Effects of straw amendment and moisture on microbial communities in Chinese fluvo-aquic soil

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Abstract
Purpose Returning crop straw into fields is a typical agricultural practice to resolve an oversupply of straw and improve soil fertility. Soil microorganisms, especially eukaryotic microorganisms, play a critical role in straw decomposition. To date, microbial communities in response to straw amendment at different moisture levels in Chinese fluvo-aquic soil are poorly understood. The aim of this study was to explore the effects of straw amendment and moisture on microbial communities in Chinese fluvo-aquic soil.

Materials and methods Two soils (one was applied with organic manure, and the other was not applied with any fertilizer) from a long-term field experiment in the North China Plain were collected. Soils with and without straw amendment at 25 and 55 % of the average water-holding capacities of the two soils were incubated at 25 °C for 80 days. All treatments were sampled 20 and 80 days after the start of incubation. Microbial biomass and community structure were analyzed by phospholipid fatty acids (PLFA) assay, and the eukaryotic diversity and community composition were assessed via barcoded pyrosequencing of the 18S ribosomal RNA (rRNA) gene amplicons.

Results and discussion PLFA analysis showed that straw amendment increased the biomass of Gram-positive bacteria, Gram-negative bacteria, actinobacteria, and fungi and shifted microbial community structure. The varied straw availability resulted in a large variation in microbial community structure. In the presence of straw, actinobacterial and fungal biomass both decreased under high moisture content. 18S rRNA gene pyrosequencing indicated that straw amendment decreased eukaryotic diversity and richness and probably restructured the eukaryotic community. Under identical moisture content, long-term organic manure-fertilized soil had higher eukaryotic diversity and richness than the unfertilized soil. In the amended soils under high moisture content, the relative abundance of dominant fungal taxa (Dikarya subkingdom, Ascomycota phylum, and Pezizomycotina subphylum) decreased.

Conclusions Straw amendment increases microbial biomass, shifts microbial community structure, and decreases eukaryotic diversity and richness. High moisture content probably has a negative effect on fungal growth in the amended soils. In conclusion, microbial communities in Chinese fluvo-aquic soil are significantly affected by straw amendment at different moisture levels.

Keywords Eukaryotic community · Microorganisms · Phospholipid fatty acids · Pyrosequencing

1 Introduction

Biological processes are of significance for the ecological functions of arable soils. Of special importance in this respect is the effect of microorganisms on the input and output dynamics of soil organic matter (SOM) content. A loss of organic carbon in agricultural soils due to mineralization and leaching can be balanced by the incorporation of crop straw (Perucci et al. 1997). Crop straw is decomposed by soil
microbial communities, which make an important contribution to the naturally occurring carbon and nutrient cycles and the maintenance of soil fertility.

Microbial processes for straw decomposition are affected by many abiotic factors, including soil salinity (Muhammad et al. 2006), straw intrinsic genotype (Lu et al. 2010), straw quality and size (Bending and Turner 1999), soil-straw contact (Henriksen and Breland 2002), and so on. These factors can affect the accessibility of straw to soil microorganisms and, thus, alter rates of colonization and patterns of decomposition. Among these factors, soil moisture content is a critical factor in governing soil microbial activity, community composition, and function (Drenovsky et al. 2004; Geisseler et al. 2011; Brockett et al. 2012). Drenovsky et al. (2004) showed that soil moisture content was highly correlated with observed differences in microbial community composition across the compost- or vetch-amended soils at four water levels (air dry, half field capacity, field capacity, and flooded). Geisseler et al. (2011) found that respiration and microbial biomass in a compost- or vetch-amended soil with higher moisture content. exocellulase activities were increased compared to an dry soil amended with oat-legume straw were reduced, while protease, beta-glucosidase, beta-glucosaminidase, and exocellulase activities were increased compared to an amended soil with higher moisture content.

The open burning of crop straw was ubiquitous in the North China Plain. At present, with a concern for air pollution, a great deal of crop straw is returned back to the agricultural fields. Soil microorganisms are a sensitive indicator of soil fertility and quality (He et al. 2008). To maintain soil fertility and quality, it is important to monitor the effect of straw management on soil microbial communities. Up to now, in Chinese fluvo-aquic soil which is widely distributed in the North China Plain, microbial communities in response to straw amendment at different moisture levels are not well understood. Moreover, eukaryotic microorganisms play a critical role in straw decomposition. Previous studies have dealt with the effect of straw amendment on eukaryotic community structure using some traditional methods, such as applying polymerase chain reaction-denaturing gradient gel electrophoresis and sequencing (Cahyani et al. 2004; Sugano et al. 2007) and automated ribosomal intergenic spacer analysis (Bastian et al. 2009). These methods provide little detail about the taxonomic composition and phylogenetic structure of the eukaryotic community.

In Chinese cropping systems, without any fertilizer input, soil fertility, quality, and productivity are very low. The application of organic manure can improve soil nutrient availability and microbial properties (Ge et al. 2008). We hypothesize that straw amendment, straw availability, and moisture level all have strong effects on microbial communities in Chinese fluvo-aquic soil, and their responses to straw amendment are different in the unfertilized and organic manure-fertilized soils. To test our hypotheses, the unfertilized and organic manure-fertilized soils were amended with maize straw and incubated at two moisture levels. Overall microbial community and the eukaryotic community structure at two incubation time points were analyze by PLFA and 18S ribosomal RNA (rRNA) gene pyrosequencing approaches, respectively. Finally, the objective of our study is to evaluate the effects of straw amendment at different moisture levels on microbial communities in Chinese fluvo-aquic soil.

2 Materials and methods

2.1 Soils

A long-term fertility experiment was conducted at Fengqiu Agro-ecological Experimental Station, Chinese Academy of Sciences, Fengqiu County, Henan Province, China (35°00′ N, 114°24′E). The soil, with a profile of sandy loam (40.7% silt, 13.7% clay) in the plough layer and loam (55.8% silt, 35.9% clay) in the subsoil, has developed from alluvial sediments of the Yellow River and was classified as Aquic Inceptisol (a calcaric fluvo-aquic soil) according to the US Department of Agriculture classification system. Seven treatments with four replicates in a randomized plot design were established in 1989 (Ge et al. 2008). Soils from organic manure treatment (OrgMan) and no fertilizer treatment (NoFer) were used in this study. The organic manure was a composted mixture of wheat straw, oil cake, and cotton cake in a weight ratio of 100:40:45. The oil cake and cotton cake were the machine-dried residues of oil-harvested rapeseed and cottonseed, respectively. These materials were ground into 3–5-mm lengths mixed completely with limited water and composted for 2 months. The organic manure, with 422 g kg⁻¹ dry weight (DW) of total C and 54 g kg⁻¹ DW of total N, was regularly applied to winter wheat (Triticum aestivum L.) and summer maize (Zea mays L.) at a rate of ~2,758 kg ha⁻¹ DW (equivalent to 150 kg N ha⁻¹). The NoFer had not received any application of mineral or organic fertilizer for either crop since 1989.

In October 2012 (at maize harvesting stage), soil samples were collected from four replicated plots of the OrgMan and NoFer at the depth of 0–20 cm. After plant materials and stones were removed, soil samples were homogenized, air-dried, and passed through a 0.25-mm mesh sieve (to lessen the influence of different aggregate distribution between the two soils). Analysis showed that the OrgMan soil contained 9.3 g kg⁻¹ of organic C, 1.3 g kg⁻¹ of total N, 0.6 g kg⁻¹ of total P, 17.5 g kg⁻¹ of total K, and a pH of 8.0; the NoFer soil contained 3.3 g kg⁻¹ of organic C, 0.5 g kg⁻¹ of total N, 0.4 g kg⁻¹ of total P, 18.1 g kg⁻¹ of total K, and a pH of 8.3.

2.2 Incubation experiment

Experimental units consisted of 200.0 g DW of sieved soil treated with 4.0 g of maize straw and placed in 500 ml plastic
The hexane was evaporated under N2 flow, and the FAME fatty acid methyl esters (FAME) were extracted with hexane. Following alkaline methanolysis of polar lipids, the resulting solid-phase extraction columns (Waters, Milford, MA, USA) separated from neutral lipids and glycolipids using Sep-Pak (10:20:8), and polar lipids (including phospholipids) were chloroform-methanol-citrate buffer mixture (volume ratio of 2.3). Phospholipid fatty acids (PLFA) were extracted by a molecular analysis software (MIDI, Newark, DE, USA). The concentration of each individual PLFA was calculated from nmol PLFA g\(^{-1}\) DW soil=(P\(_{\text{FAME}}\times C_{\text{ISTD}}\))/(P\(_{\text{ISTD}}\times W\)) (where P\(_{\text{FAME}}\) and P\(_{\text{ISTD}}\) are the mole percent of each fatty acid methyl ester and the internal standard, respectively, C\(_{\text{ISTD}}\) is the content of the internal standard, and W is the oven-dried weight of soil). The PLFA nomenclature used was that of Petersen and Klug (1994). The characterization of microbial groups followed that Gram-positive bacteria were indicated by the branched chain fatty acids: i15:0, a15:0, i16:0, i17:0, and a17:0; Gram-negative bacteria were indicated by the monounsaturated fatty acids (e.g., 16:1ω5c, 16:1ω7c, 17:1ω8c, 18:1ω7c, and 18:1ω9c), the hydroxyl fatty acids (e.g., 14:0ωH and 16:0ωH), and the cyclopropyl fatty acids (e.g., cy17:0 and cy19:0); the double-unsaturated 18:2ω6,9c was used as a biomarker of fungi; and the methyl branched fatty acids (e.g., 10Me17:0 and 10Me18:0) were ascribed to actinobacteria (Zelles 1999; Bååth and Anderson 2003; Pietri and Brookes 2009; Huygens et al. 2011).

2.4 Soil DNA extraction, PCR amplification, and pyrosequencing

Soil DNA was extracted from 0.5 g of moist soil using a FastDNA SPIN Kit for soil (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer’s instructions. The extracted soil DNA was dissolved in 50 μl of TE buffer and stored at −20°C.

To produce eukaryotic amplicon libraries for 18S rRNA gene pyrosequencing, partial eukaryotic 18S rRNA gene fragments were amplified using the primer set Euk1A (5'-CTGG TTGATCTTGCAG-3')/Euk516r (5'-ACCAGACTTGGC CTCC-3') (Diez et al. 2001). To discriminate each sample, a unique 7-bp barcoded sequence coupled with forward primer Euk1A was fused to the 454 adaptor ‘B’: adaptor ‘B’ (GCCT TGCCAGCCCCGCTCAG) + barcode + Euk1A. Reverse primer Euk516r was fused to the 454 adaptor ‘A’: adaptor ‘A’ (GCCTCCCTCGCCGCATCG) + Euk516r. The amplification mix contained 0.5 μl (125 pmol) of each forward/reverse primer, 1 μl of DNA template, 23 μl of ddH2O, and 25 μl of Premix Taq (Takara, Shiga, Japan), which contained 1.25 U of DNA polymerase, 2× dNTP mixture (0.4 mM), 2× Taq buffer (3 mM Mg\(^{2+}\)), and the marker (Tartrine/Xylene Cyanol FF). The thermal pattern was an initial denaturation at 94°C for 5 min followed by 30 cycles of 94°C for 30 s, 54°C for 30 s, 72°C for 45 s, and a final 10 min extension at 72°C. Triplicate reaction mixtures per sample were pooled and purified using an E.Z.N.A. Cycle-Pure Kit (OMEGA, Norcross, GA, USA), and quantified using a NanoDrop ND-1000 (Thermo Scientific, Waltham, MA, USA). Finally, the amplicons of all of the samples were pooled at equimolar concentrations, and pyrosequencing was completed using a Roche 454 GS-
FLX system (Roche, Branford, CT, USA). The pyrosequencing data have been deposited at NCBI Sequence Read Archive with accession number SRR1139793.

2.5 Processing of pyrosequencing data

Pyrosequencing data were processed using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline (http://qiime.sourceforge.net) (Caporaso et al. 2010a); QIIME 1.7 was used to process pyrosequencing data. In raw input sequences, length outside bounds of 200 and 1,000 bp, number ambiguous bases exceeding limit of 0, missing quality score, mean quality score below minimum of 25, max homopolymer run exceeding limit of 6, and number mismatches in primer exceeding limit of 0 were filtered out. Sequences of 38.7 % were lost during the process. The trimmed sequences were assigned to each sample based on their barcodes. Similar sequences were clustered into operational taxonomic units (OTUs) using a 97 % identity threshold with cd-hit (Li and Godzik 2006). The most abundant sequence from each OTU was selected as a representative sequence for that OTU, and the representative OTU sequences were aligned using the Python Nearest Alignment Space Termination (PyNAST) tool (Caporaso et al. 2010b). A taxonomic designation was assigned to each representative sequence using the Ribosomal Database Project (RDP) classifier (Wang et al. 2007). We assessed eukaryotic alpha diversity using four metrics: the phylogenetic diversity (PD) index (Faith 1992), the Shannon index (Hill et al. 2003), the Chao1 index (Chao and Bunge 2002), and the observed OTU richness. We obtained at least 500 quality sequences per sample for all 48 soil samples. To correct for survey effort (number of sequences analyzed per sample), we used a randomly selected subset of 500 sequences per sample to compare relative differences in eukaryotic diversity among different treatments. Even though it is highly unlikely that we have surveyed the full extent of diversity in the treated soils, previous studies suggest that we can quantitatively compare overall community composition and general diversity patterns among different treatments (Shaw et al. 2008; Chu et al. 2010; Rousk et al. 2010).

2.6 Statistical analysis

One-way analysis of variance (ANOVA) was used to detect significant differences between the treatments, and Bonferroni’s test was used for comparison of means at a significance level of $\alpha<0.05$. Full factorial analysis was performed to detect the effects of soil, straw, moisture, and their interactions. The data of PLFA concentration and eukaryotic relative abundance, which were not all normally distributed, were transformed using Van der Waerden’s formula before ANOVA was performed. Principal component analysis (PCA) was performed using the concentrations of all detectable PLFA to observe the separation in microbial community structure between the treatments and independent samples’ $T$ test using PC scores was done to check the differences between different groups. These analyses were carried out with SPSS 16.0 software.

Non-metric multidimensional scaling (NMDS) analysis of the unweighted (i.e., presence or absence of taxa) and weighted (i.e., taking into account relative abundance of taxa) UniFrac (Lozupone and Knight 2005) distance matrix was performed to observe the separation in eukaryotic community composition between the treatments, and analysis of similarities (ANOSIM) using OTU data was performed to quantitatively compare the differences between different groups. These analyses were completed in the Vegan package of the R v.3.0.1 project (R Development Core Team 2010).

3 Results

3.1 Microbial biomass and community structure

Full factorial analysis showed that straw amendment had a pronounced ($P<0.01$, Table S2, Electronic Supplementary Material) effect on microbial biomass indicated by PLFA concentrations. Due to straw amendment, microbial biomass was increased to varying degrees. Especially, actinobacterial biomass in the NoFer and OrgMan soils had a significant ($P<0.05$, Table 1) increase at day 80, and fungal biomass in soils except the OrgMan soil with a WHC of 55 % had a significant ($P<0.05$, Table 1) increase at days 20 and 80. Straw amendment drastically increased fungal/bacterial PLFA ratio in the two soils at days 20 and 80 (Fig. 1). Straw amendment also increased the number of the detectable PLFA (Table S3, Electronic Supplementary Material).

In the presence of straw, actinobacterial biomass relatively decreased under high moisture content at days 20 and 80, and fungal biomass followed the same trend (Table 1). In the amended soils with identical moisture content, the biomass of Gram-positive bacteria, Gram-negative bacteria, and actinobacteria at day 80 was higher than that at day 20 (Table 1).

PCA showed that the first principal component (PC) accounting for 52.5 % of the data variance differentiated microbial communities in the non-amended soils from those in the amended soils (Fig. 2), and the difference in PC1 score between the treatments with and without straw amendment was highly significant ($P<0.001$, Table S4, Electronic Supplementary Material), which suggests that straw amendment maybe shifts microbial community structure. Along the axis of PC2 which explained 10.7 % of the variance, the separation in
microbial community structure in the amended soils between day 20 and day 80 was clearly observed (Fig. 2), and the difference in PC2 score between day 20 and day 80 was very significant \((P < 0.001, \text{Table S4, Electronic Supplementary Material})\), indicating that the varied straw availability (due to different sampling time) results in a large variation in microbial community structure. Relative to straw amendment and availability, moisture had a weak influence on microbial community structure (Fig. 2, Table S4, Electronic Supplementary Material).

### 3.2 Eukaryotic community composition and diversity

A total of 312,107 quality sequences, which corresponded to 153–2,769 OTUs for each soil sample, were obtained from 48 soil samples. The fungi represented a significant proportion of eukaryotic microorganisms and composed an average of 41.7 % of the eukaryotic community in the amended soils. In the presence of straw, the relative abundance of fungi decreased under high moisture content (Fig. 3). Other eukaryotes, such as alveolata, viridiplantae, and rhizaria, were present in the non-amended soils at moderate abundance (Fig. 3).

The majority of fungal sequences (100,567 sequences corresponding to 32.2 % of the total) belonged to Dikarya (Table S5, Electronic Supplementary Material). Ascomycota (mainly Pezizomycotina subphylum) was the most abundant Dikarya and composed an average of 90.1 % of Dikarya (Table 2). The relative abundances of Dikarya, Ascomycota, and Pezizomycotina in soils except the non-amended NoFer soil at day 20 decreased under high moisture content (Table 2). The relative abundances of Dikarya, Ascomycota, and Pezizomycotina at day 80 were markedly affected by straw and moisture, and their interaction had a significant \((P < 0.05, \text{Table S2, Electronic Supplementary Material})\) effect on the relative abundances of Ascomycota and Pezizomycotina at day 20.

NMDS analysis (two dimensions were used, and the stress values indicated the quality of NMDS analysis) revealed that eukaryotic community composition in the amended soils was distinctly different from that in the non-amended soils (observed from NMDS1, Fig. 4a; observed from NMDS2, Fig. 4b), and the difference in eukaryotic community composition between the two soils was in evidence (Fig. 4a, Table S6, Electronic Supplementary Material).

The eukaryotic alpha diversity was strongly \((P < 0.01, \text{Table S2, Electronic Supplementary Material})\) influenced by straw amendment. Straw amendment decreased the eukaryotic diversity (PD and Shannon) and richness (Chao1 and OTU richness) except the eukaryotic richness in the OrgMan soil.

### Table 1 Microbial biomass indicated by PLFA concentrations in all treatments sampled at days 20 and 80 of the incubation

| Incubation time | Treatment | Gram-positive bacteria | Gram-negative bacteria | Actinobacteria | Fungi |
|-----------------|-----------|------------------------|------------------------|----------------|-------|
| Day 20          | NoFer S L | 9.85±0.54 abc          | 6.59±0.59 ab           | 1.01±0.12 ab   | 4.50±0.19 a |
|                 | NoFer S H | 9.37±1.72 abc          | 4.85±0.48 abc          | 0.27±0.14 bcd  | 3.98±0.05 ab |
|                 | OrgMan S L | 11.76±4.44 ab        | 7.02±3.12 ab           | 1.37±0.59 a    | 4.39±1.58 a  |
|                 | OrgMan S H | 13.34±1.42 a        | 11.36±1.71 a           | 1.32±0.33 a    | 3.47±0.34 abc |
|                 | NoFer L  | 1.92±0.33 bc          | 1.03±0.09 c            | 0.24±0.04 cd   | 0.53±0.02 bc |
|                 | NoFer H  | 1.52±0.18 c           | 0.95±0.05 c            | 0.19±0.01 d    | 0.25±0.03 d  |
|                 | OrgMan L | 4.68±0.27 abc         | 3.50±0.27 bc           | 0.47±0.05 abc  | 0.43±0.04 cd |
|                 | OrgMan H | 5.46±0.14 abc         | 3.99±0.22 abc          | 0.43±0.04 abcd | 0.51±0.00 bcd |
| Day 80          | NoFer S L | 15.63±5.27 a          | 8.49±0.50 ab           | 2.40±0.24 ab   | 4.27±0.19 ab |
|                 | NoFer S H | 10.15±0.62 ab         | 11.80±2.93 a           | 1.19±0.07 bcd  | 2.85±0.76 bc |
|                 | OrgMan S L | 14.20±0.17 a         | 12.28±1.00 a           | 3.12±0.27 a    | 5.67±0.40 a  |
|                 | OrgMan S H | 14.46±1.11 a         | 14.42±1.87 a           | 2.21±0.37 abc  | 3.24±0.54 bc |
|                 | NoFer L  | 3.64±0.12 c           | 2.77±0.04 b            | 0.38±0.01 ef   | 0.61±0.02 de |
|                 | NoFer H  | 4.36±0.10 bc          | 8.33±2.70 ab           | 0.28±0.01 f    | 0.53±0.02 e  |
|                 | OrgMan L | 6.40±0.32 abc         | 6.67±1.58 ab           | 0.97±0.13 cde  | 1.07±0.30 cd |
|                 | OrgMan H | 6.92±1.02 abc         | 7.53±2.59 ab           | 0.66±0.09 de   | 1.01±0.35 cd |

NoFer and OrgMan are soils sampled from no fertilizer and organic manure sites, respectively, S denotes straw amendment, and L and H represent low and high moisture levels, respectively. The data of PLFA indicators are transformed using Van der Waerden’s formula prior to one-way ANOVA. Results are expressed with means±standard errors, and different letters within a column at the same sampling time indicate significant differences at a level of \(\alpha < 0.05\) based on Bonferroni’s test.
with a WHC of 55 % at day 20 (Table 3). In the presence of straw with identical moisture content, the OrgMan soil had higher eukaryotic diversity and richness than the NoFer soil (Table 3).

4 Discussion

4.1 Effect of straw amendment on microbial communities

Added fresh crop straw offers energetic carbon and nutrient sources to favor microbial growth and increase microbial biomass. In a long-term field observation, Spedding et al. (2004) found that microbial biomass carbon and nitrogen, respectively, increased by 61 and 96 % in the straw-returned plots compared to the straw-removed plots. The incorporation of straw into rice soils increased the abundance of the mono-unsaturated PLFA (one indicator of Gram-negative bacteria) (Bossio and Scow 1998), which has been related to the faster intrinsic growth rates of Gram-negative bacteria (Arao 1999). Fungal biomass was significantly enhanced by straw amendment in the current study (Table 1). Fungi are generally regarded as the main decomposers of plant materials (Henriksen and Breland 2002; Fontaine et al. 2011). This is ascribed to their ability to decompose easily degradable (e.g., cellulose and hemicellulose) and recalcitrant (e.g., lignin and polyphenols) organic materials by producing a variety of extracellular depolymerizing enzymes (Swift et al. 1979) and their ability to explore soil space for nutrient acquisition and reallocate energy and nutrients at the soil-straw interface.

Fig. 1  Fungal/bacterial PLFA ratio in all treatments sampled at days 20 (a) and 80 (b) of the incubation. NoFer and OrgMan are soils sampled from no fertilizer and organic manure sites, respectively, S denotes straw amendment, and L and H represent low and high moisture levels, respectively. The bars indicate ±1 standard errors (SE) of three replicates, and some bars are invisible because of the small SE.

Fig. 2  Microbial community structure estimated by principal component analysis using the concentrations of all detectable PLFA in all treatments sampled at days 20 and 80 of the incubation. NoFer and OrgMan are soils sampled from no fertilizer and organic manure sites, respectively, S denotes straw amendment, and L and H represent low and high moisture levels, respectively. The ellipses in the plot area are manually drawn.
through their filaments (Frey et al. 2000, 2003). Our study also showed that actinobacterial biomass was enhanced by straw amendment (Table 1). This is attributed to the important role of actinobacteria in the decomposition of plant residues (e.g.,

Table 2  Relative abundance of the fungal taxa in all treatments sampled at days 20 and 80 of the incubation

| Incubation time | Treatment   | Dikarya | Basidiomycota | Ascomycota | Mitosporic | Pezizomycotina |
|-----------------|-------------|---------|---------------|------------|------------|---------------|
| Day 20          | NoFer S L   | 54.0±13.0 a | 1.2±0.5 bc | 52.7±13.4 a | 0.3±0.1 de | 52.3±13.6 a |
|                 | NoFer S H   | 30.0±5.2 abc | 0.9±0.6 c | 29.1±5.7 abc | 0.2±0.1 e | 28.8±5.7 abc |
|                 | OrgMan S L  | 39.5±3.1 ab | 1.6±0.2 bc | 37.9±2.9 ab | 3.3±0.3 bcd | 34.0±2.6 ab |
|                 | OrgMan S H  | 25.1±7.4 abc | 1.6±0.4 bc | 23.5±7.0 abc | 6.7±1.8 abc | 16.1±5.2 bcd |
|                 | NoFer L     | 10.6±1.2 c  | 2.2±0.5 abc | 8.4±0.7 c  | 2.2±0.4 ede | 5.8±0.2 d   |
|                 | NoFer H     | 15.4±0.9 bc | 3.5±0.4 ab  | 11.9±0.5 bc | 3.5±0.5 bcd | 7.9±0.2 cd  |
|                 | OrgMan L    | 45.7±4.1 a  | 4.4±0.7 a   | 41.4±3.3 ab | 16.8±1.6 a | 23.4±1.8 abc |
|                 | OrgMan H    | 37.5±4.0 abc | 2.1±0.1 abc | 35.4±4.0 abc | 15.4±2.0 ab | 18.9±2.3 bcd |
| Day 80          | NoFer S L   | 70.4±6.6 a  | 0.3±0.1 b   | 70.0±6.7 a | 0.4±0.2 d  | 69.6±6.5 a  |
|                 | NoFer S H   | 33.8±4.7 bcd | 4.2±1.0 a  | 29.7±3.6 bc | 0.2±0.0 d  | 29.4±3.6 ab  |
|                 | OrgMan S L  | 43.1±2.8 bc | 4.0±0.3 a   | 39.1±3.1 ab | 7.2±0.9 bc | 30.5±2.6 ab  |
|                 | OrgMan S H  | 27.5±7.3 cd | 2.5±0.6 ab  | 25.0±6.7 bc | 3.0±0.8 bc | 21.0±5.8 bc  |
|                 | NoFer L     | 14.4±1.4 de | 2.8±0.5 ab  | 11.6±0.9 cd | 2.1±0.1 c  | 9.3±0.9 cd   |
|                 | NoFer H     | 11.2±0.2 e  | 2.3±1.0 ab  | 8.9±1.2 d  | 1.8±0.2 cd | 6.7±1.0 d   |
|                 | OrgMan L    | 48.3±1.2 ab | 3.5±0.2 ab  | 44.8±1.0 ab | 25.1±1.2 a | 18.3±2.4 bc  |
|                 | OrgMan H    | 29.3±5.0 bcd | 1.8±0.5 ab  | 27.5±4.7 bc | 11.6±2.3 ab | 14.9±2.4 bcd |

NoFer and OrgMan are soils sampled from no fertilizer and organic manure sites, respectively, S denotes straw amendment, and L and H represent low and high moisture levels, respectively. Basidiomycota and Ascomycota both belong to Dikarya subkingdom; Mitosporic and Pezizomycotina are members of Ascomycota phylum. The data of fungal taxa are transformed using Van der Waerden’s formula prior to one-way ANOVA. Results are expressed with means±standard errors, and different letters within a column at the same sampling time indicate significant differences at a level of $\alpha < 0.05$ based on Bonferroni’s test.
cellulose) (Acosta-Martínez et al. 2008). Furthermore, straw amendment slightly increased soil pH (Table S1, Electronic Supplementary Material), and this result partly explained the increase of actinobacterial biomass, as actinobacterial abundance positively correlated with soil pH (Lauber et al. 2009).

The shift in microbial community structure occurred as soils were amended with straw (Fig. 2, Table S4, Electronic Supplementary Material). Our finding is consistent with some previous studies (e.g., Pietri and Brookes 2009; Xu et al. 2013). With regard to soil bacteria, their community structure was shifted with a concomitant increase in the abundance of some genotypic groups, such as Bacillales (Xu et al. 2013). Fungal/bacterial PLFA ratio was drastically increased by straw amendment (Fig. 1), and the factor loading of 18:2ω6,9c (a biomarker of fungi) along PC1 axis was biggest among all PLFA components (Fig. S1, Electronic Supplementary Material), indicating that straw amendment drives the redistribution in fungal and bacterial populations. Relative to soil bacteria, soil fungi have a strong competitive advantage in the utilization of straw. Previous observations support a dominance of fungi in the degradation of the polymerized fraction (e.g., cellulose and lignin) and a dominance of bacteria in the degradation of the soluble fraction (e.g., sugars and amino acids) (Henriksen and Breland 1999, 2002; de Boer et al. 2005; Poll et al. 2008).

4.2 Effects of moisture and straw availability on microbial communities

In the presence of straw, fungal biomass indicated by PLFA concentrations decreased under high moisture content (Table 1), and 18S rRNA gene pyrosequencing also showed that the relative abundance of fungi decreased under high moisture content (Fig. 3). Therefore, we conclude that fungal decomposability of added straw may be better at lower moisture levels. Our suggestion is in line with the study of...
Table 3  Eukaryotic alpha diversity in all treatments sampled at days 20 and 80 of the incubation

| Incubation time | Treatment | Eukaryotic alpha diversity |
|-----------------|-----------|---------------------------|
|                 |           | PD | Shannon | Chao1 | OTU richness |
| Day 20          | NoFer S L | 16.2±1.4 b | 4.1±0.5 b | 463±100 c | 138±17 c |
|                 | NoFer S H | 14.8±0.8 b | 4.7±0.4 b | 479±62 c | 146±20 c |
|                 | OrgMan S L | 18.2±0.5 ab | 4.7±0.2 b | 581±20 c | 165±10 c |
|                 | OrgMan S H | 20.0±3.1 ab | 6.2±0.1 ab | 874±50 a | 239±8 ab |
|                 | NoFer L | 23.5±2.8 ab | 7.3±0.8 a | 605±18 bc | 266±14 a |
|                 | NoFer H | 16.1±1.5 b | 5.2±0.9 ab | 600±18 bc | 173±14 bc |
|                 | OrgMan L | 25.4±0.7 a | 6.5±0.1 ab | 878±22 a | 259±4 a |
|                 | OrgMan H | 23.8±0.9 ab | 6.4±0.0 ab | 852±33 ab | 239±2 ab |
| Day 80          | NoFer S L | 12.9±0.6 b | 3.6±0.3 b | 381±10 b | 119±5 b |
|                 | NoFer S H | 20.2±1.3 ab | 5.1±0.2 ab | 592±12 ab | 181±6 ab |
|                 | OrgMan S L | 20.0±1.0 ab | 5.2±0.1 ab | 663±50 ab | 183±6 ab |
|                 | OrgMan S H | 15.3±1.4 ab | 6.0±1.3 ab | 664±15 ab | 196±10 ab |
|                 | NoFer L | 18.5±4.1 ab | 5.7±0.8 ab | 590±150 ab | 187±40 ab |
|                 | NoFer H | 25.2±1.1 a | 7.1±0.2 a | 765±34 a | 263±14 a |
|                 | OrgMan L | 25.7±1.1 a | 6.5±0.0 a | 848±27 a | 260±1 ab |
|                 | OrgMan H | 23.0±1.8 a | 6.6±0.2 a | 896±37 a | 254±14 a |

NoFer and OrgMan are soils sampled from no fertilizer and organic manure sites, respectively, S denotes straw amendment, and L and H represent low and high moisture levels, respectively. Diversity indexes are calculated using random selections of 500 sequences per soil sample. Results are expressed with means±standard errors, and different letters within a column at the same sampling time indicate significant differences at a level of α<0.05 based on Bonferroni’s test.

Geissler et al. (2011), who reported that in oat-legume straw-amended soil, the fungal PLFA concentration at 35 % of the WHC was higher by 18 % than that at 55 % of the WHC. Furthermore, a little increase of SOC at low moisture level (Table S1, Electronic Supplementary Material) implies that low moisture content may be favorable for fungal degradation of added straw.

As amendment time increased, the reduced straw availability resulted in a large variation in microbial community structure (Fig.2, Table S4, Electronic Supplementary Material). Drenovsky et al. (2004) suggested that the availability of organic C inputs was a determinant of microbial community structure. With the varied straw availability, the successional dominance of the copiotrophic and oligotrophic groups exists in the amended soils. Previous studies have observed that copiotrophic (or r-selected) populations (e.g., Betaproteobacteria, Bacteroidetes, and Neurospora) thrive under conditions where substrate availability is high, while oligotrophic (or K-selected) populations (e.g., Acidobacteria, Gemmatimonadetes, and Basidiomycota) are relatively more abundant under substrate-limited conditions (Fierer et al. 2007; Bastian et al. 2009; Pascault et al. 2013).

Relative to straw amendment and availability, moisture had a weak influence on microbial community structure (Fig. 2, Table S4, Electronic Supplementary Material). Drenovsky et al. (2004) found that the microbial communities changed little at moisture levels below or equal to field capacity and attributed that to microbial growth in similar relative proportions. Moderate water treatment partly accounted for the weak impact of moisture on soil microbial communities. Most likely, soil microbial communities can be severely disturbed by some extreme treatments. Chowdhury et al. (2011) reported that regardless of soil salinity or straw availability, microbial community composition in soils with consistent moisture content differed from that in soils subjected to frequent alternation of wetting and drying.

4.3 Effects of soil, straw, and moisture on the eukaryotic community

To our knowledge, this is the first work providing information on soil eukaryotic community composition and diversity as affected by straw amendment at different moisture levels using a barcoded pyrosequencing approach.

18S rRNA gene pyrosequencing showed that across all treatments, the fungi were dominated by Dikarya subkingdom (Table S5, Electronic Supplementary Material), and Ascomycota was the most abundant Dikarya (Table S5). Similar observation was shown in the study of Ma et al. (2013), who found that Ascomycota dominated the fungal community during 28 days of straw decomposition in arable soil. Ascomycota and Basidiomycota represent the main classified fungal decomposers in different soils (Vandenkornhuyse et al. 2002). For example, in an arable soil across a pH gradient,
Ascomycota and Basidiomycota made up approximately 45 and 30% of fungal sequences in each sample, respectively (Rousk et al. 2010). Lundell et al. (2010) suggested that Ascomycota preferred to use the easily degradable fractions of residues for fast-growing fungal populations. In the absence of straw, higher abundance of Ascomycota in the OrgMan soil compared to the NoFer soil (Table 2) is mainly attributed to the composted organic manure. Cahyani et al. (2004) found that Ascomycota was abundantly present in the composting process of rice straw.

In this study, we observed that the difference in eukaryotic community composition between the NoFer and OrgMan soils was in evidence (Fig. 4a, Table S6, Electronic Supplementary Material). The difference in the quality of organic matter between the two soils likely explains this result. In the NoFer soil, without any fertilizer input, the organic matter originates solely from crop rhizodeposition and humification of residues and is relatively recalcitrant. In the OrgMan soil, the organic matter mainly originates from organic manure and contains more labile components. The two soils had large differences in SOC content, bioavailability, and C/N ratio. Our hypothesis was supported by Marschner et al. (2003), who reported that in a long-term fertilizer amendment, eukaryotic community structure was significantly affected by SOC content and C/N ratio.

Little difference in SOC between day 20 and day 80 (Table S1, Electronic Supplementary Material) partly explained insignificant effect of straw availability on eukaryotic community composition. Due to straw amendment, eukaryotic diversity and richness were both enhanced; meanwhile, eukaryotic diversity was enhanced under high moisture content (Table 3). Similar finding was observed by Fell et al. (2006), who reported that the eukaryotic communities in Antarctic Dry Valley soils with 0.2–1.3% moisture levels were represented by the yeast species and an unidentified clade of eukaryotes, whereas levels from 3.1 to 4.9% contained the complex communities including primary producers, symbiotic and saprophytic fungi, predators, and nematodes. In the presence of straw, the decrease of fungal biomass and fungal/bacterial PLFA ratio (except that in the NoFer soil at day 20) under high moisture content (Table 1, Fig. 1) suggests that high moisture content probably lessens fungal advantage in the degradation of straw and facilitates the growth of other eukaryotes and results in the increase of eukaryotic diversity. Under identical moisture content, higher eukaryotic diversity and richness in the OrgMan soil compared to the NoFer soil (Table 3) mainly results from the presence of a diverse eukaryotic community in the composting process of organic materials (Cahyani et al. 2004).

The combination of PLFA and 18S rRNA gene pyrosequencing approaches effectively explains the changes of microbial communities as affected by straw amendment at different moisture levels, but both methods have some shortage for identifying microbial taxa. The PLFA method has some misuse or misinterpretation to the specificity of signature PLFA and to what extent such PLFA are found in living microorganisms (Frostegård et al. 2011). 18S rRNA genes are known to be too conserved to provide good separation of fungal taxa, and fungal-specific taxa should use the primers of ITS region of rRNA genes (e.g., Blaalid et al. 2012; Xu et al. 2012; Clemmensen et al. 2013). Hence, more scientific methods will be applied in further research to make more accurate identification of microbial taxa.

5 Conclusions

Straw amendment increases microbial biomass and shifts microbial community structure. Straw availability has a strong influence on microbial community structure. Straw amendment decreases eukaryotic diversity and richness and probably restructures the eukaryotic community. High moisture content enhances eukaryotic diversity, but probably inhibits fungal growth in the amended soils. Under identical moisture content, long-term organic manure-fertilized soil has higher eukaryotic diversity and richness than the unfertilized soil. In summary, microbial communities in Chinese fluvo-aquic soil are significantly affected by straw amendment at different moisture levels.

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