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Straw return counteracts the negative effects of warming on soil microbial community and ecosystem multifunctionality

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Abstract

Climate warming is one of the most serious threats to soil biodiversity and ecosystem stability. Straw return has been extensively recommended as an environmentally friendly management to increase soil health and agricultural productivity. However, little is known about their interactive effects on soil microbial communities and ecosystem functioning. This knowledge gap limits our capacity to assess how straw management affects soil biodiversity and ecosystem services under climate warming. To this end, we investigated the effects of soil warming, straw return, and their interaction on soil microbial communities, functional genes, enzyme activities related to C, N and P cycling, and ecosystem multifunctionality in a two-factorial experiment in an agroecosystem. Soil warming decreased fungal diversity (by 20%) and functional genes associated with organic C decomposition, N fixation, nitrification, denitrification, and P mineralization compared with ambient temperature. Thereby, the ecosystem multifunctionality (EMF) mediated by soil enzyme activities were reduced under warming, attributing to the lower soil moisture, nutrient availability, and root-derived labile organic matter inputs. The close relationships of microbial diversity and functional genes with EMF highlighted the importance of soil biodiversity in maintaining agroecosystem functioning. Under soil warming and ambient temperature, straw return resulted in higher bacterial diversity (by 3.6%) and microbial functional genes abundances of C, N and P cycling compared to straw removal, and consequently raised the EMF. This was primarily because straw buffered soil temperature cycles and moisture variation, as well as the additional nutrient supply in the added straw. Overall, straw return created favorable habitats for microorganisms, and thereby mitigated the adverse effects of warming on soil microbial communities and ecosystem functionality related to nutrient cycling. Our study provided the possibility to increase soil biodiversity

and further ecosystem services related to organic matter decomposition and nutrient turnover by straw management under climate warming.

KEYWORDS: climate change, straw management, organic matter decomposition, microbial community, ecosystem functionality, agroecosystem

1. Introduction

Global warming, a consequence of the accumulation of greenhouse gases in the atmosphere due to the burning of fossil fuels and changes in land use, is an urgent political and scientific issue (IPCC, 2021). Given that temperature is the major driver of biological processes in terrestrial ecosystems, global warming has great impacts on soil biodiversity and microbial community composition (Berg et al., 2010), which may influence nutrient cycling and ecosystem functioning (Guo et al., 2018). Agricultural production generates ca. 4 Gt yr^{-1} of crop residue globally (FAO, 2017; Chen et al., 2020), and straw return has been widely recommended as an environmentally friendly management to enhance organic C sequestration and mitigate climate change (Zhao et al., 2020). Straw return may reshape the microbial community composition and diversity, and in turn alter soil biological processes and nutrient availability (Liu et al., 2014). However, to our knowledge, little information is available on the interactive effects between warming and straw return on soil biodiversity and nutrient cycling, which limited our ability to accurately assess the responses of agroecosystems to management practices under climate change.

Microbial diversity, composition, and activity are primary drivers of biogeochemical cycling, which play important roles in soil health (Mangan et al., 2010; Zhao et al., 2014) and sustainability of crop production (Fan et al., 2020). Global warming can directly alter soil temperature and moisture, and consequently change microbial community composition, physiology, and activity (Zhang et al., 2016a; Tang et al., 2019). Warming also influences root development and growth, changes the quality and quantity of rhizodeposits and litter input, thereby further altering the soil microbial community (Singh et al., 2010) and organic matter decomposition (Kuzyakov et al., 2007). However, no consensus has been reached about whether and how the microbial community respond to warming, attributing to the various durations and ranges of warming and contrasting ecosystem types studied (Zhang et

al., 2005; Karhu et al., 2010). For instance, Sheik et al. (2011) showed that + 2 ℃ warming altered microbial community composition and increased microbial biomass by 40-150%, but reduced microbial diversity in temperate grassland soil. A 3-year warming study in a Swiss alpine treeline ecosystem suggested that increased soil temperature of 3.7 ℃ strongly altered fungal community composition, although species diversity and abundances were unchanged (Solly et al., 2017). However, a 9-year warming experiment in sub-arctic Sweden showed that soil bacteria community remained stable under + 1 ℃ warming (Weedon et al., 2012).

Soil extracellular enzymes are mainly secreted by microorganisms, serving as the major drivers of organic matter decomposition (Souza et al., 2017). The responses of soil enzyme activities to warming are highly variable across studies (Zhou et al., 2013; Razavi et al., 2017). Warming can increase the β-1,4-glucosidase (BG) and β-1,4-xylosidase (BX) activities by stimulating C and N cycling. However, it can also reduce the BG, L-leucine amino peptidase (LAP), and N-acetyl-glucosaminidase (NAG) activities by inducing soil drought or reducing microbial activity (Sanaullah et al., 2011; Souza et al., 2017; Meng et al., 2020). A global meta-analysis indicated that warming increased oxidative enzyme activities, while the impacts on hydrolytic enzymes depend on the warming duration, magnitude, and environmental factors (Meng et al., 2020). Ecosystem multifunctionality (EMF) index calculated by multiple enzyme activities has been widely applied in the ecosystems studies to evaluate soil functioning and biodiversity (Luo et al., 2018), but few studies focused on the response of the EMF index to warming and management practices in agroecosystem. It is generally believed that change in soil microbial community with high biodiversity would not translate into change in soil functionality, due to the high redundancy of microbial groups (Kuzyakov et al., 2009b; Strickland et al., 2009). However, agricultural ecosystems usually have much lower biodiversity, indicating a lack of multifunctional

redundancy in intensively managed soil. Therefore, the shift of soil microbial diversity, even a smaller number of species or functional groups, will more easily and strongly mediate soil functionalities in agroecosystem (Luo et al., 2018). However, the contribution of microbial biodiversity to agroecosystem functioning is much less clear, especially under climate warming.

Accompanied by the constant increase in cereal production, the crop residue production has grown dramatically (Chen et al., 2020). Crop straw is widely used for soil amendment, animal feeds, cooking and heating, and biofuels feedstock (Li et al., 2018a; Yang et al., 2019). However, numerous studies indicated that straw burning or removal from the field would cause severe environmental pollution, lead to decline of soil fertility, as well as potentially exacerbating soil erosion (Li et al., 2018b; Battaglia et al., 2021). Straw represents a valuable source of organic matter, and returning straw to the field has been increasingly accepted by policy-makers and smallholders for the positive impacts on soil health, agricultural productivity, and climate change mitigation (Paustian et al., 2016; Ndzelu et al., 2020). Straw return alleviates soil compaction, moderates temperature and moisture regimes, increases nutrient supply and the SOC pool (Lal, 2008; Said-Pullicino et al., 2014; Mu et al., 2016). Thus, straw return benefits for maintaining a diverse soil microbial community and enzyme activities (Akhtar et al., 2018; Jensen et al., 2021). Zhang et al. (2016c) reported that soil microbial activity was increased with straw return, resulting in higher activities of soil urease (URE), invertase, and phosphatase. Similarly, a 30-year experiment in a temperate agroecosystem showed that straw return modified soil microbial community compositions and increased the BG, BX, and NAG activities (Liu et al., 2008). However, straw return may also bring fungal pathogens into the soil system and cause serious N competition between plants and microbes, threatening the growth of succeeding crops (Yadvinder et al., 2004).

Additionally, warming and straw return may have contrary effects on soil conditions and microbial activities. Straw return reduced soil bulk density and increased total porosity, thereby maintaining a better moisture regime (Liu et al., 2014; Paustian et al., 2016). Therefore, straw return may mitigate the negative effects of warming-induced soil drying on microbial communities and enzyme activities by the moisture maintenance. On the other hand, increased soil temperature may accelerate SOM decomposition, and thereby resulted in the nutrients loss (Bastida et al., 2017; Wu et al., 2020). While straw return may offset warming effects on soil nutrients (e.g. SOC and TN) and subsequently microbial community and enzymes activities through increasing the organic matter input (Liu et al., 2014).

Our study aimed to (1) investigate the combined effects of soil warming and agricultural management practice (i.e. straw return) on soil properties, microbial communities and functional genes, and enzymes activities; (2) explore the relationships between soil microbial communities and EMF in agroecosystem, and identify their controlling factors under projected warming and straw return. We hypothesized that (a) warming and straw return would have opposite effects on the microbial communities, functional genes and EMF, and their interaction depends on the trade-offs of the diverse impacts on soil conditions (e.g. temperature, moisture, bulk density etc.) and nutrient availabilities; (b) soil microbial diversities and functional gene abundances may be strongly related with the EMF index, due to the lack of multifunctional redundancy in the intensively managed agroecosystem. A better understanding of the responses of soil microbial communities, functional groups, and ecosystem functions to warming and anthropogenic management would provide novel insights into the microbial and enzymatic mediated processes involved in the adaptations of soil to climate change.

2. Materials and methods

2.1. Experimental site, design, and sampling

The study site was located at the Wuqiao Experimental Station (37°36′N,116°21′E) of China Agricultural University in Hebei province, China. This region has a typical temperate continental climate with an average temperature of 12.9 ℃. The annual mean precipitation is ~500.0 mm with 60–80% of the precipitation occurring in July and August. Soil texture is silty loam with a bulk density of 1.5 g cm^{-3}, soil organic C (SOC) of 8.4 g kg^{-1}, total N (TN) of 1.0 g kg^{-1}, available phosphorus of 5.1 mg kg^{-1} and available potassium of 173.0 mg kg^{-1} in 0-20 cm soil layer. The site is under a long-term crop rotation with winter wheat and summer maize.

The experiment was established in Oct. 2018. A two-factorial design was applied with two levels of soil temperature (ambient temperature and warming +3.5 °C) and two levels of straw return practice (straw removal and straw return), forming four treatments (ambient, ambient-straw, warm, and warm-straw). Each treatment had three replicates, resulting in a total of 12 plots (3 × 4 m for each). Soil warming was manipulated using heating cables, which were placed at 20 cm depth with 25 cm intervals. The average soil temperature increase of 3.5 ℃ was projected based on the RCR 6.0 scenario's prediction that the global temperature will rise by about 3-4℃ by the end of this century (IPCC, 2013; Solly et al., 2017; Wu et al., 2022). The 10 cm thick plastic foam plates were inserted to 50 cm depth at the edge of each plot to minimize thermal gradients. The heating cable was installed in Oct. 2018 with an uninterrupted supply of electricity from 2018 to 2020. A "disturbance control" was conducted for ambient temperature plots, which was the same as the warming plots except that there was no electricity, to ensure equivalent physical conditions. For straw return plots, crop straws were chopped into 5-10 cm fragments after harvest of winter wheat and summer maize from Oct. 2018 to Oct. 2020. The chopped wheat straws were kept on

soil surface, and chopped maize straws were incorporated into 0-15 cm soil depth with rotary tillage. The annual incorporated amounts of summer maize and winter wheat straws were about 10000 kg ha^{-1} and 8000 kg ha^{-1}, respectively. For straw removal plots, all maize and wheat straws were manually removed after harvest.

Winter wheat (cv. Jimai 22) was sown on 14 Oct. 2018 and 15 Oct. 2019 with the seeding rate of 300 kg ha^{-1} and row spacing of 15 cm. At sowing, urea, diammonium phosphate, and potassium sulfate were applied at rates of 300 kg ha^{-1}, 300 kg ha^{-1}, and 150 kg ha^{-1}. All plots were irrigated before seeding (75 mm) and again at jointing stage (75 mm). Summer maize (cv. Zhengdan 958) was sown on 14 June 2019 and 19 June 2020 with the row spacing of 60 cm and seeding rate of 68000 plants ha^{-1}. At sowing, the urea, diammonium phosphate, and potassium sulfate were applied at rates of 100 kg ha^{-1}, 230 kg ha^{-1} and 210 kg ha^{-1}. Additionally, urea was top-dressed at the rate of 200 kg ha^{-1} at silking stage. All plots were irrigated before seeding (75 mm). Other managements (e.g. weeding, harvest) were consistent with the local farming practices.

Soil sampling was conducted on Oct. 2020 after the maize harvest. Five soil samples from each plot were randomly collected at 0-20 cm. At each sampling, the drill was cleaned, washed with sterile water, and then air dried. Samples from the same plot were mixed into one composite sample, packed in polyethylene bags, immediately stored in a cooler with ice packs, and shipped to the laboratory. After manually sieving through 2 mm and removing debris, the soil samples were stored at 4 °C within two weeks for the subsequent analyses of soil properties and extracellular enzymes activities. Additionally, another portion was kept at -20 °C for microbial community composition analysis.

2.2. Determination of soil and plant properties

Daily precipitation was recorded by the weather station (ca. 200 m away from the

study site). The T-type thermocouples (L93-5, Luge, Zhejiang, China) were placed between two cable lines at the center of each plot to record the temperature in 0-20 cm soil layer hourly. Soil water content was measured weekly by gravimetric method and expressed as water-filled pore space (WFPS). SOC was determined by $K_2Cr_2O_7$-H_2SO_4 oxidation following Kan et al. (2020b). Soil TN was determined with the Kjeldahl digestion method (Bao et al., 2000). Soil mineral N (i.e. NH_4^+ and NO_3^-) and dissolved organic C (DOC) were extracted by 0.5 M K_2SO_4 and then analyzed following Wu et al. (2020). Soil microbial biomass C (MBC) was determined following Beck et al. (1997). A quadrat sample of 6 m^2 was taken manually to estimate aboveground biomass on Oct. 2020 when the maize was harvested. The roots within 0-50 cm were taken by the core method at maturity stage of winter wheat (Kan et al., 2020a). The plant and root samples were dried at 75 ℃ by 48 hours for dry biomass determination.

2.3. Quantification of enzyme activities and ecosystem multifunctionality (EMF)

The activities of C-acquisition enzymes (i.e. BG, BX, β-D-cellobiosidase [CBH] and phenol oxidase [PO]), N-acquisition enzymes (i.e. L-leucine aminopeptidase [LAP], NAG and URE) and P-acquisition enzyme (AP) were measured by the modified methods of Sinsabaugh, Reynolds, and Long (2000) and German et al. (2012). Firstly, one g soil sample was homogenized with 50 ml distilled water for the extraction of enzymes. Subsequently, 50 μl soil slurries, 100 μl of substrate solution and 100 μl of buffer were sequentially added for potential activity measurement. Standard curves of 7-amino-4-methylcoumarin for LAP and 4-methylumbelliferone for other enzymes were made. After 30 min, 60 min and 120 min incubation at 20 ℃, all plates were measured fluorometrically (excitation 355 nm, emission 460 nm) by the automated fluorometric plate reader (Fluoroskan, ThermoFisher, USA). The PO activity was analyzed by spectrophotometer with the substrate of L-3,4-dihydroxy-

phenylalanine (DeForest, 2009). The URE activity was analyzed spectrophotometrically (667 nm) with the substrate of urea (Li et al., 2018c). The units of enzyme activities were presented as nmol g soil^{-1} h^{-1}.

We normalized soil enzyme activities of nutrients belonging to the same fvunctional group using the equation as described by Luo et al. (2017). For instance, the N-acquisition enzyme activity (nmol g soil^{-1} h^{-1}) was calculated as:

$$N - acq = \sqrt[3]{(URE \times LAP \times NAG)} \quad (1)$$

where URE, LAP and NAG represented urease, L-leucine aminopeptidase, and N-acetylglucosaminidase, respectively.

We standardized eight enzyme activities with Z-score transformation to obtain quantitative multifunctionality. Those standardize rates of enzyme activities were averaged to acquire the EMF (Bastida et al., 2017).

2.4. Analyses of soil microbial community structure and composition

Total soil DNA was extracted from 0.25 g fresh soil sample with the EZNA soil DNA isolation kit (Omega Bio-tek, Doraville, GA, USA). The universal primers 515F/907R (bacteria, GTGCCAGCMGCCGCGG/CCGTCAATTCMTTTRAGTTT) were used to amplify the 16S rRNA gene within the V4–V5 hypervariable region, and the primers ITS1F/ITS2R (fungi, CTTGGTCATTTAGAGGAAGTAA/GCTGCGTTCTTCATCGATGC) were used to amplify 18S rRNA gene within the ITS1 regions (Li et al., 2018c). The amplicons of bacteria and fungi were sequenced (2 × 250) at the Illumina MiSeq PE300 platform.

The sequenced data were quality-filtered by FASTP and merged by FLASH according to Perez-Jaramillo et al. (2019). Finally, we obtained a total of 690667 and 728343 high-quality sequences for bacteria and fungi, respectively. The sequences were

clustered into operational taxonomic units with the 97% similarity threshold, and taxonomic units were assigned by the Ribosomal Database Project classifier. The phylogenetic diversity (PD) index has been widely used as an important driver of ecosystem functionality (Luo et al., 2018). Hence, we used PD index to represent microbial diversity and analyze the relationship with EMF. All sequences used in the study were deposited at the Sequence Read Archive of the NCBI database (accession number SRP344154).

2.5. High-throughput quantitative PCR-based SmartChip analysis

A total of 66 functional genes including 35 C-cycling genes, 22 N-cycling genes, 9 P-cycling genes and one 16s rRNA were quantified through high-throughput quantitative PCR-based SmartChip analysis (Zheng et al., 2018). Amplification was conducted in a 100 nL reaction system on the Wafergen SmartChip Real-time PCR system. All qPCR reactions were conducted in triplicate for each primer set, and a non-template negative control was included for each run. The detailed program was as follows: initial heating at 95 ℃ for 5 min, thereafter denaturation of 40 cycles at 95 ℃ for 30 s, followed by annealing at 58 ℃ for 30 s and extension at 72 ℃ for 30 s. The melting process was automatically generated by the SmartChip qPCR Software. Melting peaks and amplification efficiencies less than 90% and greater than 110% were rejected when analyzing data. The threshold cycle CT (less than 31) was picked out for downstream analyses (Yue et al., 2015).

2.6. Statistical analyses

The differences of environmental factors, enzymes activities, EMF, the PD index, and the functional groups among treatment were test by one-way analysis of variance (ANOVA), soil warming, straw return impacts, and their interaction were calculated by two-way ANOVA. The beta diversity of the microbial community and its significance were

calculated by the principal coordinate analysis (PCoA) and the permutational multivariate analysis of variance (PERMANOVA) using R "vegan" (https://CRAN.R-project.org/package=vegan) (Oksanen et al., 2012). The relationships between microbial diversities, functional groups and EMF index, or between functional groups of C, N, P cycling and corresponding enzymes activities were tested by linear regressions using R "ggplot2". The correlations between EMF and environmental factors were calculated by the Pearson's correlation test, and the correlations between microbial community compositions and environmental factors were analyzed by the mantel test using R "ggcor" (Huang et al., 2020). The data analyses were mainly conducted by R software version 4.0.3 and SPSS 22.0.

3. Results

3.1. Soil and plant properties

Soil warming (warm and warm-straw) increased the soil temperature by 3.5 ℃ at 0-20 cm compared to the ambient temperature, while straw return decreased the temperature by 1.0 ℃ compared to straw removal under ambient condition ($p < 0.05$, Fig. 1a, Table 1). Soil WFPS was the highest in ambient-straw, and the lowest in warm treatment. Warming decreased soil WFPS by 12% compared to ambient condition ($p < 0.01$, Fig. 1b). There was interaction between warming and straw return in soil WFPS ($p = 0.04$). Straw return increased soil WFPS under ambient condition ($p < 0.05$), while had no effect on it under warming condition ($p > 0.05$). Soil warming decreased the SOC, MBC, aboveground biomass by 18%, 40%, and 26% under straw removal condition, and decreased the root biomass by 30% under straw removal and return conditions ($p < 0.01$, Fig. 1c, e, i, j). Straw return increased soil TN by 10% under ambient and warming conditions ($p < 0.01$, Fig. 1d), but had no effects on other parameters.

3.2. Soil extracellular enzymes activities and ecosystem multifunctionality

The activities of BG, BX, CBH, LAP, and AP were increased under straw return (ambient-straw and warm-straw) compared to straw removal, while decreased under soil warming (warm and warm-straw) compared to the ambient temperature (Fig. 2). Soil warming reduced the C-acquisition enzyme activity by 26%, while straw return increased it by 13% ($p < 0.05$, Fig. 2a). There was interaction between warming and straw return on N-acquisition enzyme activity ($p < 0.05$, Fig. 2b). Soil warming reduced N-acquisition enzyme activity under straw removal ($p < 0.05$), while had no effect on it under straw return. The P-acquisition enzyme activity was reduced under warming by 13% ($p < 0.05$, Fig. 2c), while no difference was found under straw return. Finally, soil warming reduced the EMF under straw removal and straw return condition, while straw return increased it under warming condition ($p < 0.01$, Fig. 2d).

3.3. Microbial diversity and its relationships with ecosystem multifunctionality

The bacterial and fungal PD indexes responded differently to soil warming and straw return (Fig. 3a, b). Straw return (ambient-straw and warm-straw) increased the bacterial PD index by 3.6% compared to straw removal ($p < 0.05$), while warming had no effect on it. There was interaction of the fungal PD index between warming and straw return ($p < 0.05$). Soil warming reduced the fungal PD index under straw removal condition, and straw return reduced it under ambient condition ($p < 0.05$). The EMF was increased with the increasing bacterial ($R^2 = 0.35$, $p < 0.05$, Fig. 3c) and fungal ($R^2 = 0.49$, $p < 0.01$, Fig. 3d) PD indexes (Fig. 3c, d).

3.4. Microbial community composition and structure

The bacterial community was dominated by Proteobacteria (26%) and Acidobacteria (22%), and the fungal community was dominated by Ascomycota (77%), followed by Mortierellomycota (11%) (Figs. S3 and S4). Soil warming (warm and warm-straw) decreased the abundances of Cucurbitariaceae, Plectospaerellaceae, and Rhizopodaceae on fungal family level compared to ambient temperature (Fig. S6). Straw return (ambient-straw and warm-straw) increased the abundances of Xanthobacteraceae, Xanthomonadaceae, Trichocomaceae, and Pleosporales, but decreased the abundances of Vicinaminacteraceae, Nitrosomonadaceae, and Cordycipitaceae when compared to straw removal (Figs. S5 and S6).

Effects of warming and straw return on microbial community structure were determined by PCoA at OTU level. PCo1 (24%) and PCo2 (17%) accounted for 41% of bacterial community variation (Fig. 4a), and PCo1 (23%) and PCo2 (16%) accounted for 39% of fungal community variation (Fig. 4b). The bacterial communities related to straw return were separated from straw removal plots along PCo1 axis ($p < 0.001$; Fig. 4a). Soil fungal communities were also separated into straw removal and straw return along PCo1 axis ($p < 0.001$; Fig. 4a). The fungal communities associated with warming were separated from ambient temperature along PCo2 ($p < 0.01$; Fig. 4b).

3.5. Abundance of microbial functional genes

Most functional gene abundances associated to C degradation, N fixation, nitrification, denitrification, and P mineralization showed the trend of ambient-straw > ambient > warm-straw > warm in the heatmap (Fig. 5). Soil warming decreased the microbial functional genes of C-cycling under straw removal and straw return, while straw return increased it under ambient and warming conditions ($p < 0.01$, Fig. 4a). Soil warming decreased the N-

cycling genes under straw removal, while straw return increased it under ambient and warming conditions ($p < 0.01$, Fig. 4b). Microbial functional genes of P-cycling were decreased under soil warming (warm and warm-straw), while increased under straw return (ambient-straw and warm-straw) ($p < 0.01$, Fig. 4c). The C-cycling gene positively related to C-acquisition enzyme activity ($R^2 = 0.66$, $p < 0.01$), but there were no linear relationships between N-cycling gene and N-acquisition enzyme activity or between P-cycling gene and P-acquisition enzyme activity (Fig. S7). The EMF index was increased with the increasing microbial functional genes related to C-, N-, and P-cycling ($R^2 = 0.53$, $p < 0.01$, Fig. S8).

3.6. Drivers of EMF and microbial community compositions

The EMF index increased with increasing WFPS, SOC, MBC, aboveground biomass, and root biomass, while decreased with increasing soil temperature, and NO_3^- (Fig. 6, all $p < 0.05$). We further analyzed the drivers of bacterial and fungal communities through the mantel test. Soil bacterial community was strongly related with WFPS, soil temperature, NH_4^+, and root biomass (all $p < 0.05$). Fungal community was related with soil temperature, WFPS, SOC, MBC and root biomass (all $p < 0.05$). Overall, soil temperature, WFPS, and root biomass were important drivers for microbial communities and EMF. Additionally, there were strong relationships among soil and plant properties based on the pairwise comparisons (Fig. 6). For instance, soil WFPS increased with increasing SOC, TN, MBC, NH_4^+, shoot biomass, and root biomass, while decreased with higher NO_3^-.

4. Discussion

4.1. Warming decreased soil microbial diversity, functional genes, and ecosystem multifunctionality

Soil microbes are essential components of the Earth's biodiversity and play important roles for element cycling and nutrient supplying (Zhao et al., 2014). Soil warming affected the fungal community structure and decreased its alpha diversity under straw removal condition (Figs. 3 and 4). This was consistent with Heimann and Reichstein (2008) and Zhang et al. (2016b), who demonstrated that frequent extreme warming might lead to a higher decline of microbial diversity. However, bacterial community diversity and structure were independent on soil warming (Fig. 3 and 4), suggesting that the structures and diversity of bacterial and fungal community can response differently to warming. Within the fungi-bacterial communities, bacteria are frequently considered to be r-strategy microorganisms and fungi are K-strategy microorganisms. Thus, bacteria may be more resistant to environmental disturbance through fast adaptation and rapid growth than fungi in the short term (Sun et al., 2021). The multiple C cycling genes (e.g. gam, xylA, naglu) contributing to the SOC degradation, N cycling genes (e.g. nifH, nxrA, narG) regulating N fixation, nitrification and denitrification processes, and P cycling genes (e.g. ppk3, phoD, phnK) participating in the process of P mineralization (Fan et al., 2020), dropped under soil warming (Fig. 5). This suggested that increased soil temperature could create an adverse environment for microbial growth and activity, which may weaken the processes of organic matter decomposition and nutrient availability. However, inconsistent with our results, insignificant impacts of warming on functional groups were detected in previous studies conducted at stenothermal (Zhang et al., 2005) or alpine meadow (Tang et al., 2019). Soil temperature increased in these studies were approximate 1-2 ℃, lower than ours (~3.5 ℃), likely explaining such conflict. Supportively, a 3-year field experiment in Tibetan indicated that increased soil temperature of approximate 5 ℃ significantly declined the functional genes of C and N cycling (Yue et al., 2015). Soil extracellular enzymes can serve as an indicator of microbial functioning in responses to climate change, as they reflect the

metabolic requirements of the microbial community (Zhou et al., 2013). A global meta-analysis showed that the responses of C-, N-, and P-acquisition enzymes to warming depend on warming duration and magnitude (Meng et al., 2020). Soil warming (+3.5 ℃) decreased C-, N-, P-acquisition enzyme activities, resulting in the reduction of ecosystem multifunctionality (Fig. 2). This might link to warming-induced reduction of microbial activity and root biomass, which result in less enzymes synthesis and secretion (Allison & Treseder, 2008; McDaniel & Kaye, 2013).

Microbial communities and enzyme activities are strongly influenced by soil physico-chemical properties, vegetation, and substrate quantity and quality (Schindlbacher et al., 2011; Zhou et al., 2013). Soil temperature, WFPS, SOC, MBC, and root biomass were the dominant factors in altering microbial communities and EMF index in this study (Fig. 6). Firstly, warming reduced soil moisture by 12% compared with ambient condition, directly changed the habitats of soil microorganisms and enzymes, and then influenced microbial community and activity, and consequently EMF index (Singh et al., 2010). The MBC content was decreased with reducing soil moisture under warming (Fig. 6), indicating that warming combined with drought led to a reduction of microbial abundance and activity (Zhang et al., 2005). Secondly, warming reduced wheat root biomass by 24% (Fig. 1), which may result in a decrease in rhizodeposition. Consequently, less available C dropped in the rhizosphere, leading to the decreased microbial and enzymatic activity (Bai et al., 2010; Li et al., 2018; Tang et al., 2019). This was also supported by the positive relationship between wheat root biomass and MBC (Fig. 6). Finally, soil drying and root biomass reduction caused by warming limited the diffusion of nutrients and the transfer of organic matter from plants to soil (Zhou et al., 2013), resulting in the reduction of SOC and nutrient availability (Fig. 1), and thereby affected microbial and extracellular processes (e.g. soil extracelluar enzymes and EMF).

4.2. Straw return increased soil microbial community diversity, functional genes, and ecosystem multifunctionality

Crop straw contains large portions of labile C and nutrients (Navarro-Noya et al., 2013). Straw returned to the field provides energy and nutrients for soil microbes, and thereby reshape the soil microbial community (Chen et al., 2017). Previous studies have indicated that straw return would benefit for stimulating bacterial community growth and diversity (Guo et al., 2016; Jensen et al., 2021). Similarly, straw return changed bacterial community (Fig. 4) and increased its alpha diversity by 3.6% (Fig. 3). Most genes associated with C degradation, N fixation, nitrification, denitrification, and P solubilization were increased under straw return (Fig. 5), which corresponded well with the positive impacts on soil functional microorganisms (Ding et al., 2018). However, such studies mainly focused on a single functional process, targeting only a small number of microbial functional genes such as nitrifiers (Liu et al., 2016), denitrifiers (Yang et al., 2017), and methanotrophs (Zheng et al., 2008). Comprehensive understanding of straw return impacts on soil functional groups can be obtained by detecting a whole set of microbial genes related to C-, N-, P-cycling simultaneously (Fig. 5). Straw return reduced the fungal diversity under ambient temperature (Fig. 3), indicating that fungi responded differently compared with bacteria. Generally, there are two phases in the process of residue decomposition. Bacteria dominate the initial phase and fungi dominate the later phase, as bacteria grow faster and play more important roles in labile fractions mineralization (Maschner et al., 2003). Straw return may also enrich certain species of fungi, which gradually become dominant, and thus resulted in the reduction of fungal diversity (Su et al., 2020). The fungal phylum of Ascomycota, with higher relative abundance in ambient-straw (81%) than in ambient plots (77%, Fig. S3), might become dominant under straw return.

Soil extracellular enzymes mainly synthetized and secreted by microbes, are the key drivers of SOM formation and decomposition (Meng et al., 2020). Straw return increased the enzyme activities of BG, BX, and CBH (Fig. 2), which was consistent with Zhao et al. (2016) that straw return combined with N fertilizer application mainly stimulated the C-acquisition enzymes activity. The increase in biodiversity of specific microbes with C-cycling functional genes induced by straw return resulted in more synthesis and secretion of C-acquisition enzymes (Ding et al., 2018). This was supported by the strong realtionship between C-cycling gene and C-acquisition enzyme activity (Fig. S7). Finally, with the increase of C-acquisition enzyme activity, the EMF index was increased under straw return (Fig. 2), indicating that straw management is beneficial to maintain soil biodiversity and further agroecosystem functions related to nutrient cycling. However, the large amounts of straw-derived C may cause strong N competition among crop seedings and microbes through microbial immobilization (Yadvinder et al., 2004), alter greenhouse gas emissions (Chen et al., 2021), and stimulate mineralization of native SOC via priming effect (PE) (Kuzyakov et al., 2009a). Further integrated investigations of straw return are still needed to achieve a complete evaluation of its impacts on agroecosystem productivity.

Straw return decreased soil temperature, but increased soil moisture (Fig. 1), which effectively alleviated warming-induced soil drought and high temperature. Soil temperature and WFPS were dominant factors for both bacterial community and EMF index (Fig. 6). This indicated that the increases of soil bacterial diversity and EMF index induced by straw return were primarily due to the maintenances of soil temperature and moisture. Additionally, straw return increased soil TN and nutrient availability through increasing organic matter input and maintaining soil temperature and moisture (Fig. 1, Said-Pullicino et al., 2014; Mu et al., 2016), further stimulating soil microbial growth and enzymes secretion. Overall, our study provided evidences that straw return created a favorable habitat

for microorganisms, increased bacterial diversity, functional genes, and consequently raised the EMF mediated by soil enzyme activities (Fig. 7). Thereby, straw return counteracted the negative effects of soil warming on microbial community and ecosystem functionality related to nutrient cycling, partly supporting our first hypothesis.

4.3. Soil microbial biodiversity is the key driver of ecosystem multifunctionality

Soil biodiversity plays a crucial role in controlling multiple agroecosystem services, such as crop production and nutrient cycling (Fan et al., 2020). Therefore, understanding the role and importance of biodiversity in managed soils is essential to accurately project the responses of agroecosystems to climate change. In support of our second hypothesis, soil bacterial and fungal diversities were both positively correlated with the EMF index mediated by soil enzyme activities in agricultural soils (Fig. 3). Generally, the higher functional community redundancy, namely various species performing the same functions, may serve as a buffer against the impact of biodiversity loss on soil functioning (Kuzyakov et al., 2009b; Luo et al., 2018). However, agroecosystems usually have the lower biodiversity compared with natural ecosystems (Strickland et al., 2009; Peters et al., 2019). Therefore, the positive relationships between soil microbial diversities and EMF index may be attributed to the lack of multifunctional redundancy in agroecosystems (Tuck et al., 2014; Tsiafouli et al., 2015). The microbial functional genes related to C, N, P cycling were also positively correlated with EMF index (Fig. S8), pointing to a tight linkage between microbial functional groups and ecosystem functioning (Yergeau et al., 2007; Yin et al., 2015). Similarly, with the increasing diversities and abundances of C and N cycling genes, the corresponding soil enzymes activities increased under long-term fertilization (Ding et al., 2018). These findings highlight the importance of soil biodiversity of microorganism and functional genes in maintaining agroecosystem functioning related to

nutrient cycling. Warming reduced fungal diversity, functional gene abundance, and ecosystem multifunctionality, suggesting that increased temperature might create an adverse environment for microbial growth and activity, which could weaken nutrient availability and further ecosystem services. Straw return matched with higher bacterial biodiversity and the abundances of C-, N-, P-cycling genes compared to straw removal, and thereby offset the adverse effect of warming on ecosystem multifunctionality (Fig. 7). Our results demonstrated the possibility to increase soil biodiversity and further ecosystem services related to organic matter decomposition and nutrient turnover by straw management under climate warming.

5. Conclusions

Soil warming reduced the fungal diversity, microbial functional genes related to C, N, P cycling and EMF compared to ambient temperature, primarily due to the lower soil moisture, nutrient availability, and root-derived labile organic matter inputs. The strong relationships between microbial diversities, functional genes abundances and EMF further highlighted the importance of soil biodiversity in maintaining agroecosystem functions of nutrient cycling. Under warming and ambient temperature, straw return resulted in higher bacterial diversity and microbial functional genes of C, N, P cycling compared with straw removal, and subsequently increased the EMF, attributing to the maintenance of soil temperature and moisture regimes and nutrient supplement. Consequently, straw return created a favorable habitat for microorganisms, and thereby mitigated the adverse effects of warming on soil microbial communities and ecosystem functionality. Our results demonstrated the possibility to increase soil biodiversity and further ecosystem services related to organic matter decomposition and nutrient turnover by straw management under climate warming. However, the large amount of straw-derived C may result in serious N competition between

soil microbes and crop seedings, alter greenhouse gas emissions, and stimulate native SOC mineralization via PE. Further integrated investigations of straw return are urgently needed to achieve a complete evaluation of the trade-off effect in agroecosystems under warming conditions.

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Authors' contributions

Conceptualization, GW, YW, SZ; Formal analysis, GW, JL, DZ; investigation, GW, ZL, YX; Writing-original draft, GW, JL; Writing-review and editing, YK, KM, SZ, YW; Supervision, SZ and YW; Founding acquisition, SZ and YW.

Competing interests

The authors declare no competing interests.

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Figures captions

Fig. 1 Soil physicochemical characteristics of 0-20 cm depth and plant properties. Soil temperature (a); WFPS, water-filled pore space (b); SOC, soil organic C (c); TN, total N (d); MBC, microbial biomass C (e); DOC, dissolved organic C (f); NH_4^+, ammonium (g); NO_3^-, nitrate (h); Above Bio, aboveground biomass (i); Root Bio, root biomass (j). Values are means ± standard errors (n = 3). The p values of warming, straw return and their interactions were calculated by two-way ANOVA. Lowercase letter shows significant difference among treatments ($p \leq 0.05$, one-way ANOVA).

Fig. 2 Heat map indicates the changes of multiple enzymes activities, and box plots show the enzymes activities of C-acquisition (a), N-acquisition (b), P-acquisition (c) and ecosystem multifunctionality (d). BG, β-1,4-glucosidase; BX, β-1,4-xylosidase; PO, phenol oxidase; CBH, β-D-cellobiosidase; URE, urease; LAP, L-leucine aminopeptidase; NAG, β-1,4-N-acetylglucosaminidase; AP, alkaline phosphatase. The p values of warming, straw return and their interactions were calculated by two-way ANOVA. Lowercase letter shows significant difference among treatments ($p \leq 0.05$, one-way ANOVA). The solid lines in box plots represent median values.

Fig. 3 The phylogenetic diversities of bacteria (a) and fungi (b), and the relationships of bacterial (c) and fungal (d) phylogenetic diversities with the ecosystem multifunctionality. The p values of warming, straw return and their interaction were calculated by two-way ANOVA. Lowercase letter shows significant difference among treatments ($p \leq 0.05$, one-way ANOVA). The solid lines in box plots represent median values. The red lines are fitted by ordinary least square's regressions. Shaded areas are 95% confidence interval of the fitting.

Fig. 4 The principal coordinate analysis (PCoA) of bacteria (a) and fungi (b) according to Bray-Curtis distance. Lowercase letter shows significant difference among treatments ($p \leq 0.05$, one-way PERMANOVA). The p values of warming, straw return were calculated by two-way PERMANOVA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Fig. 5 Heat map showing the changes of multiple functional genes and box plots showing the relative abundances of C cycling genes (a), N cycling genes (b), and P cycling genes (c). p values of warming, straw return and their interactions were calculated by two-way ANOVA. Lowercase letter shows significant difference among treatments ($p \leq 0.05$, one-way ANOVA). The solid lines in box plots represent median values.

Fig. 6 The environmental drivers of microbial community compositions and ecosystem multifunctionality (EMF). Pairwise correlations of environmental factors are shown, with color gradient representing Pearson's correlation coefficient. EMF is related to environmental factor by Pearson's correlation analysis. Bacterial and fungal community composition on OTU level is correlated to environmental factors by Mantel test. Edge width represents the Pearson's and Mantel's coefficients, and edge color relates to the statistical significance. SOC, soil organic C; TN, total N; WFPS, water-filled pore space; Tem, temperature; MBC, microbial biomass C; DOC, dissolved organic C; Above bio, aboveground biomass; Root bio, root biomass.

Fig. 7. Conceptual model for understanding the linkages between environmental factors, soil biodversity, and ecosystem multifunctionality under soil warming and straw return. Red and blue arrows indicate positive and negative influence, respectively. SOC, soil organic C;

TN, total N; MBC, microbial biomass C; Above bio, aboveground biomass; Root bio, root biomass.

Figure captions

Fig. 1

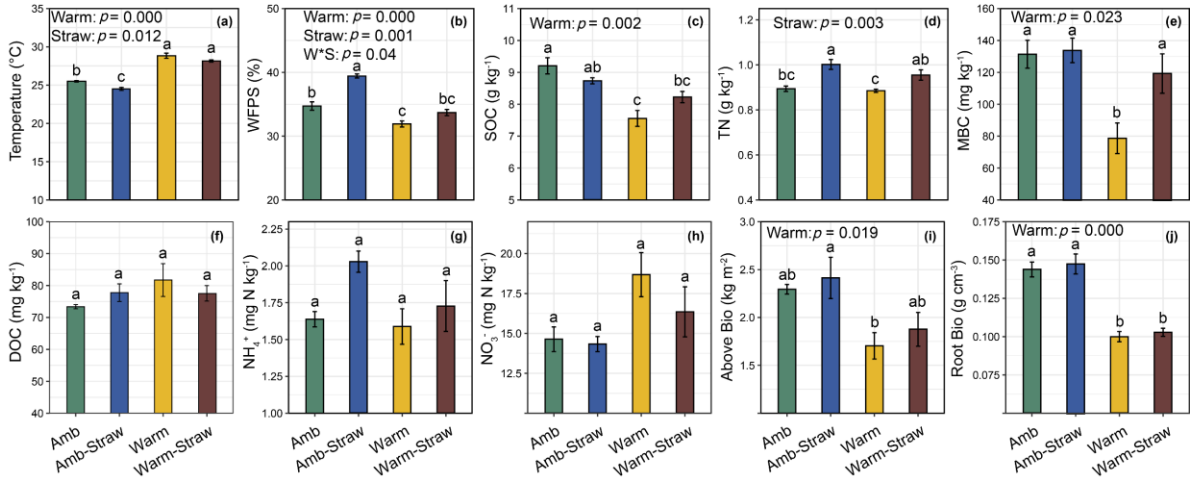

Fig. 2

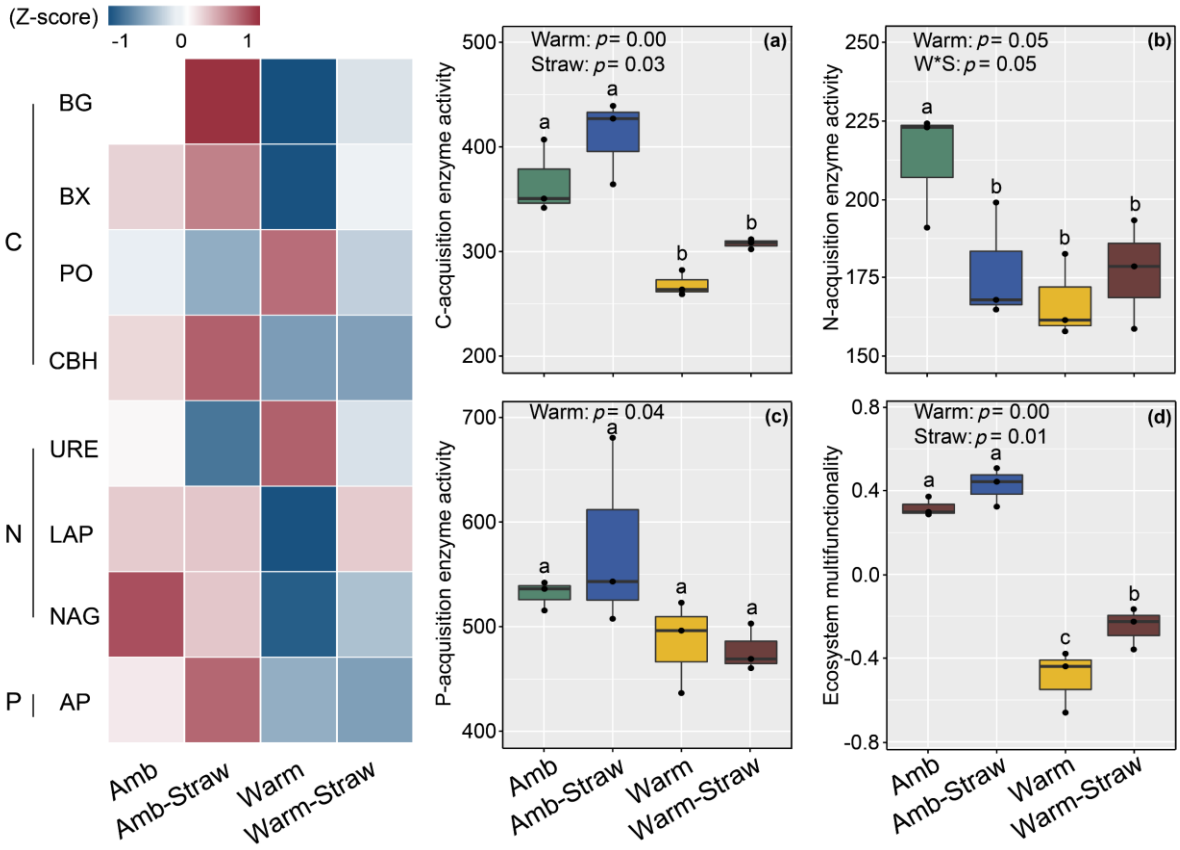
(Z-score)
-1
0
1
BG
BX
PO
CBH
C
URE
LAP
NAG
N
AP
P
Amb
Amb-Straw
Warm
Warm-Straw
Warm: p= 0.00
Straw: p= 0.03
(a)
C-acquisition enzyme activity
Warm: p= 0.05
W*S: p= 0.05
(b)
N-acquisition enzyme activity
Warm: p= 0.04
(c)
P-acquisition enzyme activity
Warm: p= 0.00
Straw: p= 0.01
(d)
Ecosystem multifunctionality

Fig. 3

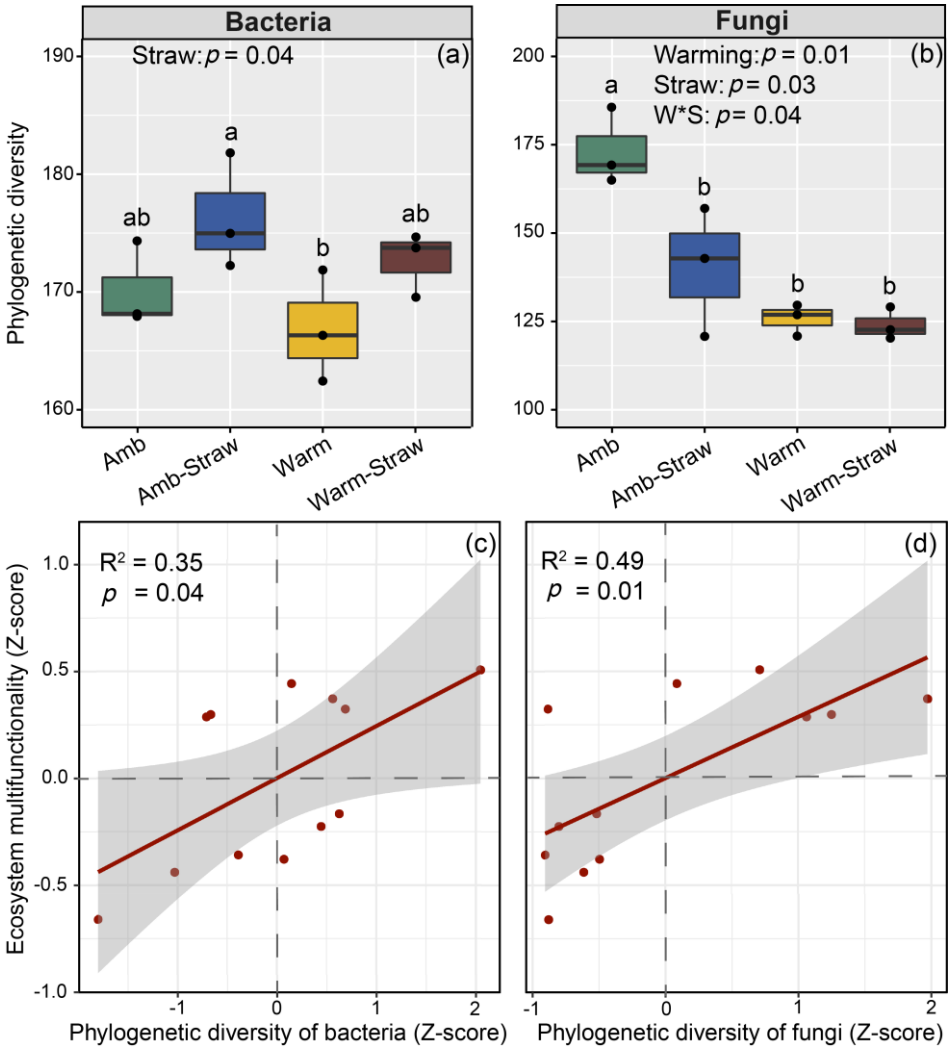
Bacteria
Straw: p = 0.04
(a)
Phylogenetic diversity
190
180
170
160
ab
a
b
ab
Amb
Amb-Straw
Warm
Warm-Straw
Fungi
Warming: p = 0.01
Straw: p = 0.03
W*S: p = 0.04
(b)
200
175
150
125
100
a
b
b
b
Amb
Amb-Straw
Warm
Warm-Straw
(c)
R² = 0.35
p = 0.04
Ecosystem multifunctionality (Z-score)
1.0
0.5
0.0
-0.5
-1.0
-1
0
1
2
Phylogenetic diversity of bacteria (Z-score)
(d)
R² = 0.49
p = 0.01
-1
0
1
2
Phylogenetic diversity of fungi (Z-score)

Fig. 4

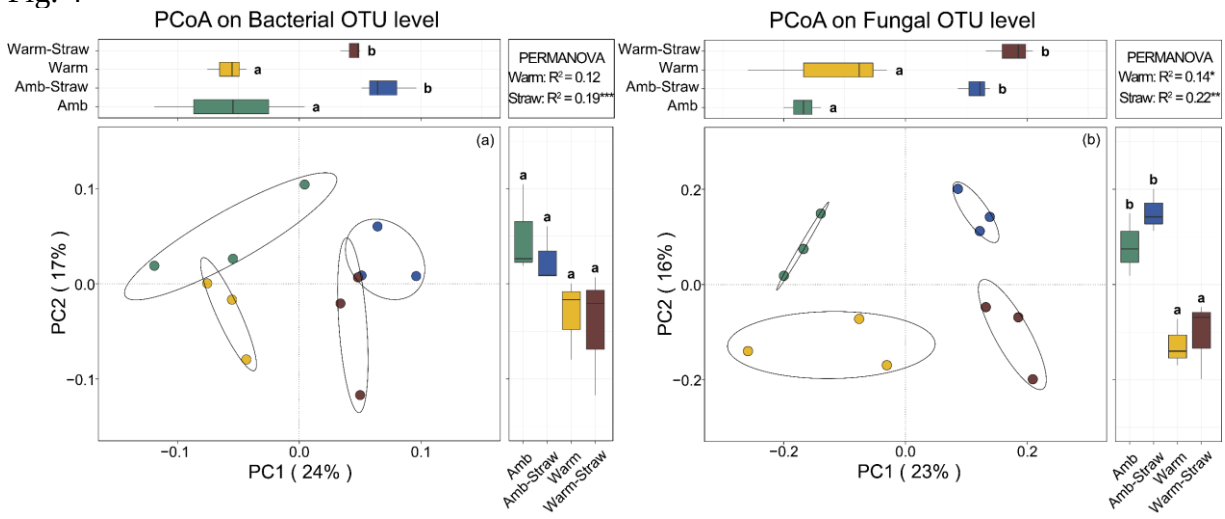
PCoA on Bacterial OTU level
Warm-Straw
Warm
Amb-Straw
Amb
PERMANOVA
Warm: R² = 0.12
Straw: R² = 0.19***
(a)
PC2 (17%)
PC1 (24%)
PCoA on Fungal OTU level
PERMANOVA
Warm: R² = 0.14*
Straw: R² = 0.22**
(b)
PC2 (16%)
PC1 (23%)

Fig. 5

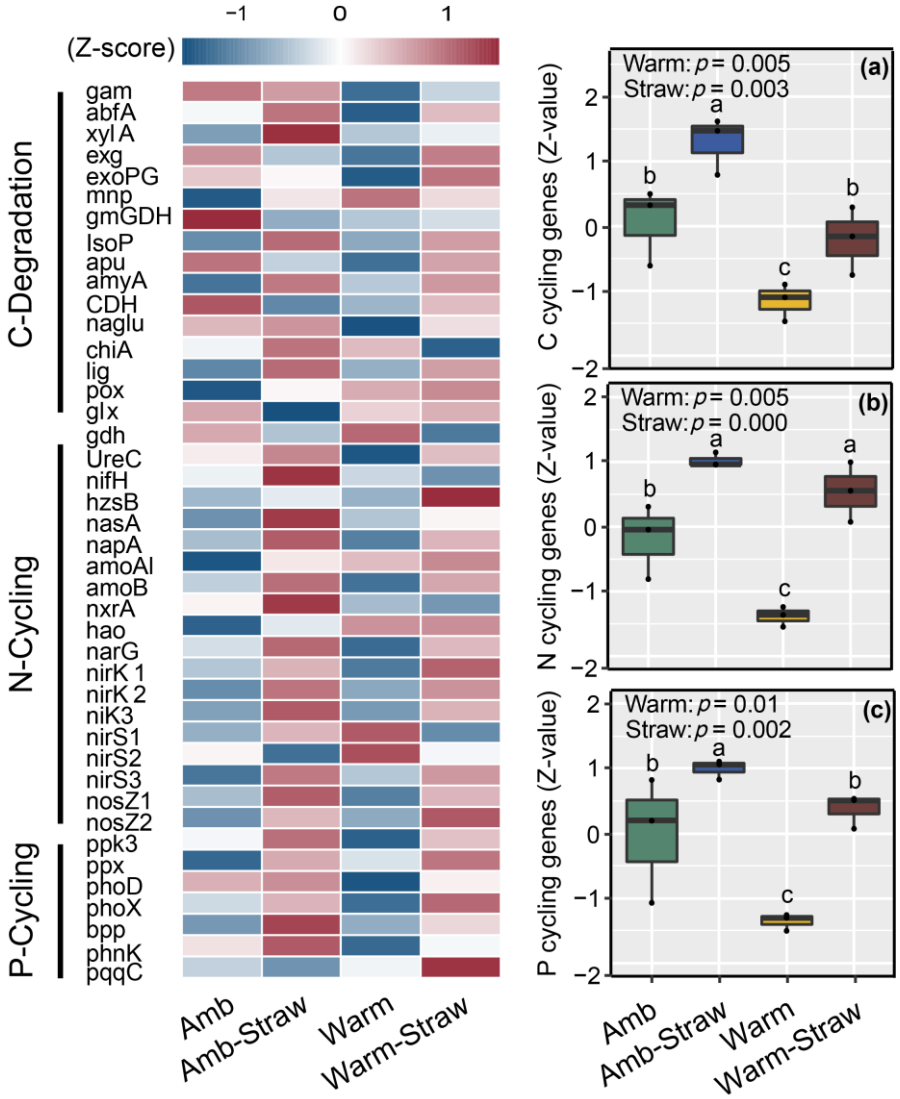
(Z-score)
−1
0
1
C-Degradation
N-Cycling
P-Cycling
gam
abfA
xylA
exg
exoPG
mnp
gmGDH
IsoP
apu
amyA
CDH
naglu
chiA
lig
pox
glx
gdh
UreC
nifH
hzsB
nasA
napA
amoAI
amoB
nxrA
hao
narG
nirK1
nirK2
niK3
nirS1
nirS2
nirS3
nosZ1
nosZ2
ppk3
ppx
phoD
phoX
bpp
phnK
pqqC
Amb
Amb-Straw
Warm
Warm-Straw
Warm: p = 0.005
Straw: p = 0.003
(a)
C cycling genes (Z-value)
Warm: p = 0.005
Straw: p = 0.000
(b)
N cycling genes (Z-value)
Warm: p = 0.01
Straw: p = 0.002
(c)
P cycling genes (Z-value)

Fig. 6

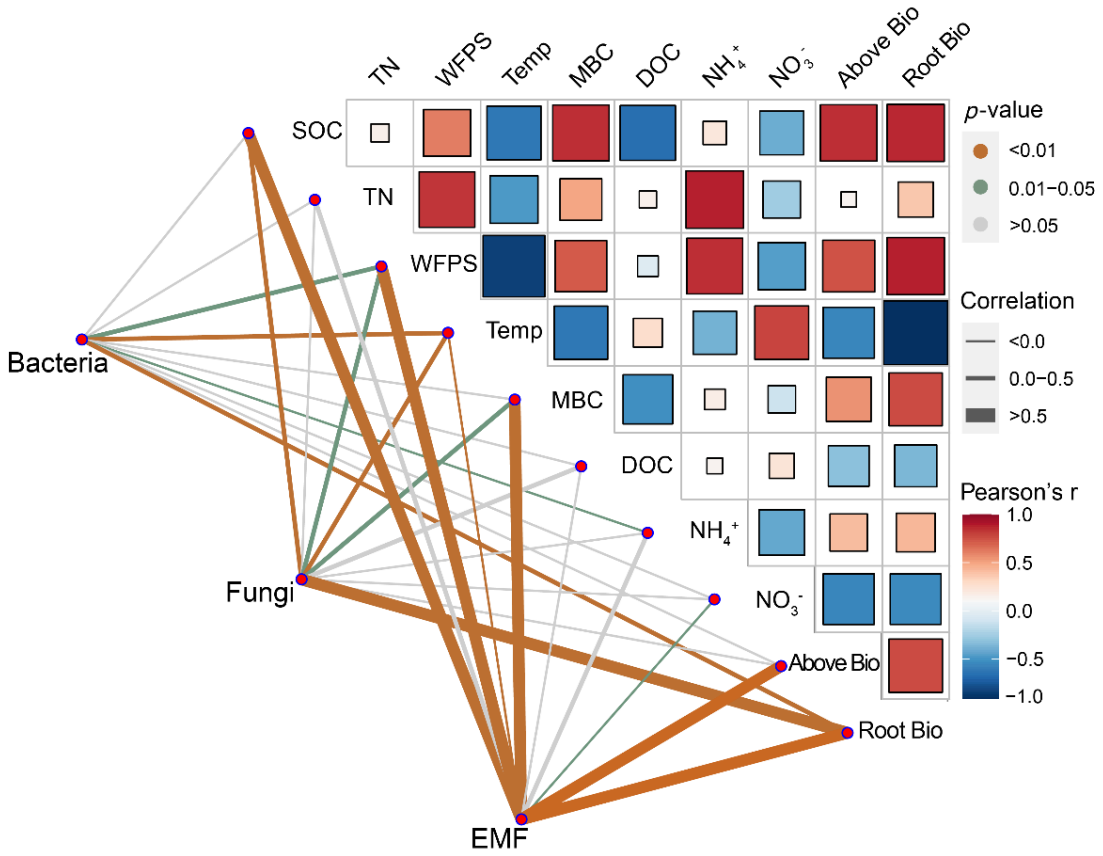
TN
WFPS
Temp
MBC
DOC
NH_4^+
NO_3^-
Above Bio
Root Bio
SOC
TN
WFPS
Temp
MBC
DOC
NH_4^+
NO_3^-
Above Bio
Root Bio
Bacteria
Fungi
EMF
p-value
<0.01
0.01–0.05
>0.05
Correlation
<0.0
0.0–0.5
>0.5
Pearson's r
1.0
0.5
0.0
−0.5
−1.0

Fig. 7

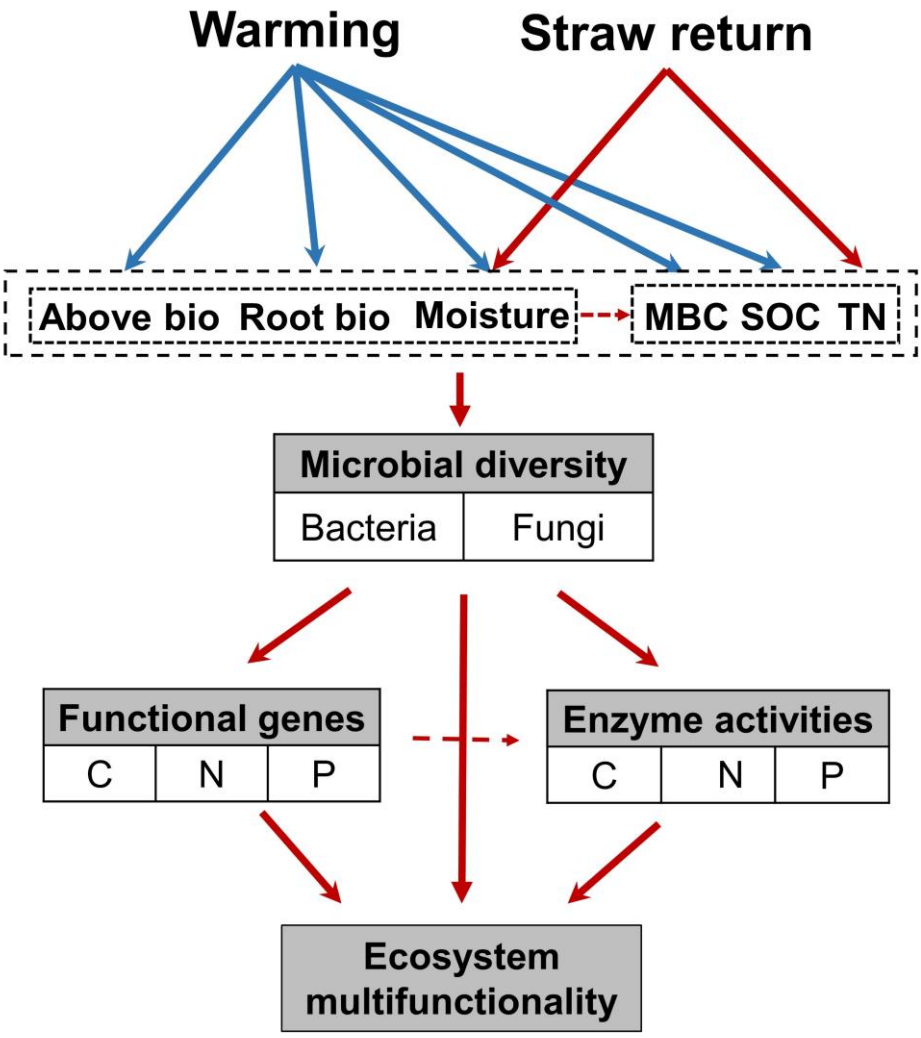
Warming
Straw return
Above bio
Root bio
Moisture
MBC
SOC
TN
Microbial diversity
Bacteria
Fungi
Functional genes
C
N
P
Enzyme activities
C
N
P
Ecosystem multifunctionality

Table 1 Warming (W), straw return (S), and their interactive effects on soil properties, microbial diversities, enzyme activities, and ecosystem multifunctionality

Variables	Factors		
	W	S	W*S
Temperature	0.000***	0.012*	0.552
Water-filled pore space	0.000***	0.001**	0.04*
Soil organic C	0.002**	0.699	0.052
Total N	0.215	0.003**	0.496
Microbial biomass C	0.023*	0.108	0.148
Dissolved organic C	0.326	0.976	0.304
Soil NH_4^+	0.239	0.094	0.400
Soil NO_3^-	0.061	0.373	0.488
Above biomass	0.019*	0.484	0.903
Root biomass	0.000***	0.524	0.524
Bacterial diversity	0.226	0.04*	0.938
Fungal diversity	0.001**	0.026*	0.04*
C-cycling enzyme activity	0.000***	0.031*	0.881
N-cycling enzyme activity	0.05*	0.230	0.05*
P-cycling enzyme activity	0.043*	0.544	0.403
Ecosystem multifunctionality	0.000***	0.01*	0.283
C-cycling genes	0.005**	0.003**	0.534
N-cycling genes	0.005**	0.000***	0.140
P-cycling genes	0.01*	0.002**	0.263

*, ** and *** indicates significant differences at 0.05, 0.01 and 0.001 levels based on two-way ANOVA.