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ORIGINAL PAPER

Plant growth stages and fertilization regimes drive soil fungal community compositions in a wheat-rice rotation system

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Abstract

Fungal communities may have different response to fertilization under different conditions. Therefore, it is necessary to reveal the effects of fertilizations on fungal communities considering temporal heterogeneity at different crop stages. To address this, soil samples were collected across eight typical plant growth stages of a wheat-rice rotation system under four fertilization regimes: no nitrogen fertilizer (NNF), chemical fertilizer (CF), organic-inorganic mixed fertilizer (OIMF), and organic fertilizer (OF). Soil temperature and moisture that co-vary with plant growth stages were the strongest predictors of fungal community compositions; meanwhile, fertilization regimes also played important roles. Shannon index and the relative abundance of Ascomycota were consistently increased when compared OF treatment with CF and OIMF treatments, while CF treatment had a higher relative abundance of Zygomycota when compared with NNF and OF treatments. For the functional guilds, application of urea-nitrogen fertilizers (CF and OIMF treatments) significantly decreased the relative abundance of saprotrophs and symbiotrophs, while soil treated with OF had less relative abundance of pathotrophs when compared to inorganic fertilizers. Our study provided a detailed picture of how fungal community composition responded to fertilization regimes across different plant growth stages.

Keywords Fungal communities · Plant growth stage · Fertilization regime · Dynamic variation

Introduction

Fungi are one of the most important decomposers in soil due to their ability to degrade complex organic matters and due to high biomass, and therefore, the shift in fungal community compositions can also influence the dynamics of carbon (C) and nitrogen (N) cycles (McGuire et al. 2010). However, compared to bacteria, there are fewer researches concerning fungal ecology and function.

Due to anthropogenic disturbance, excess N enters into ecosystems, which can impact ecosystem processes and microbial communities (Allison et al. 2007). Fungi are important player in C and N cycling, and changes in N availability caused by fertilizations can feed back to influence fungal community compositions in arable soils (Koranda et al. 2014). Making use of the rapid development of high-throughput sequencing, the entire fungal community can now be characterized at quite an accurate level compared with single gene survey. However, characterization is limited by the difficulty of parsing fungal phylogenetic taxa into ecologically meaningful categories, and researchers generally discuss changes in the abundance and compositions of fungal taxa and referred to their trophic strategies. Specific composition of fungi community is generally organized into modes by their trophic traits; these modes are saprotroph, symbiotroph, and pathotroph (Nguyen et al. 2016). Most species of soil fungi are saprotrophs, which obtain C from degradation of organic

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matters (Cuadros-Orellana et al. 2013). Symbiotrophs (symbionts) receive nutrients from host cell assimilation products and supply mineral nutrients to host cells in exchange (Lin et al. 2012). Pathotrophs (pathogens) receive nutrients by harming host cells. These three trophic traits often have different response to fertilizations. For example, it is often reported that saprotrophic fungi were stimulated by the addition of N in arable soils (Rousk and Bååth 2011; Sun et al. 2016), whereas a meta-analysis found a decrease in mycorrhizal abundance by 15% due to N fertilization (Treseder 2004). In addition, fungi of different trophic strategies appear to have different response to organic and inorganic N fertilizations. Application of chemical fertilizers has been shown to increase the proportion of pathogenic fungi (Cwalina-Ambroziak et al. 2010; Paungfoo-Lonhienne et al. 2015), whereas application of organic fertilizers could enhance the suppression of pathogens (Ghorbani et al. 2008; Zinati 2005). Additionally, the application of organic fertilization can provide C and N sources that stimulate the abundance and diversity of saprotrophic fungi (Boddy et al. 2007; Song et al. 2015). Trophic strategies can be shared by many different species and are not conserved within taxa. Characterization of the response of fungal community composition to fertilizations by taxa is problematic due to some fungal taxa have two or three trophic strategies (e.g., Basidiomycota and Ascomycota) (Jones et al. 2009). Grouping of taxa into functional guilds could reveal niche differentiation and specific ecosystem functions of fungal community. For the reason that different functional guilds transform soil nutrients in different ways, we could understand the prevalence of different functional guilds under different fertilization regimes (Sterkenburg et al. 2015).

Wheat and rice rotation agricultural systems are one of the highest yielding production systems in the world. Due to difference in water management between wheat and rice seasons, this ecosystem frequently alternates between oxic and anoxic soil conditions. This makes it an attractive model for analyzing microbial ecology and biogeochemical processes (Noll et al. 2005). As described above, application of different fertilizers could influence the compositions of soil fungal communities. However, researches based on a single time point only provide a snapshot of potential responses of fungal community compositions to fertilizations (Xue et al. 2018). To our best knowledge, little information is available regarding the time course of changes in fungal communities and functional groups under different fertilization practices. We therefore lack a detailed picture of how the compositions of fungal communities respond to fertilization regimes in different plant growth stages, and this limits our understanding of the roles of fungi in agricultural ecosystem functioning.

In our study, we investigated the effects of fertilization regime on the compositions of fungal community of soils under long-term fertilization so as to compare our previous studies about the compositions of the soil bacterial and ammonia-oxidizers' communities in the same sites. Fertilization had stronger effect on the compositions of ammonia-oxidizing bacterial community than plant growth stages, whereas it was opposite for the compositions of the whole bacterial community (Wang et al. 2016, 2017). To analyze the response of fungal trophic strategies to fertilizations, we grouped fungal taxa using a database of fungal functional guilds in the present study (Nguyen et al. 2016). We hypothesized that (1) plant growth stages also play a critical role in driving the variation of the compositions of the whole fungal community; (2) different functional groups of fungi are likely to respond differently to plant growth stages and fertilizations; and (3) different types of N fertilizations have distinct impacts on fungal diversity and composition.

Materials and methods

Experimental design, soil samples collection, and determination of soil properties

Soil samples were collected from a long-term experimental field site which was established in 2005 (Wang et al. 2016). The paddy soil is situated in Changshu, Jiangsu Province, China ((31°18′ N, 120° 37′ E, 6 m asl). The site has a humid subtropical monsoon climate with an average annual rainfall of ≈ 1063 mm (Wang et al. 2016).

This study concerned the following four fertilization regimes with three replicated plots for each treatment: (1) no N fertilizer treatment (NNF) with 750 kg ha^{-1} superphosphate (12% P_2O_5) and 183 kg ha^{-1} potassium chloride (60% K_2O) inputs; (2) chemical fertilizer treatment (CF) with application of 391 kg ha^{-1} urea (46% N), 750 kg ha^{-1} superphosphate, and 183 kg ha^{-1} potassium chloride; (3) organic fertilizer treatment (OF) with 4500 kg ha^{-1} pure organic fertilizer (26.4% organic C, 2.5% total N, 1.6% P_2O_5, and 1.3% K_2O, made of composted rice straw and pig manure by Tianniang Ltd. of Changshu, China); and (4) organic-inorganic mixed fertilizer treatment (OIMF) with application of 1500 kg ha^{-1} organic-inorganic mixed fertilizer (11.0% organic C, 12.0% total N, 4.1% P_2O_5 and 4.1% K_2O). One thousand five hundred kilograms of OIMF was pelleted with 625 kg organic fertilizers, 357 kg urea, 428 kg superphosphate, and 89 kg potassium chloride, which was made by Tianniang Ltd. (Changshu, China). An extra application of 28.5 kg ha^{-1} superphosphate and 48.5 kg ha^{-1} potassium chloride was also applied in OIMF treatment to reach the equal quantities of N, P_2O_5, and K_2O as CF plots.

Same quantity of fertilizers was applied twice as basal fertilizer on both October 25, 2012, for wheat and on June 5, 2013, for rice crops.

Soil samples were collected with four cores (5 cm diameter × 20 cm deep) on each of 8 typical crops growth stages in

2013: wheat tillering stage in March 1 (Mar), wheat jointing stage in April 1 (Apr), wheat heading stage in May 1 (May), wheat ripening stage in June 4 (Jun), rice tillering stage in July 7 (Jul), rice jointing stage in August 16 (Aug), rice heading stage in September 15 (Sep), and rice ripening stage in October 31 (Oct). Soil samples were placed in plastic bags and kept with drikold for transportation. To remove the plant materials, roots, and stones, all the 96 soil samples (4 fertilizations × 8 stages × 3 plots) were sieved.

Data of soil available phosphorus, available potassium, available N (exchangeable NH_4^+ and NO_3^-), soil organic matter, soil total N, soil pH and EC, and soil moisture can be found in Supporting Information Table S1. These data were derived from our previous study (Wang et al. 2016).

DNA extraction and fungal ITS gene high-throughput sequencing

Soil DNA was extracted using MoBio Power Soil™ DNA Isolation Kits (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions, which has been described previously (Wang et al. 2016). The PCR reaction of fungal ITS1 region was performed using 5 μL of Q5 Reaction Buffer (5×), 5 μL of Q5 GC High Enhancer (5×), 1 μL (10 μM) of ITS1F primer (5′- CTTGGTCATTTAGA GGAAGTAA -3′) (Gardes and Bruns 1993), 1 μL (10 μM) of ITS2 primer (5′- GCTGCGTTCTTCATCGATGC -3′) (Baldwin 1992), 2 μL of dNTP (2.5 mM), 1 μL of DNA template (20 ng μl^{-1}), 0.25 μL of Q5 Polymerase (5 U μl^{-1}), and 9.75 μL of ddH_2O for a final volume of 25 μL. The PCR ran with a hot start for 5 min at 98 °C, 27 cycles of 98 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s with a final extension for 5 min at 72 °C. The Illumina sequencing adapter ligated reverse primer contained a 6-bp barcode specific for sample identification (Caporaso et al. 2012). After amplification, the triplicate PCR products were pooled and purified using the PCR cleanup Kit (Axygen Biosciences, Union City, CA, USA). Sequencing was performed on a single lane of Illumina MiSeq platform at Personal Biotechnology Co., Ltd. (Shanghai, China).

Bioinformatics and statistical analysis

The obtained sequences were processed following the Quantitative Insights into Microbial Ecology (QIIME) pipeline (Caporaso et al. 2010). Barcodes and the standard primer set sequences were excluded. Low-quality sequences (quality score below 25, read length below 200 bp) were removed from the dataset. Chimera sequences were identified and removed using UCHIME (Edgar et al. 2011). The number of sequences was resampled with 36,255 sequences per sample. Sequences were clustered and assigned to operational taxonomic units (OTUs) using the implementation of QIIME according to a threshold of 97% pairwise identity. For taxonomic assignment of the OTUs, the representative sequences were identified using the UNITE database (Abarenkov et al. 2010) with higher than 80% similarity, and the non-fungal OTUs were discarded.

Fungal taxa were assigned a putative functional guild if they matched a genus that belonged to a single functional group using the FUNGuild v1.0 (Nguyen et al. 2016), the current database for assigning fungal functional guilds. FUNGuild assigns function based on matches at the genus and species level along with a confidence level (Kivlin and Hawkes 2016; Nguyen et al. 2016). All 2221 OTUs were assigned to 36 different guilds. We removed 1073 OTUs that were assigned to "Unassigned" and used the rest of 1148 OTUs for further analysis. The details of the fungal functional guilds can be found in Supporting Information Table S2.

The richness and Shannon indexes were calculated to estimate α-diversity of each sample using R software (Team 2014). Multivariate ANOVAs based on the Shannon and richness indexes were performed to determine the effects of stage and fertilization regimes on the fungal diversities, followed by Tukey's HSD as a post hoc test using SPSS 18.0. Permutational multivariate analyses were performed to determine the effects of plant growth stage, fertilization regimes, and their interactions on fungal compositions and functional guilds based on the OTU relative abundance and functional database of 36 guilds, respectively. Non-metric multidimensional scaling (NMDS) analyses were performed to determine the fungal β-diversity and functional guilds, which were based on the "bray" distance of OTU relative abundance and functional database after Hellinger transformation, respectively. Mantel tests were used to analyze the correlations between the Euclidean distance of soil properties and OTU compositions and functional guilds. Permutational multivariate analyses, NMDS analyses, and Mantel test were performed using "vegan" package in R software (Team 2014). One way ANOVAs with Duncan's tests using SPSS 18.0 were performed to compare the relative abundance of saprotroph, symbiont, and pathogen across eight plant growth stages or under four fertilization regimes, respectively.

We performed linear discriminant effect size (LEfSe) analyses to identify significant differences in fungal taxa between fertilization regimes based on the combined data across all times points. In LEfSe analysis, the Kruskal-Wallis sum-rank test was used to detect the features which were significantly different between assigned classes, and then the effect size of each differentially abundant taxon was estimated using linear discriminant analysis (Segata et al. 2011). The default threshold value (2.0) was tried in LEfSe analysis but contained too much redundant information; therefore, 3.0 was used as threshold value to reduce the redundant information and make the results brief in our present study.

Sequencing data were deposited in the NCBI Sequence Read Archive (SRA) database under the accession number SRX2987082.

Results

Fungal α-diversity

As shown in Table 1, the Shannon index of soil fungi was significantly influenced ($P < 0.001$) by different plant growth stages and different fertilization regimes. As shown in Fig. 1a, the Shannon index ranged from 4.2 to 5.0 across the eight stages. The rice stages (Jul–Oct), as compared with the wheat stages (Mar–Jun), had a significantly higher Shannon index (Tukey's HSD, $P < 0.01$). NNF had the lowest Shannon index, and when CF treatment was compared with OF treatment, its Shannon index was significantly (Tukey's HSD, $P < 0.05$) decreased (Fig. 1c). The richness index was also significantly changed ($P < 0.001$) by plant growth stages (Table 1). As shown in Fig. 1b, the later growth stages (May and Jun for wheat, and Sep and Oct for rice) of wheat and rice have significantly (Tukey's HSD, $P = 0.011$) higher average richness than that of the early growth stages (Mar and Apr for wheat, and Jul and Aug for rice), while different fertilization treatments had no significant effect on fungal richness (Table 1, Fig. 1d).

Fungal community compositions

The most abundant fungal phyla in the soil samples were Ascomycota and Basidiomycota, which accounted for 68.9 and 19.9% on an average, respectively. As shown in Fig. S1, the wheat stages (Mar–Jun) had a higher relative abundance of Ascomycota than the rice stages (Jul–Oct). The relative abundance of Basidiomycota increased with the growth of wheat (Supporting Information Fig. S1) but fluctuated with the growth of rice. The other five phyla of Zygomycota, Rozellomycota, Glomeromycota, Chytridiomycota, and Neocallimastigomycota accounted for 4.2, 1.6, 1.3, 1.2, and 0.05% of the relative abundance, respectively.

Different fertilization regimes had different fungal compositions. Compared with the NNF treatment, N fertilizations inputs increased the relative abundance of the Zygomycota phylum (Supporting Information Fig. S1) and Incertaesedis and Tremellomycetes orders (Fig. 2a). Compared with the CF treatment, partial substitution of organic fertilizer for chemical fertilizer decreased the abundance of the Sordariomycetes order (Fig. 2b). Soils treated with organic fertilizer, when compared with the application of urea-N fertilizers (CF and OIMF), had a significantly higher abundance of the Ascomycota phylum (Supporting Information Fig. S1) and the Pezizomycates order (Fig. 2c, d) across all plant growth stages, whereas the abundance of the Zygomycota phylum and the Tremellomycetes order decreased after OF treatment. In addition, the abundance of Basidiomycota was significantly decreased by the OF treatment when compared with the OIMF treatment (Fig. 2c; Supporting Information Fig. S1); however, this decrease was not observed when compared with CF treatment.

As revealed by permutation test (Table 1), fungal community compositions were significantly influenced by plant growth stages ($P = 0.001$). NMDS analysis also revealed that fungal compositions were apparently dispersed by eight plant growth stages, and that temporally separated stages had more differences in fungal community compositions than more adjacent stages (Fig. 3a). Although the effect of fertilization regimes was less than that of plant growth stages, the effect on fungal community composition was also significant (Table 1). As displayed in Fig. 3b, in the second and third dimensions of NMDS, different fertilization regimes had distinctive community compositions, with OF treatment being more apart from the other three treatments.

Mantel analysis revealed that soil moisture and temperature were the strongest drivers of fungal community composition, which could explain 20.7 and 16.2% for the variation, respectively. In addition, soil available potassium and available phosphorus contents also significantly influenced fungal community composition (Table 2). For the wheat stages, soil available potassium content significantly

Table 1 Effects of fertilization and sampling time on fungal Shannon indexes, Richness, compositions and functional guilds

	Shannon[a]		Richness		Compositions[b]		Functional guilds	
	F	*P*	*F*	*P*	R^2	*P*	R^2	*P*
Fert[c]	38.121	< 0.001**	2.558	0.063	0.211	0.001**	0.187	0.015*
Stage	99.615	< 0.001**	10.176	< 0.001**	0.263	0.001**	0.269	0.001**
Fert × Stage	72.365	< 0.001**	21.171	< 0.001**	0.473	0.003**	0.508	0.784

[a] Impacts on Shannon indexes and richness were determined using two-way ANOVA

[b] R^2 value (effect size) shows the percentage of variation explained by the category by permutational multivariate analysis

[c] Fert indicates fertilization regimes; Stage indicates plant growth stages

*$P < 0.05$; **$P < 0.01$

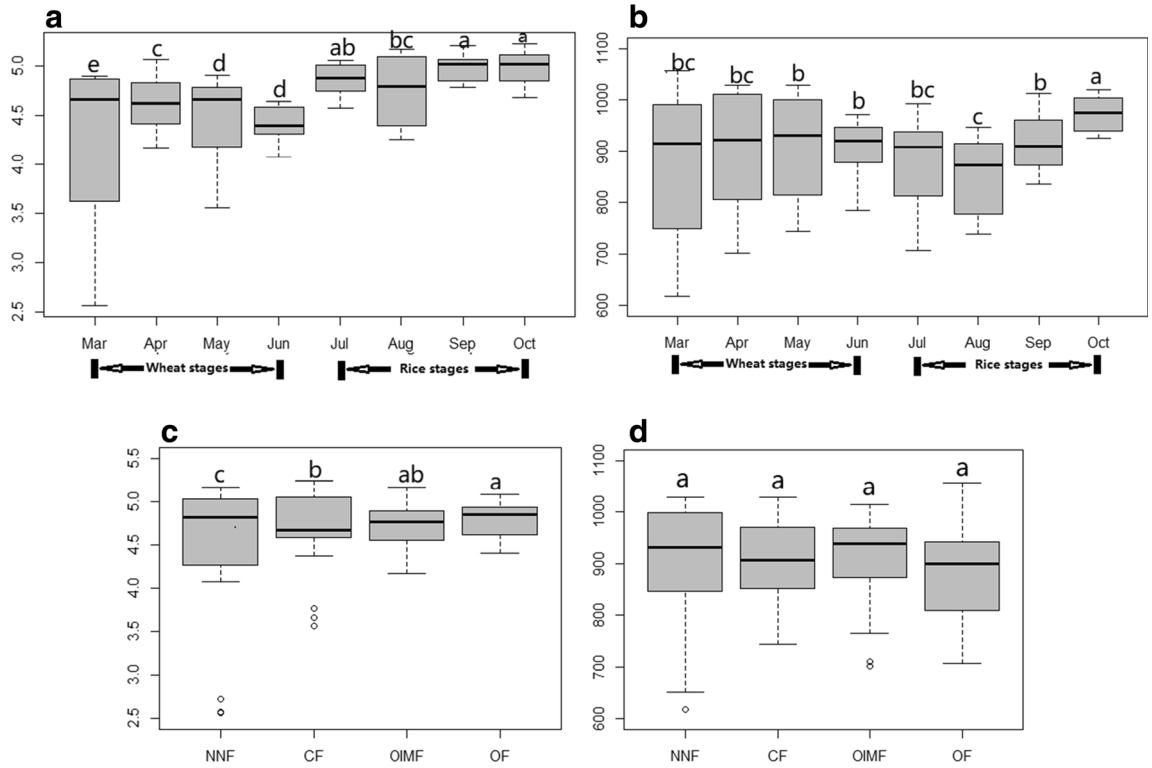

Fig. 1 Fungal Shannon index (**a**) and richness (**b**) across eight plant growth stages. Shannon index (**c**) and richness (**d**) under four fertilization regimes. Significant difference is indicated with different letters (ANOVA followed by Tukey HSD test, $P < 0.05$). In the box plot, the band inside the box is the median; outlier is plotted as individual point. *NNF* no N fertilizer, *CF* chemical fertilizer, *OIMF* organic-inorganic mixed fertilizer, *OF* organic fertilizer. Mar, wheat tillering stage; Apr, wheat jointing stage; May, wheat heading stage; Jun, wheat ripening stage; Jul, rice tillering stage; Aug, rice jointing stage; Sep, rice heading stage; Oct, rice ripening stage

affected fungal community compositions and functional guilds, soil moisture also played a significant role in affecting fungal functional guilds. For the rice stages, soil temperature had a significant effect on fungal community compositions (Table 2).

Fungal functional guilds

In all 96 samples, sequences from the high-throughput sequencing were clustered into 2221 OTUs. Most of the OTUs, 50.1% were grouped as saprotrophs. Pathotrophs and symbiotrophs were the next most common functional guilds accounting for 5.3 and for 4.1% of the OTUs, respectively. As shown in Fig. 4, 59.2% of Ascomycota, 22.6% of Basidiomycota, 98.8% of Zygomycota, and 59.5% of Chytridiomycota phyla were assigned as saprotrophs, respectively, whereas 6.3% of Ascomycota, 4.2% of Basidiomycota, and 9.9% of Chytridiomycota phyla were assigned as pathotrophs, respectively. The percentage assigned as symbiotrophs was 1.2% of Ascomycota, 9.9% of Basidiomycota, and 100% of Glomeromycota phyla, respectively.

Similar to fungal community composition, plant growth stages and fertilization regimes also had significant influence on the fungal functional guilds (Table 1). As with fungal community composition, the impact of plant growth stages was greater than the impact of fertilization regime (Table 1, Fig. 5). Wheat growth stages (Mar–Jun) had a higher abundance of saprotrophs but a lower abundance of symbiotrophs when compared to rice growth stages (Jun–Oct) (Fig. 6a, b). In terms of pathotrophs, the early growth stages (Mar and Apr, Jul and Aug) of wheat and rice had higher abundance than the later growth stages (May and Jun, Sep and Oct) (Fig. 6c). With regard to the influence of different fertilization regimes on the functional guilds, application of urea-N fertilizers (CF and OIMF treatments) significantly ($P < 0.05$) decreased the abundance of saprotrophs and symbiotrophs when compared with the other two N fertilization regimes (Fig. 6d, e). Application of OF significantly decreased the relative abundance of pathotrophs as compared with other fertilization regimes (Fig. 6f). Compared with the CF treatment, OIMF and OF treatments decreased the relative abundance of plant pathogens by 78.8 and 68.3% on an average, respectively (Supporting Information Fig. S2B).

Mantel tests demonstrated that soil moisture and temperature were the factors having the greatest influence on fungal functional guilds (Table 2). Additionally, soil exchangeable ammonium and available potassium contents also significantly influenced the functional guilds.

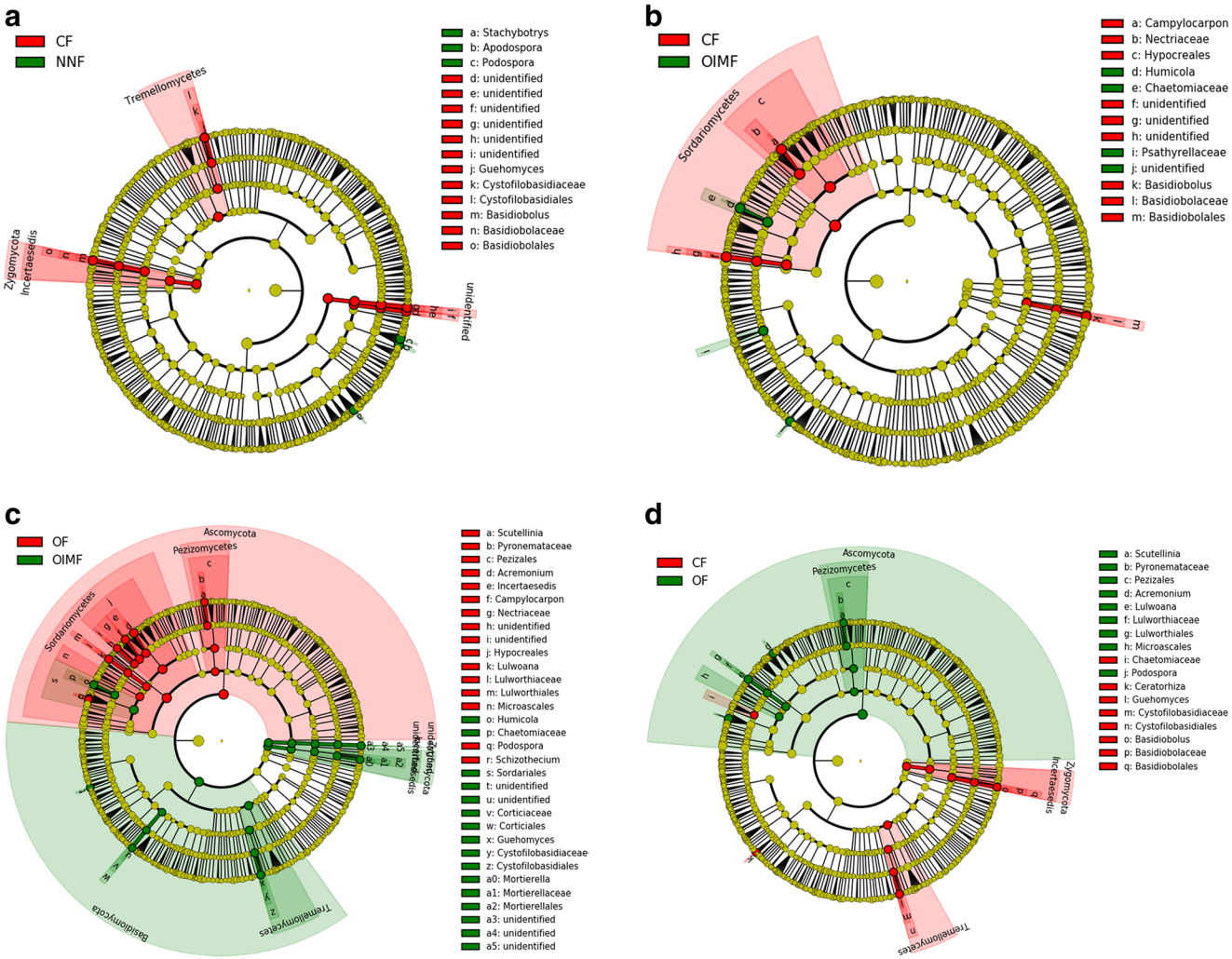

Fig. 2 Cladogram indicating the phylogenetic distribution of fungal lineages. LDA coupled with effect size measurements identifies the different abundant taxa between fertilizer regimes: NNF versus CF treatments (**a**), CF versus OIMF treatments (**b**), OF versus OIMF treatments (**c**), and CF verses OF treatments (**d**). Lineages with LDA values higher than 3.0 are displayed. Significantly ($P < 0.05$) abundant taxa are represented in the color of red or green. Circles represent phylogenetic levels from domain to genus from the inside outwards. *NNF* no N fertilizer, *CF* chemical fertilizer, *OIMF* organic-inorganic mixed fertilizer, *OF* organic fertilizer

Discussion

Plant growth stages determined the variation of fungal communities

The plant growth stage played the most important role in affecting the compositions of bacterial (Wang et al. 2016) and fungal communities in the studied soils, which supported our first hypothesis, whereas the fertilization regimes had higher effects than the plant growth stage in affecting the compositions and abundance of ammonia-oxidizing bacterial communities than different plant growth stages (Wang et al. 2017) probably because the fertilization affected soil N availability (e Silva et al. 2013; Kowalchuk and Stephen 2001). However, for the whole bacterial and fungal communities, employed by 8-year fertilization regimes were less powerful than the plant growth stages in the wheat-rice rotation system, which has been practiced for thousands of years in the experimental area. It should be mentioned that soil fungal community compositions and diversity were also varied in different years (Pickles et al. 2010), thus addressing seasonal changes should also consider the month × year interactions (Mundra et al. 2015). Therefore, a longer term fertilization regime across different years is needed to observe the fertilization effect for the further study.

Higher Shannon indexes demonstrated that soils under rice rotation had higher α-diversities than that of wheat rotation. Rice plots were flooded with 5 cm of standing water from July 10 to September 30, which caused a deficiency of soil oxygen (Lee et al. 2009), with the exception of the uppermost 2 mm

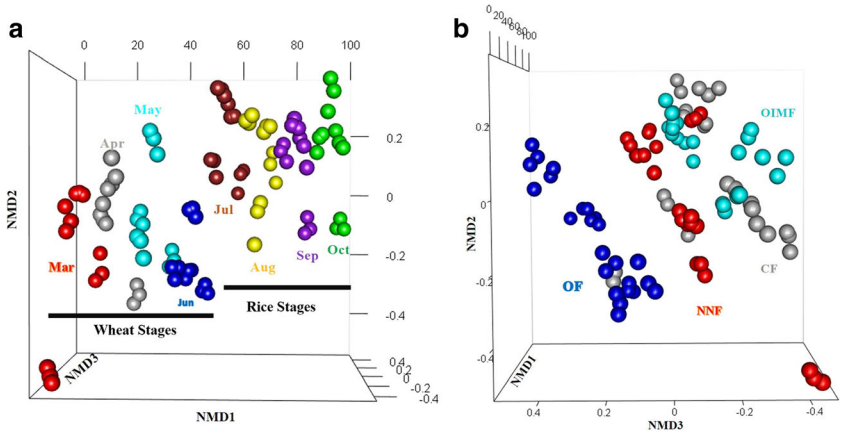

Fig. 3 Fungal community compositions as analyzed by non-metric multidimensional scaling analysis (NMDS) based on OTU relative abundance. Plots of axes 1 and 2 are colored with eight sampling stages (**a**) and axes 2 and 3 are colored with four fertilization regimes (**b**). The stress was 0.136, the non-metric fit R^2 value was 0.981, and the linear fit R^2 value was 0.915. *NNF* no N fertilizer, *CF* chemical fertilizer, *OIMF* organic-inorganic mixed fertilizer, *OF* organic fertilizer. Mar, wheat tillering stage; Apr, wheat jointing stage; May, wheat heading stage; Jun, wheat ripening stage; Jul, rice tillering stage; Aug, rice jointing stage; Sep, rice heading stage; Oct, rice ripening stage

and in the shallow zone around the roots of rice, where an oxygen gradient is established (Noll et al. 2005; Revsbech et al. 1999). Therefore, we speculate that rice growth stages soil provided more heterogeneous niches with oxygen gradients, and different fungal compositions occupied more different niches, as rice soils had more numbers of fungal taxa or lineages than those of wheat soils. In addition, the differences in the eight growth stages of wheat and rice, including different quality and quantity of plant-derived labile C, litter, and root exudates, may also affect the diversity and richness of fungi (Broeckling et al. 2008; Thormann 2006). This could explain our result of higher fungal richness in the heading stage when the plants had their most vigorous growth and also in the ripening stage when plants produce the highest amount of litter (Yoshida 1981).

Additionally, the plant growth stage is not a separate variable explaining the observed variations. Mantel test showed that soil temperature and moisture that co-vary with plant growth stage were the most important factors influencing the compositions of fungal communities. Soil temperature and/or moisture have been demonstrated to be the predominating environmental factors in influencing the activity, composition, and diversity of microbial communities in soil (Bell et al. 2008, 2009; Lauber et al. 2013; Lee et al. 2011). Indeed different fungal species have different temperature and moisture requirement for optimal growth, and this may explain the effect of these two environmental factors on the compositions of fungal communities (Barcenas-Moreno et al. 2009; Frey et al. 1999; Pietikäinen et al. 2005). It should be noted that soil temperature and moisture were nested with the type of crops (wheat and rice). Actually, soil temperature only significantly affected the fungal compositions in rice growth stages, and soil moisture only significantly affected fungal functional guilds in wheat growth stages (Table 2). Therefore, it is

Table 2 Mantle analysis of the relationships between fungal compositions and functional guilds with soil properties

	Whole stages		Wheat stages		Rice stages	
	Compositions	Functional guilds	Compositions	Functional guilds	Compositions	Functional guilds
Temperature	*0.001*	*0.001*	0.374	0.598	*0.002*	0.284
Soil moisture	*0.001*	*0.001*	0.052	*0.008*	0.350	0.420
Organic matter	0.333	0.078	0.892	0.504	0.012	0.044
Total N	0.303	0.718	0.276	0.672	0.410	0.384
NH_4^+	0.241	*0.007*	0.200	0.070	0.104	0.074
NO_3^-	0.719	0.642	0.574	0.312	0.416	0.210
Available potassium	*0.002*	0.030	*0.002*	*0.002*	0.838	0.958
Available phosphorus	0.024	0.136	0.028	0.078	0.254	0.558
EC	0.961	0.957	0.938	0.996	0.518	0.588
pH	0.170	0.181	0.388	0.058	0.454	0.438

Significant differences ($P < 0.01$) are indicated in italics

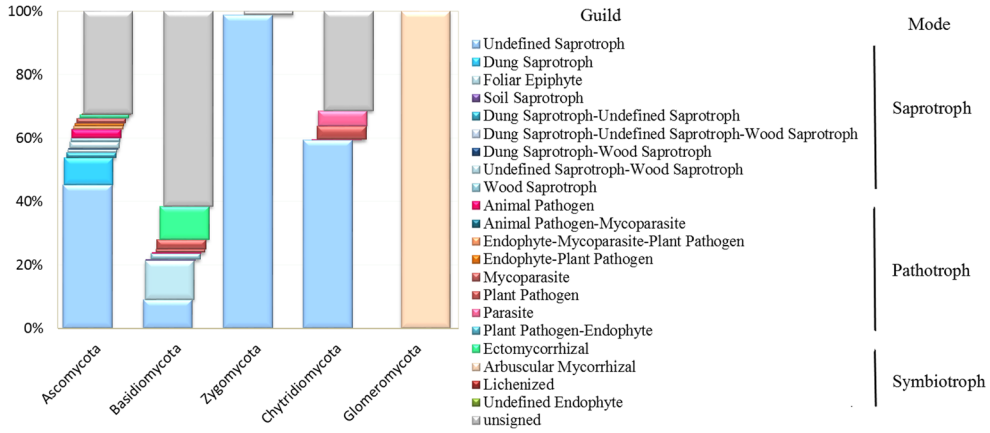

Fig. 4 Fungal functional guild assignments for the five phyla using FUNGuild

needed to be careful in considering the effects of soil temperature and moisture on fungal communities.

Different functional groups of fungi responded differently to plant growth stages, which supported our second hypothesis. Fungal pathogens were more abundant in the early stages of plant growth, indicating that crops might suffer a higher risk of the infection from soil-borne fungal diseases during the early than late growth stages (Ou 1985).

Fertilization regimes played an important role in the variation of fungal diversity and compositions

Our third hypothesis which predicted that different types of N fertilizations have distinct impacts on fungal diversity and compositions was also proved in the present study. Consistent with the previous studies (Cwalina-Ambroziak and Bowszys 2009; Kamaa et al. 2012; Mäder et al. 2002; Song et al. 2015), the organic input to soil increased soil fungal α-diversity (Shannon index) compared to the mineral fertilizer treatment, and probably this depended on the fact that organic fertilizers contain polymers such as polysaccharide, lignin, polyaromatics, etc., which are degraded and used as energy sources by fungi (Leifeld et al. 2002; Hanson et al. 2008); therefore, organic fertilizations improved the fungal α-diversity by stimulating and providing more niches for saprotrophs (Moll et al. 2015; Sun et al. 2016). Despite the N application can decrease the diversities of fungal communities and plants (Arnolds 1991; Allison et al. 2007; Borer et al. 2014; Suding et al. 2005), the α-diversity of whole fungal communities and the Shannon index were lowest in the NNF treatment. The reasons for these inconsistent results are not clear and probably climate and edaphic factors might contribute to this discrepancy. There was no difference of Shannon index between

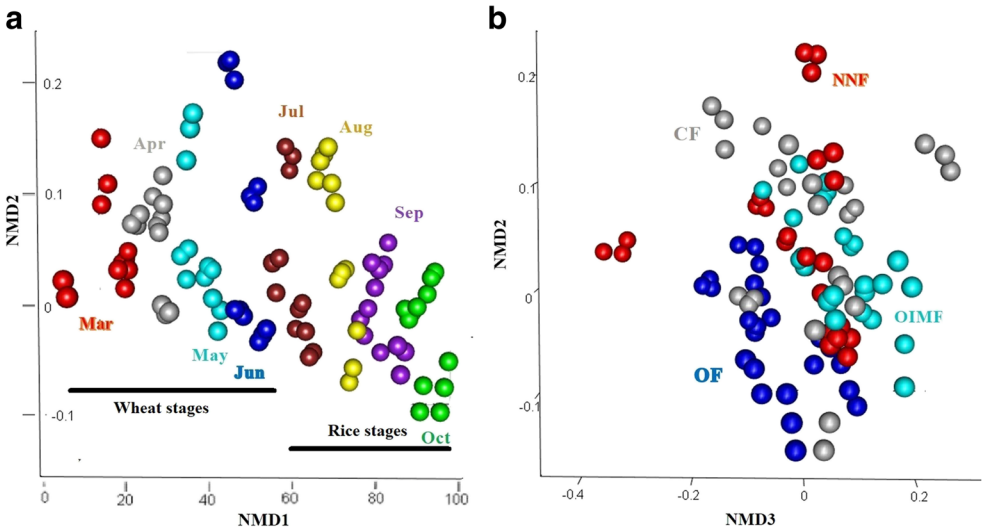

Fig. 5 Fungal community compositions as analyzed by non-metric multidimensional scaling analysis (NMDS) based on functional guilds. Plots of axes 1 and 2 are colored with eight sampling stages (**a**) and axes 2 and 3 are colored with four fertilization regimes (**b**). The stress was 0.198, the non-metric fit R^2 value was 0.96, and the linear fit R^2 value was 0.848. *NNF* no N fertilizer, *CF* chemical fertilizer, *OIMF* organic-inorganic mixed fertilizer, *OF* organic fertilizer. Mar, wheat tillering stage; Apr, wheat jointing stage; May, wheat heading stage; Jun, wheat ripening stage; Jul, rice tillering stage; Aug, rice jointing stage; Sep, rice heading stage; Oct, rice ripening stage

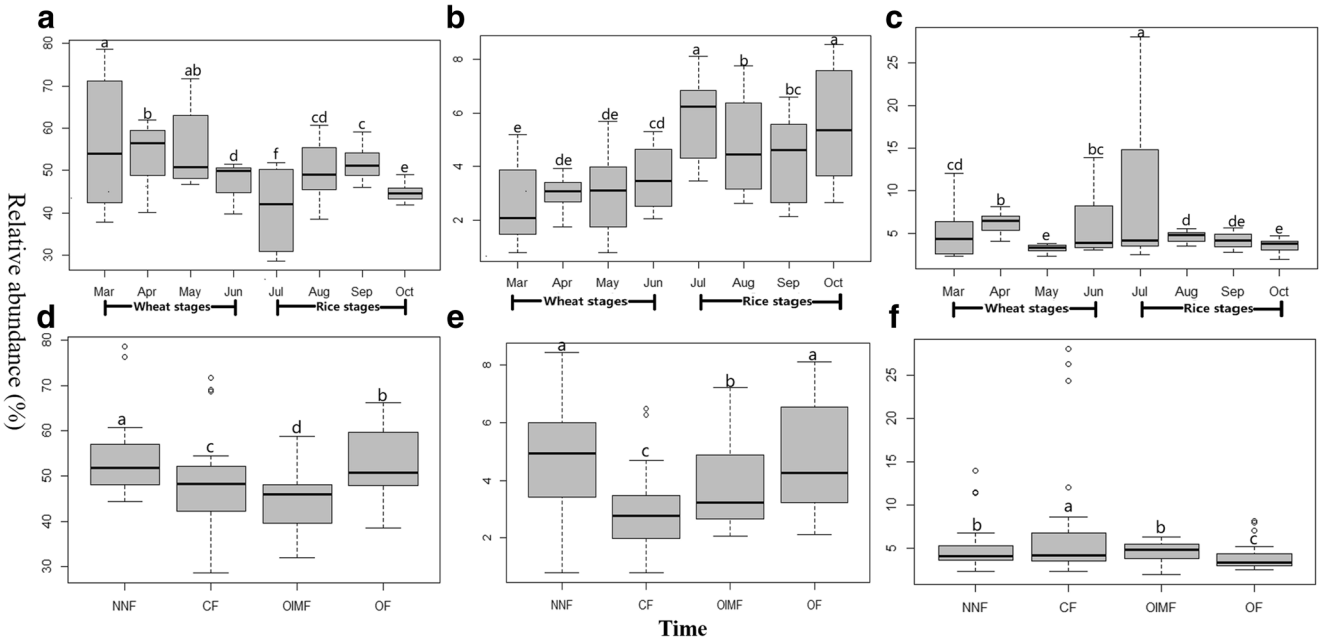

Fig. 6 Relative abundance of fungal guild of saprotrophs (**a**), symbiotrophs (**b**), and pathotrophs (**c**) across eight plant growth stages. Relative abundance of fungal guild of saprotrophs (**d**), symbiotrophs (**e**), and pathotrophs (**f**) under four fertilization regimes. Significant difference is indicated with different letters (ANOVA followed by Tukey HSD test, $P < 0.05$). In the box plot, the band inside the box is the median; outlier is plotted as individual point. *NNF* no N fertilizer, *CF* chemical fertilizer, *OIMF* organic-inorganic mixed fertilizer, *OF* organic fertilizer. Mar, wheat tillering stage; Apr, wheat jointing stage; May, wheat heading stage; Jun, wheat ripening stage; Jul, rice tillering stage; Aug, rice jointing stage; Sep, rice heading stage; Oct, rice ripening stage

CF and OIMF treatment, which was consistent with a previous study based on paddy soil (Chen et al. 2016).

Most saprotrophic fungi are Ascomycota, and previous studies have demonstrated that the abundance of Ascomycota were flourished in response to organic matter additions to soil (Ma et al. 2013; Lentendu et al. 2014), which agree with the findings that soils treated with OF had higher abundance of Ascomycota phylum compared with CF and OIMF soils. However, FUNGuild revealed that the predominant species of the Zygomycota phylum (98.8%) and the Tremellomycetes order (42.7%) are saprotrophs, and their abundance was significantly increased by mineral N fertilizers when compared with no N and organic N inputs. Zygomycota are the first colonizers fungi of fresh substrate, utilize the readily available nutrients for a short time, and their growth can be suppressed by metabolic byproducts (Moore-Landecker 2008). Therefore, Zygomycota preferred CF and OIMF soils with higher contents of available N. FUNGuild showed that all the saprotrophic Tremellomycetes group, as foliar epiphytes (Nakase et al. 2004), and the higher abundance of Tremellomycetes in CF and OIMF than the other two treatments could be explained by the higher crop biomass of these treatments (Wang et al. 2016), which had higher foliar litters compared with NNF and OF treatments.

The relative abundance of symbiotic fungi, including arbuscular mycorrhizal and ectomycorrhizal fungi, was decreased by the application of inorganic N fertilizers. It should be noted that neither rice nor wheat should form symbiosis with ectomycorrhizal fungi, and probably ectomycorrhizal fungi live as saprotrophic lifestyle (Martin et al. 2008). Higher N availability in soils will lead to less dependence of plants on mycorrhizal fungi to take soil nutrients (Treseder 2004; Treseder et al. 2018). These symbiotic fungi received less root-derived C for their growth (Johnson et al. 2010; Sterkenburg et al. 2015; Wei et al. 2013). However, the degradation of organic fertilizers could stimulate the activity of mycorrhizal fungi by the release of some growth-stimulating substances and/or nutrients (Gavito and Olsson 2003; Gryndler et al. 2006; Ravnskov et al. 1999). The Glomeromycota phylum only included arbuscular mycorrhizal fungi (Fig. 4), and our results showed that OF soil had a higher abundance of Glomeromycota than the CF soil across the wheat-rice rotation (Supporting Information Fig. S1). Inconsistently, LEfSe analysis showed that there was no significant difference of Glomeromycota abundance between CF and OF (Fig. 2); however, when the threshold value of LEfSe analyses decreased from 3.0 to 2.0 (default value), the abundance of Glomeromycota was significantly higher in OF and OIMF than in CF (data not shown). Higher abundance of Glomeromycota was also found when N and P inputs to grassland soils decreased (Leff et al. 2015).

Previous studies reported a decrease of plant pathogenic fungal abundance after application of organic inputs to soil (Mokhtar and El-Mougy 2014). Bailey and Lazarovits (2003) suggested that the application of organic matters could release

allelochemicals to reduce soil-borne diseases and Zinati (2005) suggested that compost amendments could suppress diseases by stimulating beneficial microorganisms acting through antibiosis, competition, and predation hyperparasitism or by inducing systemic resistance in the plant. On the contrary, high N fertilizer inputs can increase plant diseases as reviewed by Walters and Bingham (2007). Consistently, OF significantly decreased the relative abundance of plant and also animal pathogens, while CF had the highest relative abundance of pathogens (especially plant pathogens). Therefore, organic managements can potentially improve and cause a moderate degeneration of arable soil qualities, while application of urea-N fertilizers can be potentially harmful to agricultural ecosystem. However, our study was based on relative abundance and diversity of fungal communities and studies on fungal activities should be carried out to confirm the findings of this study.

Conclusion

As already observed for the bacterial communities (Wang et al. 2016), the present study showed that plant growth stages, covarying with the changes of climate and crops growth, played a more important role in affecting the compositions of the fungal communities than fertilization regimes. This study also provides evidence for the effect of fertilizer on fungal community compositions. For instance, OF treatment increased the relative abundance of Ascomycota phylum but decreased that of Zygomycota phylum when compared with CF and OIMF treatments. In addition, different fertilizations changed the abundance of fungal taxa grouped by their trophic strategies. Probably the application of urea-N fertilizers can be harmful to agricultural ecosystems by increasing the relative abundance of pathotrophs and decreasing that of symbiotrophs. Compared to the studies based on individual time points, our study provides a more complete picture of the successional response of fungal communities to fertilization and plant growth stages.

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