bioavailable C fraction in biochar. Furthermore, Gram-positive bacteria and actinomycetes are effective in mineralizing recalcitrant C (Fontaine et al. 2011; Waldrop and Firestone 2004) due to their extracellular enzymes degrading resistant compounds more readily (Brant et al. 2006).

NPK application provides sufficient nutrients for microbial proliferation in the short term, increasing the decomposition of exogenous C and SOC. However, as the proportion of recalcitrant C in root increases during mineralization, microorganisms gradually shift to excavating native SOC and this may create uncertainty about C storage in soil. In contrast to the abundance of bioavailable C fractions in plant residue-amended soil, which is lower in soil with perennial biochar application. Moreover, due to the low biomass, the uptake of native SOC by microorganisms is quite limited. We suggest that the scarcity of bioavailable C fractions in biochar and the soil amended with biochar over time is the fundamental reason why biochar can maintain the stability of microbial communities and steadily increase soil C sequestration. These results provide new insights into understanding microbial community dynamics and fertilizer effects on the decomposition of root, biochar and native SOC.

5 Conclusions

This study highlighted the response of microbial community structure to newly added organic materials in soils amended with diverse organic matter for 8 consecutive years. The differential soil PLFA index stemmed chiefly from the contrasting chemical stability of C and the addition of NPK. Maize root mineralisation was significantly associated with fungi and Gram-positive bacteria, whereas biochar mineralisation was significantly associated with actinomycetes and Gram-positive bacteria. NPK addition increased the mineralization of exogenous C and native SOC by increasing the proportion of bacterial community.

Limited by the bioavailable C fractions in soil, biochar is more effective than root at buffering drastic changes caused by NPK, promoting a balanced state of microbial communities, and minimizing disturbance to native SOC. This promises the soil C pool to increase steadily over time. Future studies ought to incorporate both ^{13}C-DNA-SIP and ^{13}C-RNA-SIP methodologies in extended field trials to pinpoint microbial species that exploit plant residues or biochar. The relationship between changes in native SOC and microbial community dynamics needs to be further clarified. Additionally, it is crucial to analyze biochar's substitution impact on its precursors, with a focus on C capture and emission reduction.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s42773-024-00363-w.

Additional file 1.

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Author contributions
Xiaori Han conceived, designed and financially supported the study; Zonglin Lu analyzed the data and wrote the paper, and conducted the analytical work with Tong Lu, Junmei Shi and Hangming Guo; Na Li guided and supervised the trial; Kun Chen reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Data availability
All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Declarations

Competing interests
We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work. There is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, this manuscript.

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