Fungi Outcompete Bacteria for Straw and Soil Organic Matter Mineralization

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Research

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Abstract

Background: Understanding the effects of straw return and nitrogen (N) fertilization on soil organic matter (SOM) transformations will help to mitigate climate change and maintain crop production and soil function. A 100-day soil incubation experiment was conducted using a two-factorial design with three fertilization levels and four ^{13}C -labeled maize straw and N addition treatments. The competition and contributions of the bacterial and fungal communities were assessed with relation to straw mineralization.

Results: Mineral fertilizer alone and with straw increased straw decomposition by 59% and 55% and SOM mineralization by 27% and 37%, respectively, compared with the unfertilized soil, due to raised β -N-acetylglucosaminidase and cellobiohydrolase activities. Conversely, priming effect was decreased by 59% and 39%, respectively. Priming effect increased with higher N additions and decreased with lower N additions because an improved C:N ratio for microorganisms. Straw additions increased bacterial and fungal abundance by 1.4 and 4.9 times. Fungal diversity decreased with N fertilization because lower C:N ratios increased the bacterial competition. Bacterial abundance decreased but diversity increased with the duration of incubation as bacteria preferred to utilize labile organic compounds abundant in the initial stages. Along with labile organic compounds depletion, fungal abundance was increased. Firmicutes, Actinobacteria, and Proteobacteria bacterial as well as Ascomycota, Basidiomycota, and Mucoromycota fungi dominated straw and SOM decomposition. Firmicutes were mostly involved in straw and SOM mineralization on day one because of their capacity for labile compound decomposition. Integrated co-occurrence networks revealed that fungal taxa had a stronger correlation with straw decomposition than bacterial groups. Straw and N addition increased the number of negative edges among bacterial taxa but these decreased within fungal groups when compared to trials without straw and N. The ratio for pairwise correlations between abundant fungal taxa, straw, and SOM mineralization (29.9%) was greater than with bacteria (1.2%).

Conclusions: Straw with low N additions increased soil C sequestration by decreasing priming effect. Straw alone and with N addition decreased competition for C and N among fungal groups, but increased competition within bacterial taxa. Fungi outcompete bacteria for straw and soil organic matter mineralization in long-term fertilized soils.

Background

Fresh organic carbon (C) (e.g., straw) inputs accelerate soil organic matter (SOM) decomposition via biochemical priming mechanisms [1–3]. Priming intensity is strongly influenced by nitrogen (N) availability because of the close interactions between C and N cycling [4]. Straw (high C/N ratio) returns cause a N shortage, leading to intense competition among microorganisms for N as well as between microorganisms and plants [5], and this consequently retards microbial growth [6]. If N availability with the addition of straw meets microbial demands and stoichiometry, it facilitates the microbial decomposition of straw and reduces the priming effect (PE) of SOM [7–9]. Excessive mineral N

fertilization inhibits maize straw decomposition because high N levels repress ammonia metabolites and microbial decomposers [10]. Despite SOM mineralization being strongly correlated with microbial activity and abundance, little is known of how bacteria and fungi function regarding organic matter decomposition during its initial and later stages.

Bacteria and fungi have unique preferences for straw decomposition and respond unequally to N additions [11]. Generally, bacteria are mainly involved in the utilization of easily available compounds at the initial stage of straw decomposition, while fungi have an advantage in the decomposition of recalcitrant organics (e.g., lignocellulose and cellulose) in the later stages [7, 12, 13]. The Proteobacteria and Firmicutes bacteria phyla were found to increase after the addition of straw and decrease with incubation time, as they are copiotrophs [14, 15]. Conversely, the abundance of the Basidiomycota and Glomeromycota fungi were declined 1–3 days after straw additions and increased after 75 days [15]. The C/N ratio in fungi is higher than in bacteria, resulting in fungi being more active in low nutrients conditions when compared to bacteria, because of their lower N demands and higher C utilization efficiencies [16, 17]. Consequently, straw return combined with mineral N fertilizers decreased most fungal taxa and diversity but increased bacterial groups (e.g., Actinobacteria) [18–20]. Fungi dominate organic C decomposition with increasing soil C/N [16, 17]. To date, considerable uncertainty remains regarding the sequences and competition between bacterial and fungal functional groups (e.g., rare or abundant taxa) during organic matter mineralization in agricultural ecosystems.

Co-occurrence networks provide insights into the complex interactions among microbial groups and determine the relative contributions of bacterial and fungal taxa to SOM decomposition [21–24]. Low nutrient (C and N) contents result in strong competition between and within bacterial and fungal species for resources [11, 25, 26]. Crop residue with N additions altered the co-occurrence patterns with low negative associations by decreasing competition among microbial groups [27]. SOM mineralization is involved with a wide range of microbial groups. Some microbial groups vary with environmental conditions, which emphasized the specific roles of bacterial and fungal taxa in SOM mineralization [28]. The *Acidobacteria*, *Frateriia* and *Gemmatimonas* bacteria and *Chaetomium*, *Cephalotheca* and *Fusarium* fungi, were found to be the keystone taxa for strong associations with organic matter decomposition in acidic soils [27]. Soil pH is a key factor for regulating microbial community compositions. Compared to bacteria, fungi can handle a broader range of soils with high pH values [29]. Consequently, bacterial and fungal co-occurrence patterns during straw and SOM decomposition differed between acid and alkaline soils [16]. Unfortunately, it is still unclear whether similar microbial groups regulate SOM decomposition, particularly for rare or abundant taxa of bacteria and fungi in neutral or alkaline soils.

Straw and SOM is mineralized by bacteria and fungi in karst ecosystems, which differs from non-karst areas [30, 31]. The special binary hydrogeological structure of the karst in southwest China is conducive to the rapid loss of soil nutrients and water [32]. Consequently, excessive mineral and organic fertilization is used in this region to increase fertility [33]. Long-term mineral fertilizers aggravate soil acidification in the non-karst region [34]. N fertilizers that counteract soil acidification in karst ecosystems are neutralized by CaCO_3 and Ca released from rock dissolution [30, 35], consequently, decreasing the pH had little effect

on the bacterial and fungal activity. High Ca levels in the karst soils can stabilize and protect SOC by inhibiting its decomposition [36, 37]. Microbial activity and abundance are relatively higher in karst soils that contain abundant organic matter than in the same region of red soil with low SOC [38]. Consequently, straw additions to karst soils were more easily decomposed than in non-karst ecosystems, due to their neutral or alkaline pH and high microbial biomass [39–42]. However, the biological mechanisms that keystone species of bacteria and fungi utilize to regulate organic matter decomposition in karst soils have not yet been investigated.

This study aimed to evaluate how microorganisms respond to straw returns coupled with N additions in the long-term unfertilized and fertilized soils of the karst ecosystem. Microbial properties included microbial abundance, biomass; and composition: phospholipid fatty acids (PLFA), bacterial and fungal community compositions; and extracellular enzyme activities. We hypothesized that: (i) The initial SOM decomposition phase was dominated by an increasing bacterial abundance (e.g., Firmicutes) that consumes labile organic C at the initial stages, but increasing fungal biomass (e.g., Ascomycota) decomposes recalcitrant material in the later stages; (ii) Straw with low N levels decreased the priming effects (PE) because of a good C/N balance for the microorganisms, but increased PE with high N additions; (iii) straw alone and coupled with N increased enzyme activity (β -D-glucosidase, β -N-acetylglucosaminidase, and Cellobiohydrolase) as well as bacterial and fungal abundance, resulting in raising SOM decomposition; and (iv) Fungi played a more important role in straw and SOM decompositions than bacteria in the karst cropland because of their strong adaptability to soils with high levels of Ca.

Materials And Methods

Study site and experimental design

A field experiment for maize-soybean rotations was established in April 2006 at the Huanjiang Observation and Research Station for Karst Ecosystems of Academy of Sciences (24°44'N, 107°51'E), Huanjiang County, Guangxi Zhuang Autonomous Region, China on a typical Calcic Chromic Lamelic LUVISOL (Loamic, Cutanic) (WRB, 2014). The study site is a peak-cluster depression area with a subtropical monsoon climate. The mean annual temperature and precipitation are ~ 20 °C and ~ 1380 mm, respectively. The wet and hot season occurred from late April to the end of September and the dry season was from October to March.

Maize-soybean rotations are the main crop-cultivation system in this region. The experiment site included three fertilization treatments, based on a randomized complete block design with four replicate plots (each plot was 4 × 7 m). The fertilization treatments were: (i) no fertilization (Control); (ii) mineral fertilizers only (NPK): 200 and 22.5 kg N ha⁻¹ (urea), 39 and 26 kg P ha⁻¹ (superphosphate), as well as 100 and 56 K ha⁻¹ (potassium chloride) during the maize and soybean planting seasons, respectively; (iii) mineral fertilizers plus maize and soybean straws (NPK+Straw): 107 and 9 kg N ha⁻¹, 30 and 25 kg P ha⁻¹, as well as 40 and 52 K ha⁻¹, during the maize and soybean planting seasons, respectively. At the

same time, soybean straw (5.4 Mg ha^{-1} , that is, about 93 kg N ha^{-1} , 9 kg P ha^{-1} , and 60 kg K ha^{-1}) and maize straw (1.48 Mg ha^{-1} , that is, about $13.5 \text{ kg N ha}^{-1}$, 1 kg P ha^{-1} , and 4 kg K ha^{-1}) were applied during the maize and soybean planting seasons, respectively.

In the maize planting season, mineral and organic fertilizers were added three times: before planting the maize, during maize shoot growth, and during the reproductive growth stage. During the soybean planting season, mineral and organic fertilizers were applied two times, once before planting the soybeans and once at the pre-flowering stage. Organic and mineral fertilizers were added next to the plant by hand and covered with soil during each fertilization event. The experimental site was previously described in great detail [33, 43].

Soil samples (0-20 cm) were collected in November 2016 from plots with three fertilization treatments (Control, NPK, and NPK+Straw) using a soil corer with a diameter of 10 cm. The impurities, including animal (e.g., earthworm) and plant residues were removed using a 2-mm mesh sieve. The soil samples were stored at 4°C in a refrigerator until the start of the incubation experiment. The soil properties for the three fertilization methods are presented in the Additional File: Table S9.

Preparation of ^{13}C -labeled maize straw

The ^{13}C -straw preparation was conducted according to a previous study [44]. Briefly, at the seedling stage, maize was transferred to an automatically controlled gas-tight growth chamber system that was $1.1 \times 2.5 \times 1.8 \text{ m}$. ^{13}C -labeled CO_2 was introduced into the chamber, and was generated by acid base reactions with H_2SO_4 (0.5 M) and $\text{Na}_2^{13}\text{CO}_3$ (99 atom % ^{13}C ; Cambridge Isotope Laboratories, Tewksbury, MA, USA). The CO_2 concentration was monitored using an infrared analyzer (Shsen-QZD, Qingdao, China). When the CO_2 concentration was higher than $450 \mu\text{L L}^{-1}$, the excess CO_2 was absorbed automatically by pumping the chamber air through NaOH solution. There were temperature and humidity sensors (SNT-96S, Qingdao, China) inside and outside the chamber. The air-conditioning system controlled the internal air circulation and maintained the temperature between the inside chambers and ambient environment within 1°C . Maize was grown for 60 days in the closed transparent chamber to label the maize with $^{13}\text{CO}_2$ continuously. The preparation was completed when the maize was harvested. The maize straw was dried at 60°C and ground to $< 0.5 \text{ mm}$. The ^{13}C atom percent of the maize straw was 2.65% ^{13}C (C, 35.1%; N, 2.14%; C/N, 14.7%).

Incubation experiment

The following four treatments were conducted for each of the unfertilized and fertilized soils (Control, NPK, and NPK+Straw soil): (1) control with neither straw nor N fertilizer (S0+N0); (2) addition of ^{13}C -maize straw (2 g kg^{-1} dry soil) (S+N0); (3) addition of ^{13}C -maize straw (2 g kg^{-1} dry soil) and low N (0.07

g kg⁻¹ dry soil) (S+N1); and (4) addition of ¹³C-maize straw (2 g kg⁻¹ dry soil) and high N (0.2 g kg⁻¹ dry soil) (S+N2). There are 12 treatments (three soil fertilization levels × four addition treatments), with three replications. The soils from the three fertilization levels were pre-incubated at 25 °C for 7 days, before the incubation experiment.

The soil (200 g dry weight) samples for each fertilization condition (Control, NPK, and NPK+Straw) were placed in 1 L jars. An ammonium nitrate solution was prepared (low N: 0.07 g kg⁻¹; high N: 0.2 g kg⁻¹) and added to each jar together with or without the ¹³C-maize straw. The jars were then incubated in the dark at 25 °C for 100 days. Soil samples were collected after 0, 1, 5, and 100 days. Subsamples were stored at 4 °C in a refrigerator for the microbial biomass and soil enzyme activity analyses. Subsamples were further freeze-dried and stored at -80 °C for PLFA and DNA extraction. The rest of the soil samples were dried for analysis of the soil physical and chemical properties. The gas samples for the CO₂ and ¹³CO₂ were collected after 0, 2, 5, 7, 10, 15, and 20 days, and then collected every 10 days until 100 days.

Soil physicochemical analyses

Microbial biomass

The microbial biomasses of the samples were measured using a chloroform-fumigation extraction method. A 20 g sample of each soil, fumigated and non-fumigated, was extracted with 80 mL K₂SO₄ (0.5 M). A conversion factor of $k_{EC} = 0.45$ was used to determine the microbial biomass C (MBC) and microbial biomass N (MBN) concentrations. The contents of the dissolved organic C (DOC) and dissolved organic N (DON) were determined using the non-fumigated soils with a total C and N analyzer (Phoenix 8000, USA). Additionally, 6 mL of filtrate was absorbed and freeze-dried to measure the ¹³C-MBC and ¹³C-DOC using an isotope ratio mass spectrometer (Thermo Scientific MAT 253, USA).

Soil enzyme activities

The activities of three enzymes with β-D-glucosidase (BG), β-N-acetylglucosaminidase (NAG), and cellobiohydrolase (CBH) were measured. Fresh soil (1 g) was shaken off and added to 125 mL acetate buffer (50 mM, pH 5.0) to prepare the soil suspensions. Then, 200 μL aliquots of the suspension and 50 μL 200 μM substrates (BG: 4-MUB-β-D-glucoside; NAG: 4-Methylumbelliferyl N-acetyl-β-D-glucosaminide; CBH: 4-MUB-β-D-cellobioside) were added to 96-well microplates. The microplates were incubated in the dark at 25 °C for 4 h, and then 10 μL NaOH (1.0 M) was added to stop the reactions. Fluorescence was determined by 365 nm excitation and 450 nm emission filters.

Phospholipid fatty acid analyses

The microbial community compositions of the soils were characterized using PLFA analysis, as previously described [45]. Total soil lipids were extracted from approximately 8 g of the freeze-dried soil using a single-phase chloroform-methanol-citrate buffer (1:2:0.8) system. Concentrations of each PLFA were calculated relative to the methyl nonadecanoate (19:0) internal standard concentrations. The fatty acid signatures (15:0, i15:0, a15:0, i16:0, 16:1w7, 17:0, i17:0, a17:0, cy17:0, 18:1w7, and cy19:0) were chosen to represent bacterial PLFA and the fatty acid signatures 18:2w6 and 18:1w9 were considered to represent fungal PLFA [46, 47].

DNA extraction and Illumina MiSeq sequencing

Total DNA was extracted directly from 0.5 g of soil using a Fast DNA[®] SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA), according to the manufacturer's protocol. A NanoDrop ND-2000 spectrophotometer (USA) was used to determine the soil DNA concentration and quality [48].

Real-time polymerase chain reaction

The 16S and 18S rRNA gene copy numbers were analyzed using a Real-Time PCR System (ABI 7900HT[®], Foster City, CA, USA). The primer pairs 1368F (5'-CGGTGAATACGTTTCYCGG-3')/R1492 (5'-GGWTACCTTGTTACGACTT-3') and Fung (5'-GTAGTCATATGCTTGTCTC-3')/NS1 (5'-ATTCCCGTTACCGTTG-3') were used to amplify the 16S and 18S rRNA genes, respectively. The 10 μ L real-time PCR reaction mixture for the 16S and 18S rRNA contained: 1 μ L of the DNA sample, 0.4 μ L of each primer, 5 μ L of 2 $\times$ SYBR Premix Ex Taq[™], 0.2 μ L of Rox (Takara), and 3 μ L of double distilled water (ddH₂O). The PCR cycling of 16S was performed at 95 °C for 35 s, followed by 35 cycles at 95 °C for 5 s, 58 °C for 20 s, and 72 °C for 20 s. The PCR cycling of 18S was performed at 95 °C for 30 s, followed by 40 cycles at 95 °C for 10 s and 60 °C for 30 s. The amplification efficiency and the standard curve R^2 were higher than 95 % and 0.99, respectively.

MiSeq sequencing and bioinformatic analysis

The 16S and 18S rRNA genes were amplified using the primer pairs 515F (5'-GTGCCAGCMGCCGCGGTAA-3')/ 806F (5'-GGACTACHVGGGTATCTAAT-3') and Fung/ NS1 with barcodes, respectively. Both the 16S and 18S rRNA genes for the PCR reaction system contained 1 μ L of template DNA, 1 μ L of forward or reverse primer, 2 $\times$ PCR Ex Taq (TaKaRa, Japan) 10 μ L, and was then made up to 20 μ L with ddH₂O. Amplification of the 16S rRNA gene was performed with an initial denaturation at 95 °C for 5 min, with 28 cycles of 95 °C for 30 s, 50 °C for 28 s, and 72 °C for 30 s, and extension at 72 °C for 10 min. Amplification of the 18S rRNA gene was performed with an initial denaturation at 95 °C for 10 min, with 40 cycles of 95 °C for 1 min, 58 °C for 1 min, and 72 °C for 2 min, and extension at 68 °C for 10 min. MiSeq sequencing was performed at the Novogene Company (Tianjin, China).

The quality of the raw sequences was assessed in FastQC v0.11.5. Adapters and sequences with low quality were trimmed with Trimomatic v0.36. Then, clean data for the 16S and 18S were processed using the QIIME2-2020.2 environment. The amplicon sequence variants (ASV) and ASV table were first predicted using the DADA2 plugin with “-p-trunc-len” set as 250 bp or 310 bp for bacteria and fungi, respectively. Taxonomic analysis of the ASV was performed using the q2-feature-classifier, with the corresponding taxonomic classifiers, which were trained with the sequenced regions of the 16S/18S rRNA gene sequences, from the Silva rRNA reference database, release 132. The taxonomies of each ASV were further verified according to the taxdmp database (<ftp://ftp.ncbi.nlm.nih.gov/pub/taxonomy/taxdmp.zip>). After discarding the ASV that were not annotated as fungi or bacteria, the total sequence number of each sample in the ASV table was rarefied to 10,000 in R.

Calculations and statistical analysis

Calculations of CO₂ fluxes, organic carbon mineralization, and priming effects

To calculate the fraction of the CO₂ derived from the straw and SOC, the end-member mixing model was used. We used the methodology described previously to calculate the CO₂ flux, C mineralization, and priming effect (PE) [49, 50]. Added organic matter (e.g., straw) input induced microbial mineralization of the soil organic matter, generally causing a “priming effect” (PE), described as positive and negative PE [2].

$$C_{\text{total}} = \text{CO}_2 \text{ flux} \times \text{incubation time} \quad (1)$$

$$C_{\text{straw}} = ((\delta^{13}\text{C}_{\text{total}} - \delta^{13}\text{C}_{\text{control}}) / (\delta^{13}\text{C}_{\text{straw}} - \delta^{13}\text{C}_{\text{soil}})) \times C_{\text{total}} \quad (2)$$

$$C_{\text{SOC}} = C_{\text{total}} - C_{\text{straw}} \quad (3)$$

$$\text{PE} = C_{\text{total}} - C_{\text{straw}} - C_{\text{control}} \quad (4)$$

Where C_{total} is the total CO₂ flux, derived from the straw-treated soil; C_{straw} and C_{SOC} are the proportions of CO₂ derived from the straw and SOC, respectively; $\delta^{13}\text{C}_{\text{total}}$ is the $\delta^{13}\text{C}$ value (‰) of the total CO₂ (include the decomposition of the soil and straw); $\delta^{13}\text{C}_{\text{straw}}$ is the $\delta^{13}\text{C}$ value (‰) of the straw-derived from CO₂; $\delta^{13}\text{C}_{\text{control}}$ is the $\delta^{13}\text{C}$ value (‰) of CO₂ in the untreated soil; $\delta^{13}\text{C}_{\text{soil}}$ is the $\delta^{13}\text{C}$ value (‰) of the initial soil.

Statistical analysis

Significant differences were estimated based on Duncan's multiple-range test ($p < 0.05$). The effects of soil fertilization, straw with increasing N addition levels, and incubation time on the straw decomposition, SOC mineralization, PE, and soil microbial profiles were performed using multivariate analysis of variance (MANOVA) in R. The Pearson correlation coefficients were calculated in R to investigate the relationships between the soil properties or microbial indexes and C turnover (straw decomposition, SOC mineralization, and PE). The response of the bacterial and fungal community compositions to the soil fertilization, straw with increasing N addition levels, and incubation time, were conducted by using non-metric multidimensional scaling (NMDS) ordination and permutation multivariate analysis of variance (PERMANOVA), in R using the vegan package.

Structural equation modeling (SEM) based on the IBM SPSS Amos 21 was conducted to test the cause and effect relationships between fertilization soils, straw with increasing N addition levels, soil physicochemical properties, microbial profiles, and SOC mineralization. To simplify the SEM, we used principal component analysis (PCA) of the first component (PC1) to represent soil properties (MBC, MBN, DOC, DON, NH_4^+ , and NO_3^-), PLFAs (including total, bacteria, and fungi), enzyme activities (BG, CBH, and NAG), and microbial communities (bacterial and fungal NMDS axes 1 and 2).

We constructed an integrated network with all samples from each fertilization level (Control, NPK, and NPK+Straw), and evaluated the main ecological clusters with three modules, for both bacteria and fungi. For each module, nodes were highly inter-connected as a set and the rate of inter-module edges was lower than the intra-module edges, which were determined to be sub-units or communities [24]. The network analysis was constructed using a similar method to that described in previous studies [22, 23]. First, all pairwise correlations between the bacterial or fungal taxa and environmental factors (including soil properties and SOC mineralization, straw decomposition, and PE) were calculated with an SAPRCC algorithm using Fastspar software. The significant level of SPARCC correlation was determined based on 1000- permutations. Only the top 10000 and 1800 robust ($|r| > 0.3$) and significant correlations ($p < 0.05$), were included in the network construction for bacteria and fungi, respectively. Second, the networks were visualized using the software Gephi v0.9.1, and further divided into clusters using a Louvain algorithm. Similarly, sub-networks for the bacteria or fungi in each fertilization level, for each treatment (S0+N0, S+N0, S+N1, and S+N2), were also constructed. Furthermore, the ASVs were classified into abundant and rare taxa, dependent on the relative abundance in the network analysis. To accurately estimate the diversity and taxonomic compositions of the samples, the ASVs (i.e. 100 % of the operational taxonomic units (OTUs)) were identified from amplicon sequences in QIIME2 with the DADA2 pipeline. We defined ASVs with relative abundances above 0.1 % for all sequences as "abundant" and those with a relative abundance below 0.1 % as "rare" [51, 52]. In order to demonstrate the relationships between the bacterial or fungal ASVs and three indices (straw decomposition, SOC mineralization, and PE), subnetworks were also constructed and the top 1000 edges between the ASVs and these three indices were subset and illustrated using the R package igraph.

Results

Effects of straw and nitrogen additions on microbial biomass carbon and dissolved organic carbon

Straw alone and with the N addition increased MBC and DOC. The contents of the MBC, ^{13}C -MBC, DOC, and ^{13}C -DOC increased rapidly and peaked after 3 to 5 days of incubation, followed by a gradual decrease with the straw and N additions (Additional File: Figure S1). For the three fertilization types, MBC and DOC increased in S+N0, S+N1, and S+N2 compared with S0+N0, with the highest MBC and DOC levels in S+N2 (Additional File: Figure S1). The ^{13}C -MBC and ^{13}C -DOC in the NPK+Straw soils were higher with the S+N2 than with the others throughout the first month of incubation (Additional File: Figure S1). These results highlighted that the effect of the straw and N addition on MBC and DOC occurred in the initial decomposition stage and then disappeared until the end of the incubation.

CO₂ emissions, straw decomposition, soil organic matter mineralization, and priming effects

Long-term fertilization increased CO₂ and $^{13}\text{CO}_2$ fluxes but decreased PE. Regardless of the straw and N additions, the cumulative CO₂ flux and $^{13}\text{CO}_2$ fluxes increased (CO₂: 50%; $^{13}\text{CO}_2$: 69%) with the NPK and NPK+Straw soils when compared with the unfertilized control soil. The CO₂ and $^{13}\text{CO}_2$ fluxes rapidly increased during the first 3 days of incubation, followed by decreases, and then stabilization after 30 days. An increase in the PE of 48.8%, however, was observed for the unfertilized control soil, when compared with the NPK and NPK+Straw soils (Additional File: Figure S2). Consequently, priming intensity was found to decrease as mineral and organic fertilizers increased.

CO₂ fluxes were increased in the treatments with the straw alone and with N additions for the three fertilization levels. Furthermore, the cumulative $^{13}\text{CO}_2$ fluxes as well as the straw decomposition were increased with high N levels only in the unfertilized control soil. The straw decomposition and cumulative $^{13}\text{CO}_2$ showed no variation with the straw and N additions for the NPK and NPK+Straw soils (Fig. 1a; Additional File: Figure S2). Following this, SOM mineralization was found to be increased by 124%, 41%, and 44% by the straw alone and with N additions when compared with the S0+N0 with the control, NPK, and NPK+Straw soils, respectively (Fig. 1b). Straw with high N addition levels increased the PE by 30% compared with the S+N0 and S+N1, for the three soil fertilization levels. The lowest PE value was found for S+N1, with the unfertilized control and NPK+Straw soils at the end of the 100-day incubation period (Fig. 1c). Overall, the CO₂ emissions derived from the SOM were higher (1.2-2.0 times) than those from the straw (Fig. 1d).

Enzyme activities

Straw additions increased enzyme activities compared with S0+N0, with similar levels between S+N0, S+N1, and S+N2. Straw alone and with N additions compared to trials without straw and N increased β -D-glucosidase (BG), Cellobiohydrolase (CBH), and β -N-acetylglucosaminidase (NAG) activities by 3.9, 9.6, and 4.9 times, respectively. Enzyme activities of BG, CBH, and NAG increased during the first 5 days, but decreased after 100 days of incubation compared to day 5 (Additional File: Figure S3). Taken together, straw rather than the N additions was found to increase enzyme activities.

Soil microbial communities

Soil microbial communities based on phospholipid fatty acid composition

Straw additions increased the relative abundance of the phospholipid fatty acid (PLFA) composition, N levels had not effect on the PLFAs. During the whole incubation period, the total and bacterial PLFA abundances were higher in the NPK+Straw soil than the unfertilized control and NPK soils. The total, bacterial, and fungal PLFA abundances were highest in the first 1-5 days, and then decreased until the end of incubation. Straw alone and with N additions compared with S0+N0 increased the total, bacterial, and fungal PLFA abundances levels by 54%, 82%, and 107%, respectively. Similar levels for the total, bacterial, and fungal PLFA abundances between the straw alone and straw with N addition (Additional File: Figure S4). Overall, straw addition increased PLFA abundances, particularly in the first 1-5 days.

Soil bacterial and fungal abundance, diversity, and community composition

Soil bacterial and fungal abundance (based on the real time qPCR) as well as fungal diversity shifted with the straw and N additions (Additional File: Table S1). Both the straw alone and with the N additions increased the 16S and 18S rRNA gene copies by 1.4 and 4.9 times, respectively. Similar contents with 16S and 18S rRNA gene copies between straw alone and straw with N levels. Straw with increasing N levels decreased fungal richness by 4.8-49.8% and the Shanon index was 1.2-23.3%. The opposite temporal tendencies were found in 16S and 18S rRNA gene copies. 16S rRNA gene copy levels were higher in the early incubation period (1-5 days) than after 100 days in the fertilized soils. Soil bacterial diversity and 18S rRNA gene copies increased with incubation duration (Fig. 2). Consequently, straw additions increased 16S and 18S rRNA gene copies, N levels decreased fungal diversity.

Bacterial and fungal community compositions, estimated by high-quality sequences, had dominant keystone taxa. Actinobacteria (38.8-29.7%), Firmicutes (30.7-3.1%), Proteobacteria (29.4-17.6%), and Acidobacteria (21.2-10.4%) were the most dominant bacterial phylum. The phylum Ascomycota (63.3-29.9%), Mucoromycota (53.0-15.8%), and Basidiomycota (27.2-6.5%) were the dominant fungal types

(Additional File: Figure S5). The development of bacterial communities varied during the 100 days (Fig. 3a; Additional File: Table S2). The relative abundance of the bacterial Firmicutes was increased on day 1 and then decreased with the incubation duration. Actinobacteria abundance was higher during the first 1-5 days than on day 1, but the relative abundance of Proteobacteria was increased with the incubation duration (Additional File: Figure S6). Straw with N levels increased the relative abundance of Mucoromycota on day 5 but decreased the relative abundance of Blastocladiomycota and Zoopagomycota (Additional File: Figure S7). Thus, bacterial community compositions had dynamic development with the incubation duration and the N fertilization levels affected the fungal community compositions (Fig. 3; Additional File: Table S2, S3).

Soil bacterial and fungal co-occurrence networks associated with environment factors

The integrated co-occurring networks were built for three ecological clusters that closely co-occurred with each other (modules 1, 2, and 3) to detect the dominant bacterial and fungal phylotypes (Fig. 4). The relative abundance of modules 1 was strongly increased by 54% compared to those for modules 2 and 3. Bacteria phylum of Actinobacteria, Firmicutes, and Proteobacteria were highly clustered in module 1 (Additional File: Figure S8, Table S4). Ascomycota, Basidiomycota, and Mucoromycota were the most dominant phylum of fungi in modules 1, 2, and 3 (Additional File: Figure S8, Table S4). These multiple bacterial and fungal groups were attributed to the different effects on the straw and SOM decompositions. Specifically, the bacterial phylum Firmicutes dominated after 1 day of incubation, while Actinobacteria and Proteobacteria were most correlated with straw and SOM mineralization during their later stages (5-100 days) (Fig. 5). Fungal phylum of Ascomycota, Basidiomycota, and Mucoromycota had higher degrees of centrality (correlations with other taxa) with straw and SOM decompositions remained until the end of incubation (Fig. 5; Additional File: Table S4). Thus, Actinobacteria, Firmicutes, Proteobacteria Ascomycota, Basidiomycota, and Mucoromycota were the main drivers for straw and SOM decomposition.

Fungal taxa compared with bacterial groups had strong correlations with environmental factors (including soil properties, e.g., DOC, MBC, and enzyme activities; straw and SOM decomposition) (Fig. 4, 5). Edge numbers and ratio for pair-wised correlations of the bacterial community compositions were highest among abundant taxa themselves as well as being interrelated with rare groups. For the fungal communities, abundant taxa were more widely associated with environmental factors and interrelated with themselves (Table 1). Fungal taxa more than bacterial groups were interrelated with SOM mineralization, straw decomposition, PE, and soil properties, particularly for abundant taxa (ratio for pair-wised correlations: 21.9 vs. 1.2%) (Table 1; Additional File: Figure S9). Overall, abundant fungal taxa outcompeted bacteria for straw and SOM mineralization.

Table 1
Edge number and ratio for pair-wised correlations between bacterial and fungal subgroups and environmental factors.

Items		Control	NPK	NPK+Straw
Bacteria	Abundant taxa-Abundant taxa	5621 (53.2%)	6406 (64.6%)	6233 (62.8%)
	Rare taxa-Rare taxa	1025 (10.4%)	368 (3.7%)	643 (6.5%)
	Environment-Environment	15 (0.2%)	4 (0.04%)	8 (0.08%)
	Abundant taxa-Rare taxa	3293 (33.3%)	3026 (30.5%)	2940 (29.6%)
	Abundant taxa-Environment	199 (2.0%)	89 (0.9%)	72 (0.7%)
	Rare taxa-Environment	97 (0.9%)	26 (0.3%)	33 (0.3%)
Fungi	Abundant taxa-Abundant taxa	1029 (62.2%)	1054 (64.2%)	1034 (61.8%)
	Rare taxa-Rare taxa	11 (0.7%)	4 (0.2%)	12 (0.7%)
	Environment-Environment	32 (1.9%)	34 (2.1%)	36 (2.2%)
	Abundant taxa-Rare taxa	187 (11.3%)	145 (8.8%)	219 (13.1%)
	Abundant taxa-Environment	356 (21.5%)	379 (23.1%)	356 (21.3%)
	Rare taxa-Environment	39 (2.4%)	26 (1.6%)	15 (0.9%)
<p>Bold fonts suggest the values that more than 20%;</p> <p>Environmental factors include soil properties and soils organic carbon (SOC) mineralization, straw decomposition, and priming effect (PE), for the no fertilization (Control), mineral fertilizers only (NPK), and mineral fertilizers plus maize and soybean straws (NPK+Straw) soils.</p>				

Four sub-networks in each unfertilized control and fertilization soils were used to explored the linkages between straw alone or with N level additions and bacterial as well as fungal taxa (Additional File: Table S5, S6). Compared to trials without straw and N, straw alone and with N levels increased bacterial ratio of negative edges (correlation with each other among taxa) for 7.3% (Additional File: Table S5). The opposite was observed for the fungal taxa, as straw alone or with the added N levels decreased fungal ratio of negative edges by 12.6% (Additional File: Table S6). Similar results were found in the bacterial and fungal negative edges between straw alone and with addition N levels (Additional File: Table S5, S6).

The negative correlations between the bacterial taxa and organic C mineralization were higher after 100 days than after 1-5 days incubation. In contrast, negative correlations between the fungal taxa and organic C mineralization decreased over 100 days compared with the first 1-5 days, except for those without straw and N fertilizer (Fig. 5). Taken together, straw addition decrease and increase competition among fungal and bacterial taxa with low and high negative edges, respectively.

Key factors for straw decomposition, soil organic matter mineralization, and priming effects

Incubation time, soil fertilization, and straw with N additions, affected SOC mineralization, straw decomposition, and PEs (Additional File: Table S1). SOC mineralization, straw decomposition, and PEs were correlated with soil properties (MBC, MBN, DOC, DON, NH_4^+ and NO_3^-), soil enzyme activities (BG, CBH, and NAG), abundance of bacterial and fungal PLFAs, 16S and 18S rRNA gene copies, and bacterial and fungal diversity and community compositions (Additional File: Table S7).

Soil fertilization and straw with N additions indirectly affected straw and SOM mineralization by increasing abundance of bacterial and fungal PLFAs, enzyme activities (BG, CBH, and NAG), and soil properties (MBC, MBN, DOC, DON, NH_4^+ and NO_3^-). These increases all altered bacterial and fungal community compositions (especially fungal abundant keystone groups), resulting in increasing straw and SOM mineralization (Fig. 6). Overall, enzyme activities, soil properties (e.g., MBC and DOC), and fungal community compositions were the main factors determining organic matter mineralization (Fig. 6; Additional File: Table S8).

Discussion

Organic matter mineralization is linked to fungi taxa more than bacteria taxa

The co-occurring network provided empirical evidence that Actinobacteria, Proteobacteria, and Firmicutes bacteria phyla were the keystone taxa in organic matter decomposition. Actinobacteria, Proteobacteria, and Firmicutes had highest interactions with other taxa as well as straw and SOM decomposition in module 1 (Fig. 5; Additional File: Table S4). This confirms that these bacterial groups are widely considered to assimilate maize C [53-55]. The relative abundance of Firmicutes was increased on day 1 and decreased from 5 to 100 days (Additional File: Figure S6). Additionally, the correlation coefficient for Firmicutes was linked to straw and SOM decomposition was higher than with other fungal groups on day 1 (Fig. 5). Consequently, copiotrophic lineages (e.g., Firmicutes) mainly mediated the decomposition of labile organic compounds in the initial stage [53, 54]. As the labile compounds were gradually decomposed, the copiotrophic lineages of bacteria were substituted by oligotrophic lineages [19, 56], increasing the relative abundance of Proteobacteria on day 100 (Additional File: Figure S6). Therefore, the

bacteria decreased capacity to degrade recalcitrant compounds during the later incubation stages followed by increased bacterial diversity but decreasing 16S rRNA gene copies (Fig. 2).

Fungi, more than bacteria, were the key drivers regulating straw and SOM decomposition. Though nodes (represented as amplicon sequence variants) for fungi were lower than for bacteria. Soil properties, such as MBC and DOC, as well as organic matter decomposition with fungal taxa had remarkably higher connection numbers than those with bacterial groups within the integrated network (Fig. 4, 5). This was because: 1) The N content of the limestone (210-280 mg kg⁻¹) in karst area is high [37] due to the abundance of legumes fixing atmospheric N and N rich bedrock [57]. Microbial C limitation were aggravated more than N in the karst regions [38]. Fungi have high C utilization and a competitive advantage for low N nutrient acquisition over the bacteria by increasing the abundance of some abundant taxa (e.g., Ascomycota) [16, 17, 58]. Straw additions increased fungal abundance more than bacteria, as 16S and 18S rRNA gene copies increased by 1.4 and 4.9 times, respectively (Fig. 2). This confirm that fungi, when compared with bacteria, were more inspired by straw addition; 2) Soil Ca suppressed microbial cell communications and cell motility [59]. Fungi compared with bacteria could tolerate more extreme soil environments (e.g., high Ca) [14, 51]. Consequently, fungi had direct and closer correlations with organic matter decomposition compared with bacteria.

Abundant fungal groups dominated SOM decomposition. Particularly, the ratio for pairwise correlations between abundant fungal taxa and soil properties as well as organic matter decomposition increased by 20% more than those within rare groups (Table 1; Additional File: Figure S9). The Ascomycota prefer to grow in rich nutrients during organic matter and N additions [60-62]. The Basidiomycota phylum is generally most effective for lignin decomposition [63]. Straw with the increased N levels increased Mucoromycota abundance but declined fungal diversity by decreasing Blastocladiomycota and Zoopagomycota, which showed that raising N availability suppressed most fungal taxa growth (Additional File: Figure S7). Consequently, abundant fungal taxa of Ascomycota, Basidiomycota, and Mucoromycota had the highest degree numbers in the integrated networks (Additional File: Table S6). This was clearly as abundant fungal groups (e.g., Ascomycota, Basidiomycota, and Mucoromycota) had more important functions in straw and SOM decomposition than rare taxa, especially for soil organic fertilized with N.

The positive and negative correlations within the microbial groups reflect the cooperation and competition relationships, respectively [64, 65]. The ratio of negative correlations among the fungal groups (45.7%) were greater than those within bacterial taxa (5.6%), showing that bacteria was dominated by cooperation relationships while fungi had fierce competition for C and N (Additional File: Table S5, S6). Bacteria prefer nutrient rich environments [17, 19], and consequently less labile organic compounds in the later stages caused strong competition among the bacterial taxa (Fig. 5) [53, 66]. Consequently, bacterial taxa had higher negative correlations with the straw and SOM mineralization after 100 days than in the initial 1-5 days (Fig. 5). Conversely, straw alone or with N additions decreased the negative links between the fungal taxa and organic matter decomposition more after 100 days than during the first 1-5 days (Fig. 5). Straw provides more cellulose and lignin for fungi in the later stages [7,

13], increased 18S rRNA gene copies thereby decreased competition with low negative correlations among fungal taxa (Fig. 2, 5; Additional File: Table S6). Consequently, straw additions decreased fungal but increased bacterial competition relationships among taxa.

Long-term fertilization increases straw and soil organic matter mineralization

Long-term applications of mineral and organic fertilizers increased C and N availability for microbial growth [54, 67-69]. The SOC, available N, enzyme activity (CBH), and bacterial and fungal abundance were higher in the NPK and NPK+Straw soils than in unfertilized control soil before the straw and N additions (Fig. 2; Additional File: Table S9, Figure S3). Consequently, higher CO₂, ¹³CO₂ flux, and SOM mineralization were occurred in the NPK and NPK+Straw than in unfertilized control soil (Fig.1; Additional File: Figure S2). Conversely, increases in C and N limitations had consequence for microbial growth limited by these nutrients. As labile C is rapidly exhausted, increased microbes immediately and efficiently decomposed more SOM for C and N acquisition, leading to a stronger PE in unfertilized control soils than in NPK and NPK+Straw soils [4, 66]. Therefore, straw return is necessary to decrease PE as well as increase SOM accumulation. Consequently, long-term fertilization increased straw and SOM decomposition but decreased PE.

Response of organic matter mineralization and the microbial characteristics to straw and nitrogen additions

There are large CO₂ fluxes in the initial stages followed by increasing bacterial and fungal abundance as well as enzyme activity. Microbes prefer to utilize soluble substrates from straw instead of SOM, and thus increased their CO₂ and ¹³CO₂ emissions during the first 0-30 days, but they peaked after the 3rd day [66, 70, 71]. Microbial activity and biomass increased as the straw released sufficient soluble organic C for microbial growth [66, 72, 73]. The enzyme activity (BG and NAG), bacterial and fungal PLFA abundances, MBC, and DOC rapidly increased after straw additions during the initial 1-5 days and then decreased, confirmed for the temporal dynamics of CO₂ emissions (Additional File: Figure S1, S2, S3, and S4) [72, 74, 75]. Consequently, straw and N additions with greater C and N content as well as larger microbial populations and higher activity caused higher CO₂ fluxes in the karst calcareous soils during the initial 1-3 days [66, 70].

An elemental stoichiometric balance of the microbial requirements for C:N regulated straw and SOM decomposition [66, 70]. Increasing N availability causes relative deficiency of C [66]. The highest PE in the straw was with the high N (S+N2) (Fig. 1) and showed that decomposer activities increased by increasing SOM decomposition to meet the demands of the stoichiometric microbial nutrients [4, 66]. In contrast, straw coupled with appropriate N additions (S+N1) would decrease the PE for the unfertilized control and

NPKS soils [7]. Consequently, it was found that when N fertilization is split into small doses it can suitably decrease PE [70, 76].

Straw alone or with additional N, increased the bacterial and fungal abundance by increasing substrate availability (MBC, DOC, DON, NH_4^+ , and NO_3^-) (Fig. 2; Additional File: Figure S1) [14, 54, 77]. Karst soils have developed from limestone or dolomite and consequently have large Ca contents [30, 32]. Increased soil Ca promoted soil organic carbon, resulting in the low availability of organic C (e.g., DOC) in the karst soils is low when compared to non-karst soils [78, 79]. The straw additions increased the labile C compounds (MBC and DOC), causing raised enzymatic activities (BG CBH and NAG), bacterial and fungal PLFA abundances, as well as 16S and 18S rRNA gene copies (Fig. 2; Additional File: Figure S1, S3, and S4). As described above, microorganisms in karst soils were more susceptible to C than N restrictions [38]. Consequently, there was little difference in enzyme activities, and bacteria and fungi populations (both PLFAs and gene copies) between the S+N0, S+N1, and S+N2. Enzyme activity and microbial abundance were sensitive to straw rather than N additions.

Microbial characteristics and soil properties regulate organic matter decomposition

Microbe changes accompany soil nutrient changes to drive SOM decomposition [4, 6, 14, 77]. Close correlations between the microbial characteristics (bacterial and fungal PLFAs abundance as well as Shannon index) with straw and SOM decomposition, as well as PE show that microorganisms were the key drivers in organic matter mineralization (Additional File: Table S7). Straw alone and with N additions indirectly increased SOM mineralization by increasing the bacterial and fungal abundance as well as enzyme activity towards raised the labile substrates (e.g., DOC, ^{13}C -DOC, MBC, DON, NH_4^+ , and NO_3^-) [74, 80]. Bacterial and fungal community compositions (especially with fungal abundant groups) are directly altered by increases in the DOC, DON, NH_4^+ , NO_3^- , and enzyme activity (Fig. 6), and result in increased SOM decomposition [4, 66, 70].

Conclusions

Long-term mineral fertilizers increase straw decomposition and CO_2 emissions but decreased PE compared with the unfertilized control soil. Straw alone and with additional N induced SOM decomposition because of the increasing enzyme activities (BG, CBH, and NAG), as well as bacterial and fungal abundance. Straw with low N additions increase SOC accumulations by decreasing PE in the control as well as mineral fertilizers with straw soils. This provided empirical evidence that split N fertilizer additions mitigate straw decomposition with low PE. The 16S rRNA gene copies were decreased when easily available compounds were depleted with incubation time, thereby increasing the competition among bacterial taxa by raising diversity. In contrast, in the late decomposition stage, 18S rRNA gene copies were increased but competition among fungal taxa decreased due to their capacity for recalcitrant

compounds. Bacterial phyla Firmicutes (1 day), Actinobacteria (5-100 days) and Proteobacteria (5-100 days) regulated SOM decomposition. Compared with bacteria, the fungi taxa had stronger connections with straw and SOM decomposition because of their capacity to utilize C and N in high Ca and pH environments. Additionally, the ratio for pairwise correlations between abundant fungal taxa (e.g., Ascomycota, Basidiomycota, and Mucoromycota) and organic matter decomposition was higher than those with bacteria did. Consequently, fungal abundant groups rather than rare taxa outcompeted bacteria for organic matter decomposition in karst soils. In conclusion, straw and N additions indirectly increased SOM decomposition, followed by directly increasing the available nutrients (e.g., DOC and N), enzyme activities (BG, CBH, and NAG), and bacterial and fungal abundances. Straw return coupled with low N additions regulated fungal community compositions and is recommended for the management of karst soils.

Abbreviations

C, carbon; N, nitrogen; SOM, soil organic matter; SOC, soil organic carbon; PE, priming effect; Straw-M, straw mineralization; SOC-M, soil organic carbon mineralization; PLFA, phospholipid fatty acids; BG, β -D-glucosidase; NAG, β -N-acetylglucosaminidase; CBH, cellobiohydrolase; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; NH_4^+ , ammonium nitrogen; NO_3^- , nitrate nitrogen; Ca, exchangeable calcium; Mg, magnesium; Control, no fertilization; NPK, mineral fertilizers only; NPK+Straw, mineral fertilizers plus maize and soybean straws; S0+N0, control with neither straw nor nitrogen fertilizer; S+N0, addition of ^{13}C -maize straw; S+N1, addition of ^{13}C -maize straw and low nitrogen; S+N2, addition of ^{13}C -maize straw and high nitrogen.

Declarations

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Authors' contributions

HX-Y, HY-J, CX-B, SY-R, KL-W, Y-K, and D-X designed and conceived the ideas for the study. XX-C and GH-W conducted the experiment. D-X analyzed the data and wrote the manuscript. HX-Y, Z-W, and Y-K revised the manuscript. All authors reviewed and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Straw and soil organic matter (SOM) mineralization, priming effects and carbon dioxide ratios derived from straw and SOM. Straw mineralization (a), soil organic matter mineralization (b), cumulative priming effects per gram of soil organic carbon (c), and ratio of CO₂ derived from straw and SOM (d) after a 100-day incubation period. Control, soil without fertilization; NPK, mineral fertilizers only; NPK+Straw, mineral fertilizers plus maize and soybean straws. S0+N0, neither straw nor nitrogen fertilizer addition; S+N0, addition of 13C-maize straw; S+N1, addition of 13C-maize straw and low nitrogen; S+N2, addition of 13C-maize straw and high nitrogen. Uppercase letters reflect significant differences among fertilization levels (Control, NPK and NPK+Straw) for the same straw and nitrogen additions (S0+N0, S+N0, S+N1, and S+N2) ($p < 0.05$); Lowercase letters represent significant differences among the straw and nitrogen additions (S0+N0, S+N0, S+N1, and S+N2) for the same fertilization level ($p < 0.05$). Values and bars are the mean $\pm$ standard errors ($n = 3$).

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Figure 2

Abundance, richness, and Shannon index for the soil bacteria and fungi. Soil bacterial and fungal abundances (16S and 18S rRNA gene copies) (a), richness (b), and Shannon index (c) for incubation periods of 0, 1, 5, and 100 days. Control, soil without fertilization; NPK, mineral fertilizers only; NPK+Straw, mineral fertilizers plus maize and soybean straws. S0+N0, neither straw nor nitrogen fertilizer addition; S+N0, addition of 13C-maize straw; S+N1, addition of 13C-maize straw and low nitrogen; S+N2, addition of 13C-maize straw and high nitrogen. Uppercase letters reflect significant differences among fertilization levels (Control, NPK and NPK+Straw) for the same nitrogen addition level and incubation time ($p < 0.05$); Lowercase letters represent significant differences among the straw and nitrogen additions (S0+N0,

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Figure 3

Non-metric multidimensional scaling ordinations of bacterial and fungal community compositions. Non-metric multidimensional scaling (NMDS) ordinations of bacterial (a) and fungal (b) community compositions based on amplicon sequence variants for the three fertilization methods during the 100-day incubation period. S0+N0, neither straw nor nitrogen fertilizer addition; S+N0, addition of ¹³C-maize straw; S+N1, addition of ¹³C-maize straw and low nitrogen; S+N2, addition of ¹³C-maize straw and high nitrogen. MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; NH₄⁺, ammonium nitrogen; NO₃⁻, nitrate nitrogen; BG, β -D-glucosidase; CBH, cellobiohydrolase; NAG, β -N-acetylglucosaminidase; Straw-M, straw mineralization; SOC-M, soil organic carbon mineralization; and PE, priming effects. Vectors indicate soil properties significantly correlated with the bacterial and fungal community compositions ($p < 0.05$). The bend arrow (blue) shows the development of bacterial communities during 100 days. The vertical arrow (yellow) shows the effects of N fertilization levels.

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Figure 4

Network of co-occurring analysis of bacteria and fungi. Network of co-occurring analysis bacterial (top) and fungal (bottom) module features at amplicon sequence variant levels associated with environmental factors in the soil without fertilization (Control), mineral fertilizers only (NPK), and mineral fertilizers plus maize and soybean straws (NPK+Straw) soils integrated networks. Three modules (ecological clusters) are divided based on strongly inter-connected nodes. A connection between two nodes represents strong (SparCC $r > 0.3$) and significant ($p < 0.05$) correlation. The size of each node for the bacterial and fungal taxa or environmental factors is proportional to its degree, with the number of connections among a node. MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; NH_4^+ , ammonium nitrogen; NO_3^- , nitrate nitrogen; BG, β -D-glucosidase; CBH, cellobiohydrolase; NAG, β -N-acetylglucosaminidase; Straw-M, straw mineralization; SOC-M, soil organic carbon mineralization; and PE, priming effects.

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Figure 5

Networks edges linked to bacterial or fungal taxa and soil carbon and straw decomposition. Networks containing the top 100 edges that directly link the bacterial (top) or fungal (bottom) taxa that directly connected to straw decomposition, soil organic carbon mineralization, and priming effects, after 1, 5, and 100 days depending on straw and nitrogen additions. S0+N0, neither straw nor nitrogen fertilizer addition; S+N0, addition of ^{13}C -maize straw; S+N1, addition of ^{13}C -maize straw and low nitrogen; S+N2, addition

of ¹³C-maize straw and high nitrogen. Nodes (filled with dark red) in the center refer to priming effects, straw decomposition, and soil organic carbon mineralization from left to right. The size of surrounding nodes which refer to ASVs denote the absolutely value of SparCC correlation (r). The red and blue lines between each pair of nodes indicate positive and negative correlations, respectively. The numbers right below each subfigure represent ratio of positive and negative correlations.

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Figure 6

Conceptual model showing the environmental factors and microbial characteristics affecting straw and soil organic matter mineralization. PLFA, phospholipid fatty acid; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; NH₄⁺, ammonium nitrogen; NO₃⁻, nitrate nitrogen; BG, β-D-glucosidase; CBH, cellobiohydrolase; NAG, β-N-acetylglucosaminidase. The “+” at the top of black solid arrows represent positive correlation. Larger size

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