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The biogeography of fungal communities in paddy soils is mainly driven by geographic distance

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Abstract

Purpose The aim of this study is to answer how the biogeographic patterns of fungi are affected by spatial and environmental factors in paddy soils characterized by unique field management. Given the generally low C:N ratios of paddy soils, we also want to test a hypothesis that the dominant fungi in paddy soils are Ascomycota, which reportedly prefer habitats with low soil C:N ratios.

Materials and methods Using quantitative PCR and barcoded pyrosequencing, we investigated the abundance, diversity, and community composition of fungal communities in 30 surface paddy soil samples collected from 10 rice cultivation regions of China. Pearson's correlation, analysis of variance, partial least squares regression, principal coordinates analysis, and variation partition were performed for analyses of gene copy numbers, α-diversity, β-diversity, and relative abundances of fungal taxa and their relationships with environmental factors.

Results and discussion The abundance of fungal 18S rRNA gene varied from $10^{6.4}$ to $10^{8.6}$ copies g^{-1} soil, and was positive correlated with soil sand, organic matter, and total nitrogen content, and negatively correlated with soil chloride concentration. Ascomycota comprised 88% of total fungal sequences and increased in relative abundance with increasing soil pH and decreasing mean annual temperature (MAT) and precipitation (MAP). The predominance of Ascomycota in fungal communities is probably due to the low soil C:N ratios (9-15) in the paddy soils studied. The α-diversity increased with MAT, MAP, and soil nitrate-N and total nitrogen content but decreased with soil pH, clay content, chloride concentration, and C:N ratio. Variation partition revealed that fungal β-diversity was mainly driven by geographic distance.

Conclusions In paddy soils which are characterized by intensive rice cropping practices, fungal abundance is mainly influenced by soil properties, fungal α-diversity is constrained by both climatic factors and soil properties, while fungal community compositions are mainly structured by geographic distance.

Keywords Abundance • Community composition • Diversity • Fungi • Paddy soils

1 Introduction

Paddy ecosystems, composing the third largest cropland area and the largest anthropogenic wetland on Earth, are crucial for the global food security and environmental sustainability (Leff et al. 2004; Witt and Haefele 2005; Kögel-Knabner et al. 2010). Paddy fields provide food to more than 50% of the global population, with China as the largest rice-producing country (Lüke et al. 2014). Fungi are important decomposers of soil organic matter, and can form mycorrhizae with plant roots, act as pathogens, or produce greenhouse gases (Nishizawa et al. 2010; Tedersoo et al. 2014; Hu et al. 2015b). Fungi are mostly aerobic and often microaerophilic (Walker and White 2011), yet they have been reported to be numerously abundant in paddy soils with limited oxygen availability and fluctuating redox conditions (Hussain et al. 2011; Lee et al. 2011; Liu et al. 2012). Certain fungal taxa can survive in permanently waterlogged and poorly aerated soils (Hao et al. 1981), and some arbuscular mycorrhizal fungi are able to promote nutrient transportation to rice plants under flooded conditions (Solaiman and Hirata 1995; Watanarojanaporn et al. 2013). In addition, usually there is a period of drainage during rice cropping (Witt and Haefele 2005), and rice plants can transport oxygen to rhizosphere (Kögel-Knabner et al. 2010), which may facilitate the growth of aerobic and microaerophilic fungi. Despite of the important functioning provisions, however, to date far less attention has been paid to fungal communities in paddy soils compared to other terrestrial ecosystems.

Early researchers isolated and enumerated fungi in paddy soils mainly via cultivation-dependent techniques (Dutta and Ghosh 1965; Hao et al. 1981). With the advance of molecular biology techniques independent of cultivation, recent studies shed light on the total fungal community in paddy soils. The abundance and composition of fungal communities have been found to shift between rice cultivars and growth stages (Hussain et al. 2011; Lee et al. 2011). The land-management practices including organic farming, irrigation, and

application of fertilizers, manure, or rice straw (Lopes et al. 2011; Somenahally et al. 2011; Yuan et al. 2013; Ma et al. 2016; Pan et al. 2016), as well as toxic-metal pollution (Somenahally et al. 2011; Liu et al. 2012), can also trigger variation of the fungal abundance and community structure. However, due to the limited number of soil samples or parameters examined and the low throughput and resolution of methods employed, we can hardly have a panoramic view of fungal diversity and its relationships with environmental factors in paddy ecosystems. High-throughput sequencing technologies can help in overcoming this bottleneck and is increasingly being adopted in the broad-scale survey of soil fungi. Recently, using Illumina HiSeq sequencing, Liu et al. (2016a) investigated fungal community composition in a few soils with different rice cultivation history. Jiang et al. (2016) extended the scope to East Asia and explored mechanisms shaping fungal communities in paddy soils. These studies provided useful information in understanding fungal community composition and its spatial distribution, but failed to systematically address the influence of environmental factors on fungal abundance, diversity, and distribution, which is important for understanding fungal microbial ecology and relevant processes in paddy soils and prediction of their responses to environmental change.

Soil properties such as pH, water content, and nutrient levels (particularly nitrogen) have considerable influence on fungal distribution (Taylor and Sinsabaugh 2015). Intensive human manipulation introduces unique features to paddy soils such as water-logging conditions and heavy fertilizer input (Witt and Haefele 2005) that can alter soil properties. One may therefore expect unique patterns of fungal community structure and their responses to environmental gradients in paddy soils, which are not fully understood. To help filling this research gap, we collected 30 surface paddy soil samples from 10 rice cultivation areas in China with a wide range of environmental parameters and investigated the abundance, α- and β-diversity, and community composition of fungal communities. The aim is to answer how the distribution of

fungi is affected by the unique environment in paddy soils. Given the generally low C:N ratios of paddy soils (Witt and Haefele 2005), we also want to test a hypothesis that the dominant fungi in paddy soils are Ascomycota, which reportedly prefer habitats with low soil C:N ratios (Lauber et al. 2008).

2 Materials and methods

2.1 Soil sampling

A total of 30 paddy soil samples were collected from 10 rice cultivation regions in China with a wide range of latitude (N27.18°-N41.52°) and mean annual temperature (8-18°C) and precipitation (650-1500 mm) (Fig. 1 and Table 1), during the rice growth period (May to August) when paddy fields were saturated. The 10 regions were Binhai, Jiangsu Province (BH), Changshu, Jiangsu Province (CS), Hengyang, Hunan Province (HY), Jiaxing, Zhejiang Province (JX), Jingzhou, Hubei Province (JZ), Shenyang, Liaoning Province (LN), Miluo, Hunan Province (ML), Panjin, Liaoning Province (PJ), Shangyu, Zhejiang Province (SY), and Xiantao, Hubei Province (XT). At each site, three soil samples were taken 20 m apart from each other. For each of the three samples, five cores of surface soil (0-20 cm) were taken from an approximate 50 m^2 plot and mixed into a composite sample. Soil samples were transported to the laboratory on ice, and split into two parts. A part of the soil was frozen at -80°C immediately upon arrival for DNA extraction, and another part was stored at 4°C for determination of soil physicochemical properties.

2.2 Physicochemical characterization

The methods used to determine the soil physicochemical properties have been described previously (Hu et al. 2015a). Briefly, soil pH was measured with a soil to water ratio of 2.5

and soil water content was determined by oven-drying soil subsamples at 105°C for 48 hours. Soil organic matter was determined using the $K_2Cr_2O_7$ oxidation-reduction titration method, and total nitrogen was measured on an Element Analyzer (Vario EL III, Elementar, Hanau, Germany). Ammonium and nitrate were extracted from soils with 1 M KCl and measured on a Continuous Flow Analyzer (SAN++, Skalar, Breda, Netherlands). Sulfate (SO_4^{2-}) and chloride (Cl^-) ions were extracted with water (CO_2-removed by boiling) with a soil to water ratio of 2.5 and analyzed using an Ion Chromatography (ICS2500, Dionex, USA). Soil particle size was determined using the rapid sieving method (Kettler et al. 2001).

2.3 DNA extraction and quantitative PCR (qPCR) analysis

Soil DNA was extracted from 0.5 g of soil using the Fast DNA® SPIN Kit for soil (Q BIOgene Inc., Carlsbad, CA, USA) according to the manufacturer's instructions, and evaluated using a NanoDrop ND-2000c UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The copy numbers of fungal 18S rRNA gene (see discussion in Section 4.1) in the 30 paddy soil samples were quantified on an iCycler iQ 5 thermocycler (Bio-Rad Laboratories, Hercules, CA, USA) using the forward primer NS1 (5'-GTAGTCATATGCTTGTCTCPCR-3') (White et al. 1990) and the reverse primer Fung (5'-ATTCCCCGTTACCCGTTG-3') (May et al. 2001). PCR reactions were performed in a 25 µl volume containing 12.5 µl of SYBR® Premix Ex Taq™ (TaKaRa Technology, Dalian, China), 0.5 µl of each primer, and 2 µl of DNA template. Thermal-cycling conditions were as follows: 95°C for 3 min, followed by 35 cycles of 95°C for 10 s, 55°C for 30 s, 72°C for 60 s, and plate read at 83°C for 10 s. Standard curves for qPCR assays were developed using the pGEM-T Easy Vector (Promega, Madison, WI, USA) containing the correct insert of the fungal 18S rRNA gene.

2.4 Barcoded pyrosequencing and data processing

The fungal 18S rRNA gene (see discussion in Section 4.1) was amplified using the primer set EF4/Fung5 (Smit et al. 1999) for barcoded pyrosequencing. A Roche 454 adapter A followed by 10-bp sample-specific barcode sequence was added to the 5′ portion of the forward primer, and a Roche 454 adapter B was added to the 3′ portion of the reverse primer. PCR amplification was conducted in 50 μl reactions including 25 μl of Premix Ex Taq^{TM} (TaKaRa Biotechnology, Dalian, China), 1 μl of each primer, 0.5 μl of bovine serum albumin (25 mg ml^{-1}), and 3 μl of DNA template. Thermal-cycling conditions were as follows: 94°C for 15 min; 94°C for 60 s, 48°C for 60 s, and 72°C for 3 min (40 cycles); and 72°C for 10 min. The PCR products were gel-purified using Wizard SV Gel and PCR Clean Up Kit (Promega, SanLuis Obispo, CA, USA), combined into an equimolar mix, and sent to Macrogen Inc., South Korea for pyrosequencing from the end of adapter A on the Roche 454 GS FLX Titanium platform (Roche Diagnostics, Branford, CT, USA). The resultant sequences were summited to NCBI (SRP116099).

Pyrosequencing data was processed following the standard operation procedure using the Mothur platform (Schloss et al. 2009). Briefly, raw pyrosequencing reads shorter than 200 bp and with quality scores lower than 20 were removed to improve sequence quality. Putative chimeric sequences were eliminated by performing the *chimera.uchime* algorithm (Edgar et al. 2011). The resultant sequences were classified using the Bayesian method (with a bootstrap time of 1,000 and a cutoff 80%) with the SILVA reference database (Pruesse et al. 2007) and the corresponding NCBI taxonomy outline. After removing non-fungal tags, the remaining sequences were binned into operational taxonomic units (OTUs) at the 97% similarity level. To ensure the same level of sampling effort, a randomly selected subset of 189 sequences per sample was used to calculate OTU-based α-diversity and β-diversity. Samples containing

fewer sequences were excluded and consequently 27 fungal communities remained for the diversity calculation.

2.5 Statistical analyses

Pearson's correlation, analysis of variance (ANOVA) with LSD post hoc test, and partial least squares (PLS) regression were used for analyses of gene copy numbers, α-diversity, and relative abundances of fungi and their relationships with environmental factors using IBM SPSS Statistics 19 (IBM Co., Armonk, NY, USA). The copy numbers of fungal 18S rRNA gene were $\log_{10}$-transformed prior to statistical analyses. Principal coordinates analysis (PCoA) and homogeneity of molecular variance (HOMOVA) test were performed for β-diversity analyses based on the Bray-Curtis dissimilarity matrix (Stewart and Excoffier 1996; Schloss 2008) using the "pcoa" and "homova" functions in Mothur, respectively. Variation partition based on distance-based redundancy analysis (db-RDA) was conducted via the "varpart" function in the "vegan" package of R to assess the relative contributions of spatial, climate, and soil factors to Bray-Curtis community dissimilarities. Spatial factors were derived from geographic distances between sampling sites using the "pcnm" function in the "vegan" package (Dray et al. 2006). Environmental factors were pre-selected with the "bioenv" function in the "vegan" package considering the possible collinearity between them (Clarke and Ainsworth 1993). Consequently, in variation partition, climatic factors included mean annual temperature and precipitation, and soil factors comprised soil pH, water content, and concentrations of total nitrogen (TN) and Cl^-. A heatmap to present fungal community compositions was built using the Heatmap Builder software (Clifton Watt, Stanford University, USA). *P* values lower than 0.05 were considered significant.

3 Results

3.1 Soils properties

Soil physiochemical characteristics varied widely across the 10 sampling sites, with pH 5.1-8.7, water content 0.35-0.75 g g^{-1}, organic matter (OM) 15-41 g kg^{-1} soil, TN 0.6-2.2 g kg^{-1} soil, C:N ratios 9-15, sand content 0.07-0.47 g g^{-1}, clay content 0.07-0.39 g g^{-1}, NO_3^--N 0.2-4.5 mg kg^{-1}, NH_4^+-N 11-54 mg kg^{-1}, SO_4^{2-} 53-263 mg kg^{-1}, and Cl^- 7-157 mg kg^{-1} (Table 1).

3.2 Fungal abundance

The abundance of fungal 18S rRNA gene ranged between $10^{6.4}$-$10^{8.6}$ copies g^{-1} soil across the 30 paddy soil samples, with the highest value recorded in the CS site and the lowest value in the PJ site (Fig. 2). Fungal abundance was positively correlated with soil sand, OM, and TN content while negatively correlated with soil chloride concentration (Table 2).

3.3 α- and β-diversity of fungal communities

In total, 132,599 quality-filtered fungal sequences with a read length of ≥ 200 bp were recovered from the soil samples. We resampled 189 sequences per soil sample for analyzing community diversity at the same level of surveying effort. With 189 sequences the Shannon and Simpson indexes became stable (data not shown). Samples containing fewer sequences were excluded, resulting in 27 fungal communities for the α- and β-diversity analyses.

The number of observed OTUs (at the 97% similarity level) varied from 23 to 64 across the 27 paddy soils, and the Chao1, inverse Simpson, and Shannon indexes ranged from 58 to 241, 4.6 to 14.4, and 2.3 to 3.7, respectively (Table 3). The lowest fungal diversity was observed in soils from the site PJ (Table 3), which had high soil pH and concentrations of SO_4^{2-} and Cl^- (Table 1). PLS regression analysis showed that fungal α-diversity had positive relationships

with mean annual temperature and precipitation and soil nitrate-N and total N concentrations but had negative correlations with soil pH, C:N ratio, clay content, and sulfate and chloride concentrations (Fig. 3).

Separation of fungal communities at different sites was significant according to the HOMOVA test ($P < 0.05$ across sites). In the PCoA ordination (Fig. 4), fungal communities separated largely according to location, and a few clusters could be identified: sites LN and PJ from Liaoning in the Northeast of China; sites BH, CS, and JX from Jiangsu and Zhejiang in the East of China; sites JZ and XT from Hubei in Central China; and sites HY and ML from Hunan in the South of China (Fig. 1 and Fig. 4). The distance-decay curve also revealed a significant correlation between community dissimilarity and geographic distance (Fig. 5). Variation partitioning further indicated that spatial and environmental factors considered in the study together explained 42% of fungal community dissimilarity (Fig. 6). Spatial, soil, and climatic factors accounted for 36, 26, and 15% of the variation, with their exclusive percentages of explanation confined to 8, 1, and < 0.001%, respectively.

3.4 Fungal community composition and distribution

Fungal communities retrieved from paddy soils were dominated by the phylum Ascomycota (comprising 87.8% of total fungal sequences), followed by Basidiomycota (5.9%), Glomeromycota (0.8%), and unclassified sequences (4.9%) (Fig. 7). The relative abundance of Ascomycota increased with soil pH ($r = 0.382$, $P < 0.05$, $n = 27$) but decreased with mean annual temperature and precipitation (r was -0.483 and -0.558, respectively; $P < 0.05$ and $n = 27$ for both). The relative abundance of Glomeromycota had positive correlations with soil water content and the ammonium-N concentration (data not shown).

Of Ascomycota, 96% were classified into Pezizomycotina, which included Sordariomycetes

(64.0% of total fungal sequences), Dothideomycetes (5.3%), Eurotiomycetes (1.1%), Leotiomycetes (1.0%), Pezizomycetes (0.5%), and unclassified Pezizomycotina (12.0%). The relative abundance of Dothideomycetes was negatively correlated with mean annual temperature and precipitation and soil water content, OM, TN, and sand content, but positively correlated with soil clay and chloride content. The relative abundance of Eurotiomycetes had a negative relationship with soil pH. The relative abundance of Leotiomycetes decreased with mean annual temperature and precipitation and soil water content, OM, and TN content (data not shown).

The dominant class Sordariomycetes was composed of Hypocreomycetidae (17.3% of total fungal sequences), Sordariomycetes incertae sedis (1.6%), Sordariomycetidae (0.3%), Xylariomycetidae (0.4%), and unclassified Sordariomycetes (44.4%). The relative abundance of Hypocreomycetidae decreased with mean annual temperature and precipitation and soil TN content, but increased with soil pH and C:N ratio. The proportion of Xylariomycetidae increased with soil chloride concentration (data not shown).

Of the second largest phylum Basidiomycota, 93% were Agaricomycetes, whose relative abundance was not significantly correlated with any environmental parameters examined.

4 Discussion

4.1 18S rRNA gene as a marker for fungi

Both the internal transcribed spacer (ITS) region and 18S rRNA gene are used as markers for fungal community studies (Lindahl et al. 2013). The ITS region is usually preferred because it gives finer taxonomic resolution and can be useful for species separation (Bellemain et al. 2010; Lindahl et al. 2013). Nevertheless, Lindahl et al. (2013) also pointed out the ITS region is too variable for phylogenetic analysis at high taxonomic ranks like families and orders. For

assessment of fungal abundance, the reproducibility and accuracy of qPCR results are hampered by the length of the ITS region and its polymorphism as well as taxonomic bias of primers (Bellemain et al. 2010; Chemidlin Prévost-Bouré et al. 2011). Therefore, primers targeting 18S rRNA gene region have also been used in qPCR, although issues related to the length of targeted region and the specificity of primers still exist (Chemidlin Prévost-Bouré et al. 2011; Liu et al. 2016b). In this study, taxonomic resolution is not relevant to abundance analysis, and community composition was discussed at the taxonomic level of families or above. Thus, we believe using 18S rRNA gene as a marker for fungi is valid in this study.

4.2 Relationships between fungal abundance and α-diversity with climatic and soil factors

The fungal abundance in our paddy samples was influenced by several soil properties (Table 2). Fungi cannot fix carbon and nitrogen and require provision of organic matter and nitrogenous compounds for growth and energy, and most fungi prefer warm, sugary, acidic, aerobic, and moisture conditions (Walker and White 2011). This might explain the positive correlations of fungal abundance with soil OM and TN content (Table 2). The negative correlation between fungal abundance and soil chloride concentration may be attributed to the impact of salinity, because it has been reported that fungal growth needs high water activity (i.e. low solute concentration) (Walker and White 2011) and could be suppressed by excessive salt in the soil (Juniper and Abbott 1993). The positive influence of soil sand content on fungal abundance may be direct via affecting aeration and oxygen availability or indirect due to the correlations of soil sand content with OM content (r = 0.406, P < 0.05, n = 30) and chloride concentration (r = -0.579, P < 0.05, n = 30). Nevertheless, in upland soils, Chemidlin Prévost-Bouré et al. (2011) found that fungal abundance was negatively correlated with sand

content. Perhaps in dry soils fungal growth is more constrained by water, which is less withheld in sandy soils.

The number of observed OTUs (23-64) in the paddy soils studied was comparable to that reported in upland soils when estimating with the same quantity of 18S rRNA gene sequences (Allison et al. 2007). Fungal diversity was correlated with both climatic and soil factors (Fig. 3). Greater quantity and diversity of fungal OTUs were observed at sites with higher mean annual temperature and precipitation, consistent with their physiological characteristics mentioned above. In contrast to the observation in some forest ecosystems where nitrogen addition could reduce fungal diversity (Allison et al. 2007), fungal diversity in our paddy soils increased with soil TN and nitrate-N content but decreased with C:N ratio. This is probably due to, as discussed later, the dominant fungi in our paddy soils were Ascomycota which reportedly can proliferate in soils with low soil C:N ratios (Allison et al. 2007; Lauber et al. 2008). Since most fungi are acidophilic (Walker and White 2011), it is not surprising that paddy diversity also had a significant negative relationship with soil pH.

4.3 Geographic distance as the main driver of fungal community dissimilarity

Two processes shaping microbial biogeography have been proposed: contemporary environmental conditions (environmental selection) and historical contingencies which is reflected by dispersal limitation (Hanson 2016). In the paddy soils studied, variation partition showed that the dissimilarity of fungal communities was more associated with geographic distance compared to soil properties and climatic factors (Fig. 6), corroborated by the significant increase of community dissimilarity with increasing geographic distance (Fig. 5). Similarly, an investigation in paddy soils across East Asia also found better prediction by geographic distance than soil properties for fungal community composition (Jiang et al. 2016).

This suggests that in paddy soils dispersal limitation (historical contingencies) is the main driver for community dissimilarity, which contrasts with some studies in forest soils where climatic factors have been shown as the best predictors (Shi et al. 2014; Tedersoo et al. 2014). The reason requires further investigation, but the relative importance of historical contingencies and contemporary environment may vary in different types of soils or ecosystems which have distinct current environmental conditions and experienced different historic events.

4.4 Dominance of Ascomycota in paddy soils

Like in most other flooded paddy soils (Hussain et al. 2011; Lee et al. 2011; Jiang et al. 2016; Liu et al. 2016a; Liu et al. 2016b) and upland agricultural soils (Klaubauf et al. 2010; Nishizawa et al. 2010; Xu et al. 2012; Moll et al. 2016), the dominant fungi in our samples were Ascomycota (Fig. 7), which supports our hypothesis that the dominant fungi in paddy soils are Ascomycota. The finding contrasts with forest soils where Basidiomycota were often reported as the most dominant phylum (O'Brien et al. 2005; Allison et al. 2007; Lauber et al. 2008; Buée et al. 2009; Shi et al. 2014; Tedersoo et al. 2014). This divergence was also observed by Lauber et al. (2008) who found that Ascomycota (mainly Sordariomycetes) were more abundant in cultivated and pasture soils compared to forest soils which contained more Basidiomycota (mainly Agaricales). They further reported that the relative abundance of Sordariomycetes decreased while Agaricales increased with soil C:N ratio. This may explain the predominance of Ascomycota over Basidiomycota in our paddy soils and other agricultural soils, because the C:N ratio, which has been widely observed influencing fungal community structure (Lauber et al. 2008; Curlevski et al. 2010; Newsham et al. 2016), is generally lower in agricultural soils than forest soils due to the quality (C:N ratio) of litter

received (Compton and Boone 2000; Lauber et al. 2008; Chapin III et al. 2011) and N fertilizer input (Witt and Haefele 2005; Allison et al. 2007). Indeed, the C:N ratios in our paddy soils (9-15) were much lower compared to those (generally > 20) in most forest soils (Compton and Boone 2000; Lauber et al. 2008; Ross et al. 2011).

The relative abundance of Ascomycota had a positive relationship with soil pH, despite that most fungi prefer slightly acid habitats (Walker and White 2011). The response of ascomycotal distribution to soil acidity has been scarcely investigated, but alkalophilic or alkali-tolerant Ascomycota are present in soil (Nagai et al. 1995; Nagai et al. 1998; Elíades et al. 2006; Grum-Grzhimaylo et al. 2016). Especially, Grum-Grzhimaylo et al. (2016) recently studied fungi isolates from soda soils and found that the alkaliphilic trait is spread throughout Ascomycota. The results in this study call for assessment of prevalence of alkaliphilic Ascomycota in paddy soils.

5 Conclusions

Microbial abundance, diversity, and community composition and their relationships with the environment are fundamental information for understanding microbial ecology and processes in soil ecosystems. This study examined the abundance, diversity, and distribution patterns of fungal communities in 30 paddy soils sampled from 10 representative rice-growing regions of China. The results revealed Ascomycota are the dominant fungi in paddy soils, and fungal abundance, α-diversity, and β-diversity are influenced by different environmental parameters. The findings may help us better understand fungal ecology and related processes and predict their responses to environmental changes in paddy ecosystems.

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References

Allison SD, Hanson CA, Treseder KK (2007) Nitrogen fertilization reduces diversity and alters community structure of active fungi in boreal ecosystems. Soil Biol Biochem 39:1878-1887

Bellemain E, Carlsen T, Brochmann C, Coissac E, Taberlet P, Kauserud H (2010) ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. BMC Microbiol 10:189

Buée M, Reich M, Murat C, Morin E, Nilsson RH, Uroz S, Martin F (2009) 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. New Phytologist 184:449-456

Chapin III FS, Matson PA, Vitousek P (2011) Principles of Terrestrial Ecosystem Ecology. 2ed Edition. Springer

Chemidlin Prévost-Bouré N, Christen R, Dequiedt S, Mougel C, Lelièvre M, Jolivet C, Shahbazkia HR, Guillou L, Arrouays D, Ranjard L (2011) Validation and application of a PCR primer set to quantify fungal communities in the soil environment by real-time quantitative PCR. PLoS One 6:e24166

Clarke KR, Ainsworth M (1993) A method of linking multivariate community structure to environmental variables. Mar Ecol Prog Ser 92:205-219

Compton JE, Boone RD (2000) Long-term impacts of agriculture on soil carbon and nitrogen in New England forests. Ecology 81:2314-2330

Curlevski NJA, Xu ZH, Anderson IC, Cairney JWG (2010) Converting Australian tropical rainforest to native Araucariaceae plantations alters soil fungal communities. Soil Biol Biochem 42:14-20

Dray S, Legendre P, Peres-Neto PR (2006) Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). Ecol Modell 196:483-493

Dutta BG, Ghosh GR (1965) Soil fungi from orissa (India) IV. Soil fungi of paddy fields. Mycopathol Mycol Appl 25:316-322

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194-2200

Elíades L, Cabello M, Voget C (2006) Contribution to the study of alkalophilic and alkali-tolerant Ascomycota from Argentina. Darwiniana 44:64-73

Grum-Grzhimaylo AA, Georgieva ML, Bondarenko SA, Debets AJM, Bilanenko EN (2016) On the diversity of fungi from soda soils. Fungal Diversity 76:27-74

Hanson CA (2016) Microbial Biogeography. In: International Encyclopedia of Geography: People, the Earth, Environment and Technology. John Wiley & Sons, Ltd.

Hao W-y, Yao H-q, Xu Y-r (1981) Investigation on Ecological Distribution of Fungi in Paddy Soils. In: Proceedings of Symposium on Paddy Soils. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 323-329

Hu H-W, Zhang L-M, Yuan C-L, Zheng Y, Wang J-T, Chen D, He J-Z (2015a) The large-scale distribution of ammonia oxidizers in paddy soils is driven by soil pH, geographic

distance, and climatic factors. Front Microbiol 6:938

Hu HW, Chen D, He JZ (2015b) Microbial regulation of terrestrial nitrous oxide formation: understanding the biological pathways for prediction of emission rates. FEMS Microbiol Rev 39:729-749

Hussain Q, Liu Y, Zhang A, Pan G, Li L, Zhang X, Song X, Cui L, Jin Z (2011) Variation of bacterial and fungal community structures in the rhizosphere of hybrid and standard rice cultivars and linkage to CO2 flux. FEMS Microbiol Ecol 78:116-128

Jiang Y, Liang Y, Li C, Wang F, Sui Y, Suvannang N, Zhou J, Sun B (2016) Crop rotations alter bacterial and fungal diversity in paddy soils across East Asia. Soil Biol Biochem 95:250-261

Juniper S, Abbott L (1993) Vesicular-arbuscular mycorrhizas and soil salinity. Mycorrhiza 4:45-57

Kögel-Knabner I, Amelung W, Cao Z, Fiedler S, Frenzel P, Jahn R, Kalbitz K, Kölbl A, Schloter M (2010) Biogeochemistry of paddy soils. Geoderma 157:1-14

Kettler TA, Doran JW, Gilbert TL (2001) Simplified method for soil particle-size determination to accompany soil-quality analyses. Soil Sci Soc Am J 65:849-852

Klaubauf S, Inselsbacher E, Zechmeister-Boltenstern S, Wanek W, Gottsberger R, Strauss J, Gorfer M (2010) Molecular diversity of fungal communities in agricultural soils from Lower Austria. Fungal Diversity 44:65-75

Lüke C, Frenzel P, Ho A, Fiantis D, Schad P, Schneider B, Schwark L, Utami SR (2014) Macroecology of methane-oxidizing bacteria: the β-diversity of pmoA genotypes in tropical and subtropical rice paddies. Environ Microbiol 16:72-83

Lauber CL, Strickland MS, Bradford MA, Fierer N (2008) The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biol Biochem 40:2407-2415

Lee S-H, Kim C-G, Kang H (2011) Temporal dynamics of bacterial and fungal communities in a genetically modified (GM) rice ecosystem. Microb Ecol 61:646-659

Leff B, Ramankutty N, Foley JA (2004) Geographic distribution of major crops across the world. Global Biogeochemical Cycles 18. doi: 10.1029/2003gb002108

Lindahl BD, Nilsson RH, Tedersoo L, Abarenkov K, Carlsen T, Kjøller R, Kõljalg U, Pennanen T, Rosendahl S, Stenlid J, Kauserud H (2013) Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide. New Phytol 199:288-299

Liu C, Ding N, Fu Q, Brookes PC, Xu J, Guo B, Lin Y, Li H, Li N (2016a) The influence of soil properties on the size and structure of bacterial and fungal communities along a paddy soil chronosequence. Eur J Soil Biol 76:9-18

Liu Y, Wang P, Pan G, Crowley D, Li L, Zheng J, Zhang X, Zheng J (2016b) Functional and structural responses of bacterial and fungal communities from paddy fields following long-term rice cultivation. J Soils Sediments 16:1460-1471

Liu Y, Zhou T, Crowley D, Li L, Liu D, Zheng J, Yu X, Pan G, Hussain Q, Zhang X, Zheng J (2012) Decline in topsoil microbial quotient, fungal abundance and C utilization efficiency of rice paddies under heavy metal pollution across South China. PLoS One 7:e38858

Lopes AR, Faria C, Prieto-Fernandez A, Trasar-Cepeda C, Manaia CM, Nunes OC (2011) Comparative study of the microbial diversity of bulk paddy soil of two rice fields subjected to organic and conventional farming. Soil Biol Biochem 43:115-125

Ma X, Liu M, Li Z (2016) Shifts in microbial biomass and community composition in subtropical paddy soils under a gradient of manure amendment. Biol Fertil Soils 52:775-787

May LA, Smiley B, Schmidt MG (2001) Comparative denaturing gradient gel electrophoresis

analysis of fungal communities associated with whole plant corn silage. Can J Microbiol 47:829-841

Moll J, Hoppe B, König S, Wubet T, Buscot F, Krüger D (2016) Spatial distribution of fungal communities in an arable soil. PLoS One 11:e0148130

Nagai K, Sakai T, Rantiatmodjo RM, Suzuki K, Gams W, Okada G (1995) Studies on the distribution of alkalophilic and alkali-tolerant soil fungi I. Mycoscience 36:247-256

Nagai K, Suzuki K, Okada G (1998) Studies on the distribution of alkalophilic and alkali-tolerant soil fungi II: Fungal flora in two limestone caves in Japan. Mycoscience 39:293

Newsham KK, Hopkins DW, Carvalhais LC, Fretwell PT, Rushton SP, Odonnell AG, Dennis PG (2016) Relationship between soil fungal diversity and temperature in the maritime Antarctic. Nature Climate Change 6:182-186

Nishizawa T, Zhaorigetu, Komatsuzaki M, Sato Y, Kaneko N, Ohta H (2010) Molecular characterization of fungal communities in non-tilled, cover-cropped upland rice field soils. Microbes Environ 25:204-210

O'Brien HE, Parrent JL, Jackson JA, Moncalvo JM, Vilgalys R (2005) Fungal community analysis by large-scale sequencing of environmental samples. Appl Environ Microbiol 71:5544-5550

Pan F, Li Y, Chapman SJ, Yao H (2016) Effect of rice straw application on microbial community and activity in paddy soil under different water status. Environ Sci Pollut Res 23:5941-5948

Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig WG, Peplies J, Glockner FO (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res 35:7188-7196

Ross DS, Bailey SW, Lawrence GB, Shanley JB, Fredriksen G, Jamison AE, Brousseau PA

(2011) Near-Surface Soil Carbon, Carbon/Nitrogen Ratio, and Tree Species Are Tightly Linked across Northeastern United States Watersheds. Forest Science 57:460-469

Schloss PD (2008) Evaluating different approaches that test whether microbial communities have the same structure. ISME J 2:265-275:311

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537-7541

Shi L-L, Mortimer PE, Ferry Slik JW, Zou X-M, Xu J, Feng W-T, Qiao L (2014) Variation in forest soil fungal diversity along a latitudinal gradient. Fungal Diversity 64:305-315

Smit E, Leeflang P, Glandorf B, van Elsas JD, Wernars K (1999) Analysis of fungal diversity in the wheat rhizosphere by sequencing of cloned PCR-amplified genes encoding 18S rRNA and temperature gradient gel electrophoresis. Appl Environ Microbiol 65:2614-2621

Solaiman MZ, Hirata H (1995) Effects of indigenous arbuscular mycorrhizal fungi in paddy fields on rice growth and N, P, K nutrition under different water regimes. Soil Science & Plant Nutrition 41:505-514

Somenahally AC, Hollister EB, Loeppert RH, Yan W, Gentry TJ (2011) Microbial communities in rice rhizosphere altered by intermittent and continuous flooding in fields with long-term arsenic application. Soil Biol Biochem 43:1220-1228

Stewart CN, Excoffier L (1996) Assessing population genetic structure and variability with RAPD data: Application to Vaccinium macrocarpon (American Cranberry). J Evol Biol 9:153-171

Taylor DL, Sinsabaugh RL (2015) The soil fungi: occurrence, phylogeny, and ecology. In: Paul E (ed) Soil microbiology, ecology, and biochemistry. 4th Edition. Academic Press, pp 77-100

Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, Wijesundera R, Ruiz LV, Vasco-Palacios AM, Thu PQ, Suija A, Smith ME, Sharp C, Saluveer E, Saitta A, Rosas M, Riit T, Ratkowsky D, Pritsch K, Põldmaa K, Piepenbring M, Phosri C, Peterson M, Parts K, Pärtel K, Otsing E, Nouhra E, Njouonkou AL, Nilsson RH, Morgado LN, Mayor J, May TW, Majuakim L, Lodge DJ, Lee SS, Larsson K-H, Kohout P, Hosaka K, Hiiesalu I, Henkel TW, Harend H, Guo L-d, Greslebin A, Grelet G, Geml J, Gates G, Dunstan W, Dunk C, Drenkhan R, Dearnaley J, De Kesel A, Dang T, Chen X, Buegger F, Brearley FQ, Bonito G, Anslan S, Abell S, Abarenkov K (2014) Global diversity and geography of soil fungi. Science 346(6212:1256688

Walker GM, White NA (2011) Introduction to Fungal Physiology. In: Kavanagh K (ed) Fungi: Biology and Applications. 2nd Edition. John Wiley & Sons, Ltd, Chichester, pp 1-35

Watanarojanaporn N, Boonkerd N, Tittabutr P, Longtonglang A, Young JPW, Teaumroong N (2013) Effect of Rice Cultivation Systems on Indigenous Arbuscular Mycorrhizal Fungal Community Structure. Microbes Environ 28:316-324

White T, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: A guide to methods and applications. Academic Press, San Diego, pp 315-322

Witt C, Haefele SM (2005) Paddy Soils. In: Daniel H (ed) Encyclopedia of Soils in the Environment. Elsevier, Oxford, pp 141-150

Xu LH, Ravnskov S, Larsen J, Nilsson RH, Nicolaisen M (2012) Soil fungal community structure along a soil health gradient in pea fields examined using deep amplicon

sequencing. Soil Biol Biochem 46:26-32

Yuan H, Ge T, Zhou P, Liu S, Roberts P, Zhu H, Zou Z, Tong C, Wu J (2013) Soil microbial biomass and bacterial and fungal community structures responses to long-term fertilization in paddy soils. J Soils Sediments 13:877-886

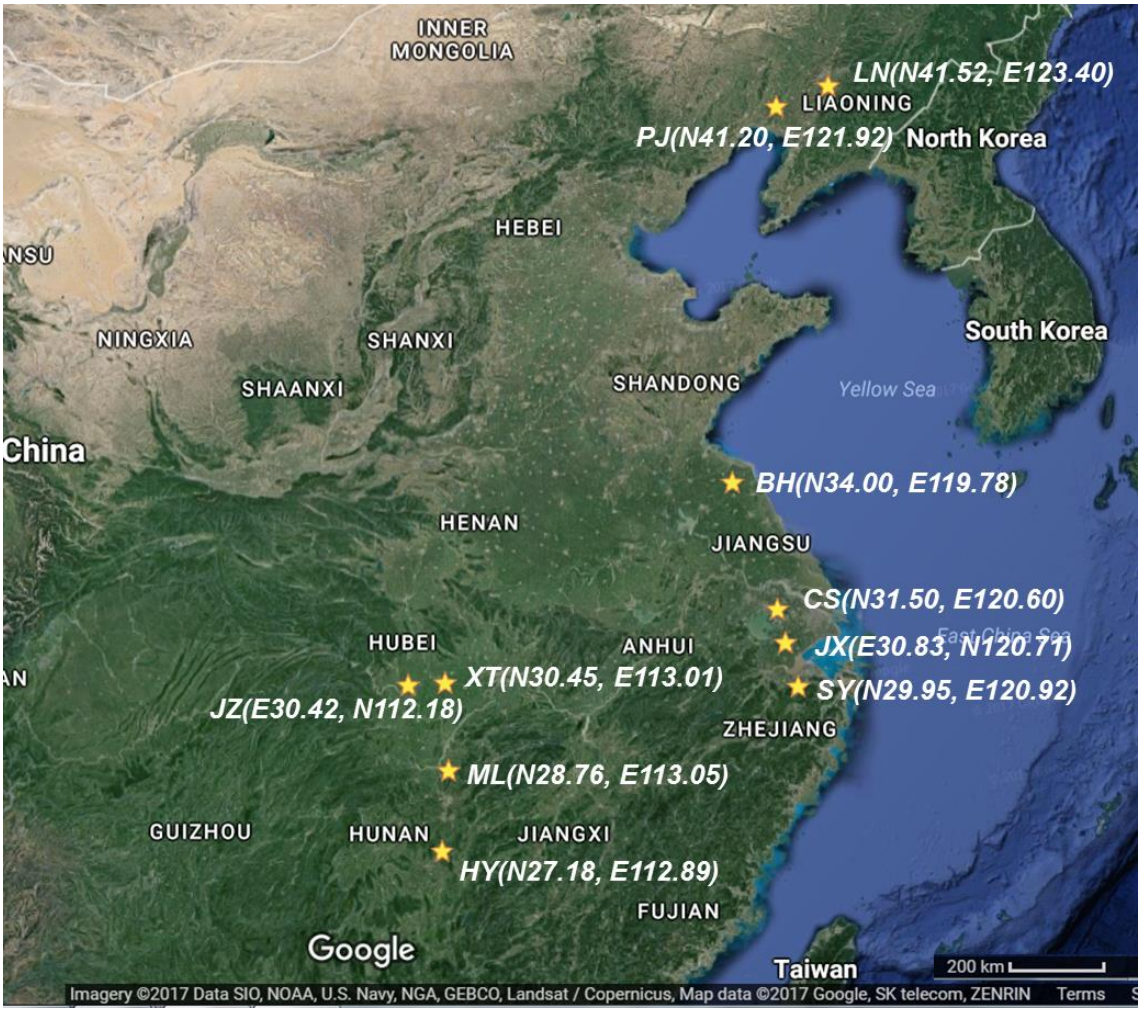

Fig. 1 Sampling sites of Chinese paddy soils indicated by stars in the map with labels showing site name and coordinates. BH, Binhai, Jiangsu Province; CS, Changshu, Jiangsu Province; HY, Hengyang, Hunan Province; JX, Jiaxing, Zhejiang Province; JZ, Jingzhou, Hubei Province; LN, Shenyang, Liaoning Province; ML, Miluo, Hunan Province; PJ, Panjin, Liaoning Province; SY, Shangyu, Zhejiang Province; XT, Xiantao, Hubei Province

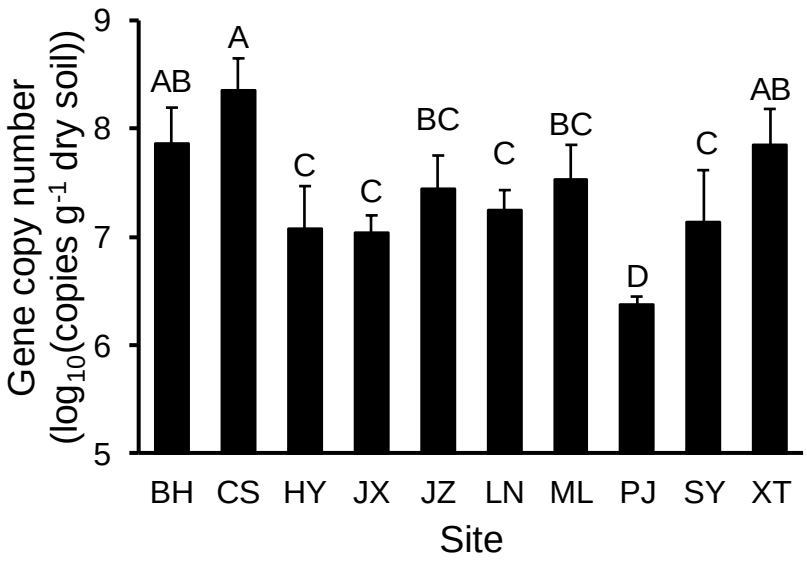

Fig. 2 Copy numbers of the fungal 18S rRNA gene in Chinese paddy soils. The error bars represent standard deviations of triplicates. Values that do not share the same letters are significantly different at $P < 0.05$. BH, Binhai, Jiangsu Province; CS, Changshu, Jiangsu Province; HY, Hengyang, Hunan Province; JX, Jiaxing, Zhejiang Province; JZ, Jingzhou, Hubei Province; LN, Shenyang, Liaoning Province; ML, Miluo, Hunan Province; PJ, Panjin, Liaoning Province; SY, Shangyu, Zhejiang Province; XT, Xiantao, Hubei Province

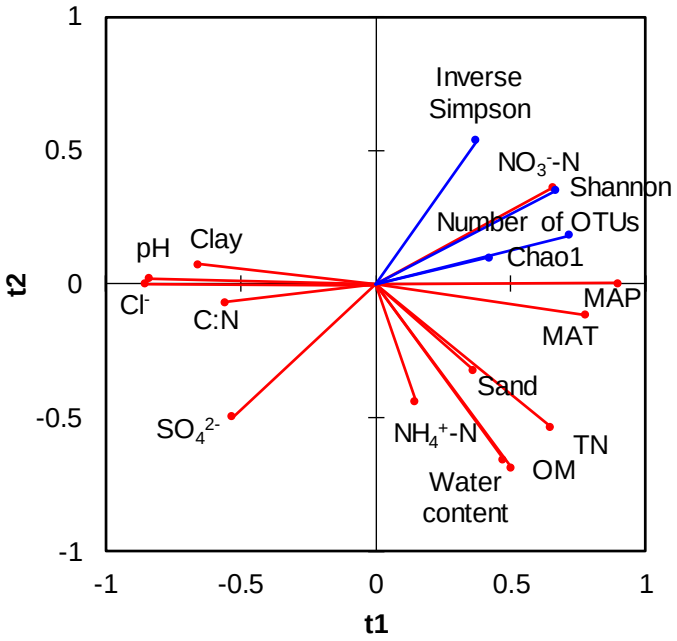

Fig. 3 PLS regression for the relationships between fungal α-diversity (blue lines) and environmental and climatic factors (red lines) (n = 27). MAT and MAP are mean annual temperature and precipitation; OM, organic matter; TN, total nitrogen. Variables pointing in opposite directions are negatively related while in the same directions are positively related, but if they point perpendicularly, their correlations are weak. Two factors were extracted in the model. The first factor explained 41.2 % of the variance in the predictors (environmental factors) and 32.1 % of the variance in the dependent variables (α-diversity). The second factor explained 14.5 % of the variance in the predictors and 11.4 % of the variance in the dependent variables

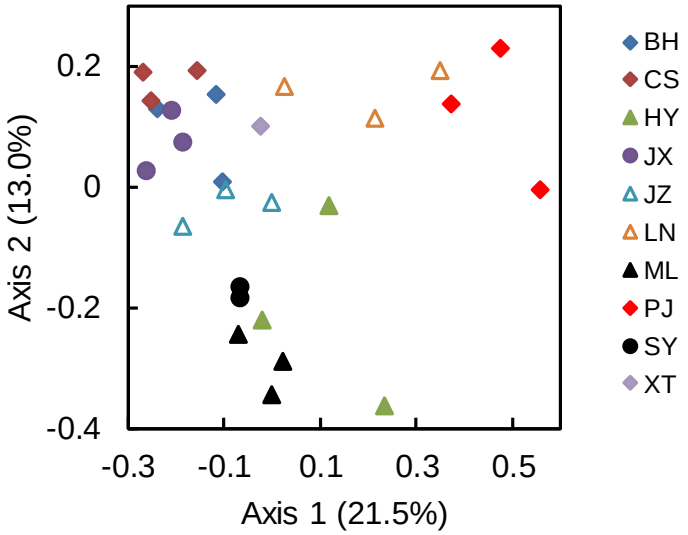

Fig. 4 Ordination by principal coordinates analysis (PCoA) for the 27 fungal communities based on the Bray-Curtis dissimilarity matrix. BH, Binhai, Jiangsu Province; CS, Changshu, Jiangsu Province; HY, Hengyang, Hunan Province; JX, Jiaxing, Zhejiang Province; JZ, Jingzhou, Hubei Province; LN, Shenyang, Liaoning Province; ML, Miluo, Hunan Province; PJ, Panjin, Liaoning Province; SY, Shangyu, Zhejiang Province; XT, Xiantao, Hubei Province

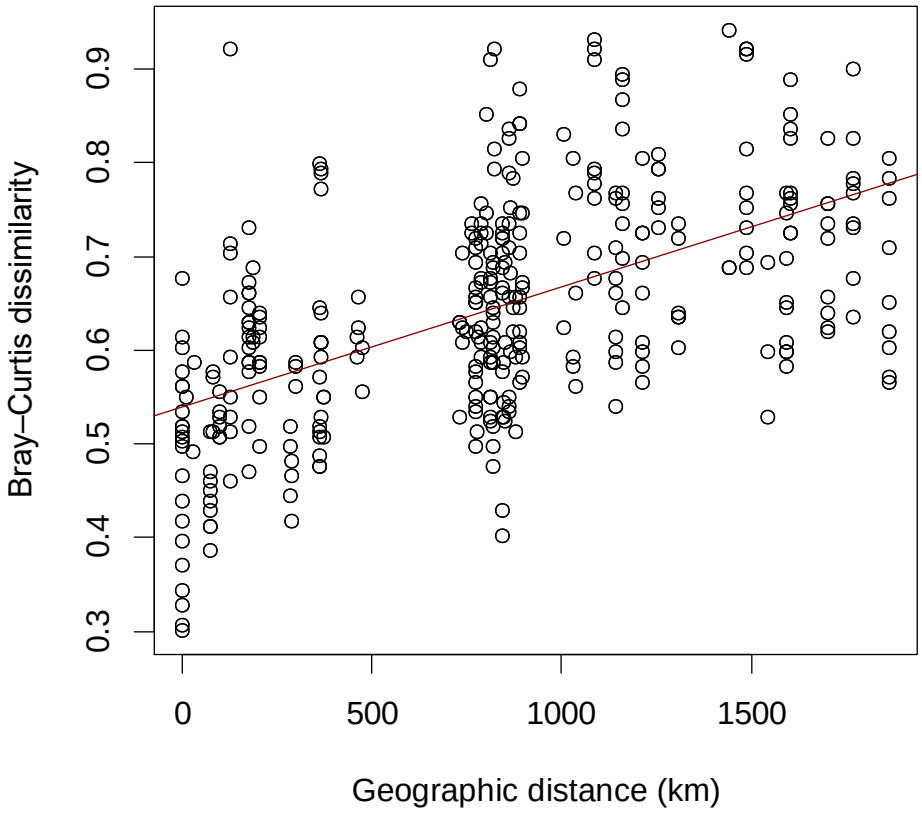

Fig. 5 Linear relationship between geographic distance and the Bray–Curtis dissimilarity of fungal communities (slope = 1.275×10^{-4}, $R^2 = 0.3114$, $P < 0.001$)

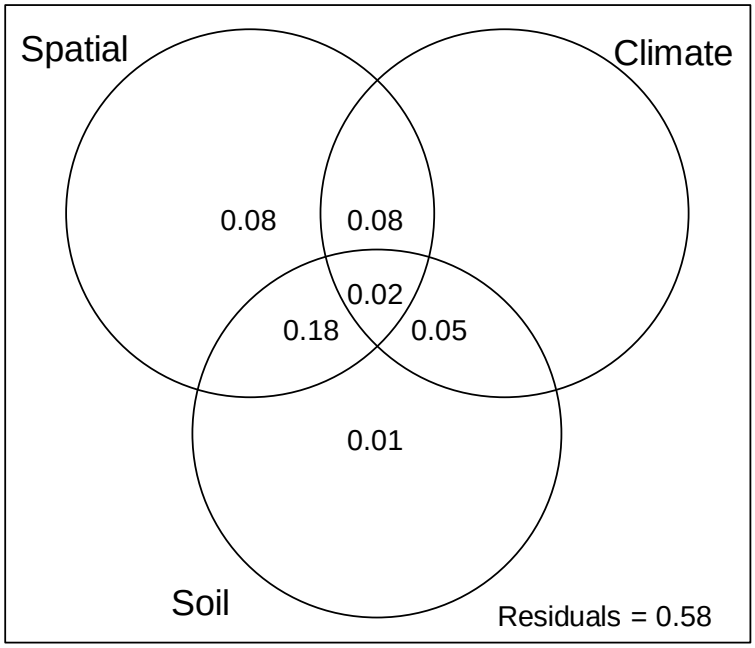

Fig. 6 Partition of variation of fungal communities into components accounted for by spatial, climatic, and soil factors. Spatial factors are PCNM eigenvectors derived from geographic distances between sampling sites. Climate factors include mean annual temperature and precipitation. Soil factors are soil pH, water content, and concentrations of total nitrogen and chloride. Figures represent variation explained by the factors (values < 0.001 are not shown; total variation is 1)

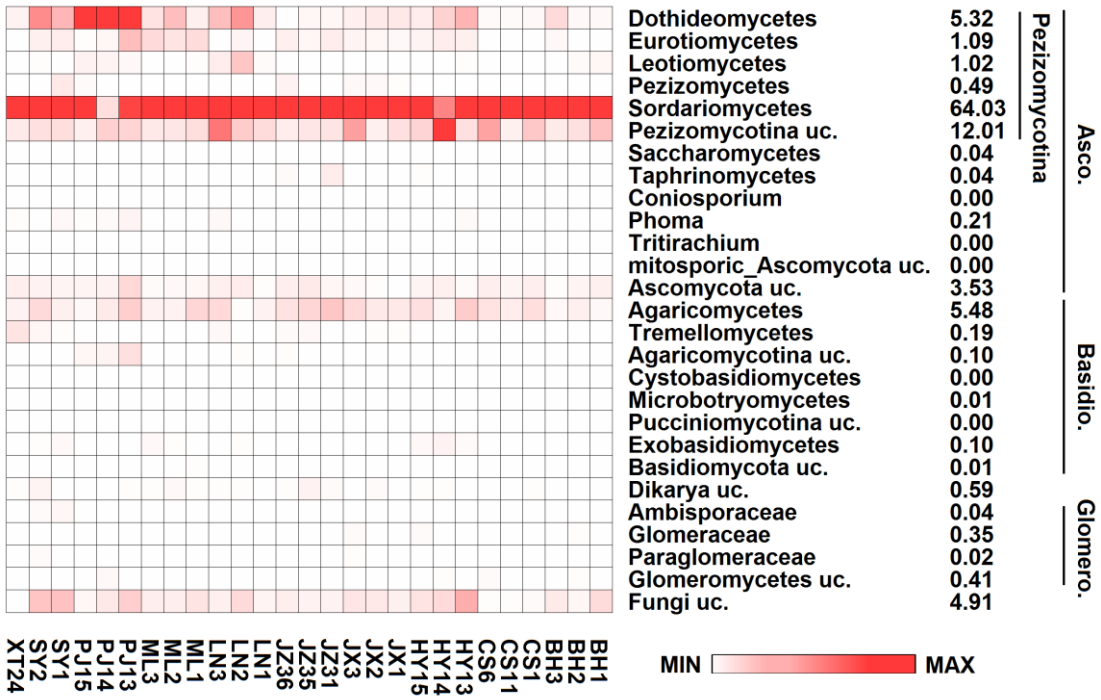

Fig. 7 Distribution of fungal phylotypes in 27 paddy soils. In each soil, red and white were given to phylotypes with the highest and lowest relative abundance, respectively. Asco., Basidio., and Glomero. indicate Ascomycota, Basidiomycota and Glomeromycota, respectively. uc. stands for unclassified. Ascomycota and Basidiomycota were assigned to the class level and Glomeromycota were assigned to the family level. Figures following taxa are their relative abundances (%). BH, Binhai, Jiangsu Province; CS, Changshu, Jiangsu Province; HY, Hengyang, Hunan Province; JX, Jiaxing, Zhejiang Province; JZ, Jingzhou, Hubei Province; LN, Shenyang, Liaoning Province; ML, Miluo, Hunan Province; PJ, Panjin, Liaoning Province; SY, Shangyu, Zhejiang Province; XT, Xiantao, Hubei Province

Table 1 Information of sampling sites and selected properties of the 30 paddy soil samples[a]

Site	Location	MAT (°C)	MAP (mm)	Soil pH	Water content (g g^{-1})	OM (g kg^{-1})	TN (g kg^{-1})	C:N	Sand (g g^{-1})	Clay (g g^{-1})	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	SO_4^{2-} (mg kg^{-1})	Cl^- (mg kg^{-1})
B	Binha	14.0	10	8.24±	0.43±0.	21.1	1.35±	9±	0.13±	0.29±	1.86±	23.1±	263.0	81.6±
C	Chan	15.4	10	6.98±	0.60±0.	41.1	2.02±	12	0.47±	0.10±	1.04±	16.1±	223.8	31.3±
H	Heng	18.0	15	5.45±	0.70±0.	25.6	1.31±	11	0.28±	0.15±	1.94±	21.8±	76.1±	7.4±2.
J	Jiaxin	15.5	11	6.51±	0.75±0.	25.5	1.51±	10	0.17±	0.20±	1.37±	53.9±	190.0	43.9±
J	Jingz	16.3	12	6.69±	0.50±0.	23.3	1.29±	11	0.17±	0.14±	3.31±	14.2±	146.5	37.8±
L	Sheny	8.1	71	6.73±	0.39±0.	15.2	0.62±	14	0.37±	0.07±	2.13±	29.6±	78.8±	21.5±
M	Miluo	17.0	13	5.06±	0.41±0.	23.8	1.50±	9±	0.34±	0.12±	2.59±	24.2±	72.0±	13.4±
P	Panjin	10.5	65	8.66±	0.35±0.	15.5	0.60±	15	0.07±	0.39±	0.23±	11.5±	208.3	156.6
S	Shang	16.4	14	5.39±	0.71±0.	33.6	2.16±	9±	0.21±	0.11±	4.47±	44.5±	74.6±	14.1±
X	Xiant	16.6	12	8.19±	0.43±0.	18.8	1.16±	9±	0.28±	0.16±	3.08±	11.2±	53.0±	15.6±

[a] Mean ± standard error for soil properties; MAT and MAP are mean annual temperature and precipitation; OM, organic matter; TN, total nitrogen

Table 2 Pearson's correlations between environmental factors and copy number of fungal 18S rRNA gene[a]

Variable	*r*
MAT	0.298
MAP	0.203
pH	0.023
Water content	0.064
OM	**0.467**
TN	**0.424**
C:N	-0.287
Sand	**0.477**
Clay	-0.357
Nitrate-N	0.242
Ammonium-N	-0.141
Sulfate	0.053
Chloride	**-0.381**

[a] $n = 30$. *r* values in bold are with $P < 0.05$. MAT and MAP are mean annual temperature and precipitation, respectively; OM, organic matter; TN, total nitrogen

Table 3 α-diversity of fungal communities in surface paddy soils[a]

Site	Number of OTUs	Chao1	Inverse Simpson	Shannon
BH	56±7ab	176±138	8.13±2.08b	3.36±0.06abc
CS	48±9b	180±93	4.62±2.58b	2.83±0.46de
HY	55±8ab	142±72	10.79±3.44ab	3.43±0.25abc
JX	45±4b	101±12	5.41±0.73b	2.94±0.04cd
JZ	59±17ab	241±113	11.74±1.21ab	3.54±0.40ab
LN	46±1b	149±22	7.98±3.21b	3.03±0.15bcd
ML	64±5a	203±27	14.44±4.77a	3.69±0.14a
PJ	23±5c	58±50	6.49±4.19b	2.32±0.46e
SY	50±16ab	129±95	7.98±0.44b	3.18±0.39abcd
XT	55ab	150	11.84ab	3.43abc

[a] Mean ± standard deviation, standard deviation was not available for XT because there was only one soil sample left after removing samples containing less than 189 sequences. Values that do not share the same letters are significantly different at $P < 0.05$ (a post hoc test was not conducted for Chao1 because ANOVA showed no significant difference between sites). Chao1, Chao1 richness estimate; Inverse Simpson, inverse of the Simpson diversity index; Shannon, non-parametric estimate of the Shannon diversity index