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ORIGINAL ARTICLE

Soil fungal community variation by large-scale reclamation in Sanjiang plain, China

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Abstract Large-scale marshland reclamation can cause substantial changes to the soil fungal community by disturbances associated with the growth of crop plants and by the addition of fertilizers and pesticides. In this study, high-throughput sequencing of the fungal-specific internal transcribed spacer (ITS) gene region was used to identify fungal taxa. We analyzed the variation in soil fungi diversity and community composition in marshland, paddy, and farmland corn soils, and investigated the relationship between soil fungal community composition and soil physicochemical characteristics to quantify the effect of large-scale reclamation on marshland soil environment in the Sanjiang Plain, northeast China. Marshland soil contained most of the 1997 operational taxonomic units (OTUs) found across all sites (1241), while paddy soil had only 614 OTUs and farmland corn soil 817 OTUs. All reclaimed lands presented a decline in richness and diversity of soil fungi at the OTU level, and soil fungal richness was significantly different between marshland and reclaimed sites ($P < 0.05$), although it did not differ significantly between marshland and farmland corn sites. Additionally, soil fungal community composition showed different trends and structure after the reclamation. One-way analysis of variance showed Basidiomycota, Zygomycota, Glomeromycota, and Chytridiomycota composition differed significantly between marshland and reclaimed sites ($P < 0.05$). Nine dominant genera (relative abundance >1.5% in at least one site) and many unclassified genera showed significant variation between marshland and reclaimed sites, including *Blumeria*, *Tomentella*, *Peziza*, *Hypholoma*, *Zopfiella*, *Mrakia*, and *Fusarium*. Soil fungal community composition and diversity were affected by soil moisture, pH, total carbon (C), available nitrogen (N), soil organic carbon, soil dissolved organic carbon, and C/N (the ratio of total carbon to total nitrogen). The present results contribute to understanding the fungal community in marshland ecosystems, and the role of environmental variability as a predictor of fungal community composition.

Keywords Marshland · Reclamation · Fungi · Diversity index · Community composition · High-throughput sequencing

Introduction

In the 1960s, Sanjiang Plain was the largest wetland in China. Because of its unique climate and geographical position, Sanjiang Plain's soil has high soil humus content and low diversity of soil microorganisms (Tong et al. 2005; Gong et al. 2015). A soil with such features is highly desirable for agricultural development, but also ecologically fragile and vulnerable to destruction. Over the last 60 years, Sanjiang Plain was reclaimed four times and most of its wetland areas were turned into cultivated land. Wetland and cultivated land areas were 352.59×10^4 and 171.34×10^4 ha in 1954 but became 95.87×10^4 and 556.88×10^4 ha in 2005, respectively (Song et al. 2005; Z.M. Wang et al. 2009), indicating that Sanjiang Plain wetland was reclaimed as farmland over those 50 years. Large farms expanded across the plain as wetland was lost, and, indeed, much marshland in northeast China is environmentally degraded due to global warming and substantial land reclamation for agriculture (Zhang et al. 2007). This degradation has severely affected normal cycling processes of matter and

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energy in the marsh ecosystem (Huo et al. 2013). The thickness of the organic-rich black soil has gradually diminished during the prolonged period of agricultural reclamation and water tables are now lowering (L.L. Wang et al. 2009).

The composition and diversity of soil microbial communities have also changed in association with soil modifications (Yu et al. 2011). Soil microorganisms are increasingly recognized as the most sensitive bioindicators in the soil ecosystem, and therefore there is growing interest in managing soil microorganisms to improve the soil environment (Zhao et al. 2011; Ludwig-Müller 2015). Thus, in recent years, changes in the communities of soil microorganisms in marshlands have received considerable attention from researchers and from the Chinese government. In particular, the effects of agricultural reclamation (Y. Wang et al. 2014) and pollution treatments (Cortés-Lorenzo et al. 2014), and the role of microorganisms in carbon (C) and nitrogen (N) cycles (Huang et al. 2008) have been the focus of several studies.

Soil fungi are important in the decomposition and mineralization of soil organic matter and are distributed widely in the soil environment. Fungi play an important role in matter cycling, pollutant degradation, and ecological restoration (Liu et al. 2015). Species within the soil fungal community not only reflect the impact of environmental changes but also directly affect community functions (Zhang et al. 2015a). Therefore, previous ecological research focused on identifying the species present in soil fungal communities and determining their functions, such as plant growth and nutrient cycling regulation, organic matter decomposition, and pesticide degradation (Cookson et al. 2008; Huang and Long 2014). However, the slow development of sufficiently sensitive assays and limitations in the resolution of the available methods has prevented the recognition and study of most fungal species and the isolation of pure cultures. These constraints make it difficult to obtain an accurate picture of soil fungal communities (Wang and Bau 2014). In recent years, however, progress in molecular biological techniques has allowed uncultured fungi to be identified and fungal diversity to be assessed using genomic information (Franzosa et al. 2015). These tools have advanced our ability to explore the relationship between the environment and the diversity of the fungal community, contributing to both a comprehensive taxonomy and our functional understanding (Zhang et al. 2015b).

In the present study, we obtained DNA sequences from soil samples from one uncontaminated marsh site and two distinct reclaimed sites and compared their fungal communities in terms of species richness, diversity, and composition, aiming to determine the extent to which fungal communities are affected by disturbances associated with marshland reclamation. These comparisons allow further understanding of the changes in the fungal community due to environmental factors, and the results obtained here can help expand our knowledge of soil ecological systems by examining the influence of reclamation and other external disturbance factors on soil biology and ecology. We identified important practical consequences of reclamation that will be valuable for the management and utilization of microbial resources in marshland, and that provide a sound scientific basis for the protection and use of wetlands in northeast China.

Materials and methods

Study sites and soil sampling

The study sites were located in the Sanjiang Plain, in the hinterland of Heilongjiang Honghe Nature Reserve, northeast China (47°42′38″–47°52′00″ N, 133°34′38″–133°46′29″ E). This nature reserve is a wetland ecosystem that was identified as internationally important in 2002. The elevation of Honghe Nature Reserve ranges mostly between 58 m and 61 m, and it has a temperate humid climate, with average monthly temperature from −23.4 °C in January to 22.4 °C in July. The average annual precipitation is 585 mm, and the average annual evaporation is 1166 mm. The marsh freezes to 1.6–1.8 m deep for about 7 months every year. The vegetation of Honghe Nature Reserve is still in the original state, consisting mainly of marsh and aquatic species, dominated by perennials such as *Carex lasiocarpa*, *C. pseudocuraica*, *C. meyeriana*, *Glyceria spiculosa*, and *Calamagrostis angustifolia* (Hao et al. 2003).

In September 2014, three study sites within Honghe Nature Reserve were selected to represent different habitats across the reserve (Table 1). Site 1 (S1), which is not affected by land reclamation, is located in pristine marshes inside the core zones of the nature reserve. These core zones are protected by law, and both development projects and marshland destruction are prohibited in these areas. Site 2 (S2) and Site 3 (S3) belong to land reclaimed for agriculture and are located on experimental fields inside the reserve's test zones (Song et al. 2005). Chemical fertilizers are applied to both sites: 205 kg ha^{-1} $year^{-1}$ in S2 [CON_2H_4: 100 kg ha^{-1}, $(NH_4)_2HPO_4$: 100 kg ha^{-1}, KH_2PO_4: 5 kg ha^{-1}] and 235 kg ha^{-1} $year^{-1}$ in S3 [CON_2H_4: 130 kg ha^{-1}, $(NH_4)_2HPO_4$: 75 kg ha^{-1}, K_2SO_4: 30 kg ha^{-1}]. Test zones were established by law to allow scientific experiments related to ecological studies. The linear distance between sampling sites was less than 800 m.

Soil samples were collected at 5–15 cm depth in June 2015, i.e., during the crop-growing season. Three standard quadrats (10 × 10 m each) were set up in each study site. We randomly deployed three smaller quadrats (1 × 1 m each) within each standard quadrat. Approximately 1 kg soil was collected in each of the smaller quadrats using a sterile soil drill. The soil drill was washed with purified water between sampling procedures at each site. Each soil sample was divided into 4:1 parts. The heavier part of each

Table 1 Details of the sampling sites

Site	Coordinates	Altitude (m)	Main vegetation species	Habitat types
S1	133°40′28″E, 47°45′46″N	58.5	*Calamagrostis angustifolia*, *Carex meyeriana*	Pristine marshland (non-disturbance)
S2	133°34′38″E, 47°42′39″N	59.0	Paddy	Paddy field (large-scale reclamation)
S3	133°35′24″E, 47°44′12″N	60.0	Corn with Soybean	Corn farmland (large-scale reclamation)

sample was placed in a sterile valve bag and stored in an electronic refrigerator at 4 °C. Overall, 27 samples of about 0.8 kg each were collected from all sites for measuring soil physicochemical properties and microbial biomass. The lighter part of each sample was pooled with two samples from the same standard quadrat, placed in a sterile valve bag, and stored in a refrigerator with dry ice at −80 °C until DNA extraction. Nine samples were collected using this procedure for identifying soil fungi (Rousk et al. 2010; Zou et al. 2014).

Soil physicochemical properties and microbial biomass

Soil pH was determined with a pH meter (PHS-3C; Rex, Shanghai, China) using a soil-to-water ratio of 1:2.5. Soil moisture content (Mc) was measured gravimetrically. Soil total carbon (TC) and total nitrogen (TN) were determined using an elemental analyzer (VarioEL III; Elementar Analysensysteme, Hanau, Germany). Fresh soil samples were oven-dried at 105 °C for 12 h and then ground and sieved through a 150 μm mesh. Each soil sample (25 mg) was placed in the combustion furnace of the elemental analyzer, and the manufacturer's instructions were followed to determine sample elements and their ratios (Wang et al. 2013). Soil organic carbon (SOC) was measured using a non-dispersive infrared method in an automated total organic carbon (TOC) analyzer (TOC-VCPH; Shimadzu, Shimane-ken, Japan). Soil samples were treated with HCl solution to remove inorganic carbon and then placed with the platinum salt catalyst in the combustion furnace of the automated TOC analyzer following the manufacturer's instructions (Kong et al. 2013). Soil dissolved organic carbon (DOC) was extracted using high purity water, and also measured using the automated TOC-VCPH analyzer (Shimadzu) after filtrating the solution through a 0.45 μm membrane (Kong et al. 2013). Alkali nitrogen (AN) was determined using the alkali N-proliferation method (Sun et al. 2007). Soil microbial biomass carbon (MBC) and nitrogen (MBN) were estimated using the chloroform-fumigation-extraction method and calculated as MBC = E_C/0.45 and MBN = E_N/0.54, where E_C and E_N are the differences in organic carbon and nitrogen between chloroform-fumigated and unfumigated soil samples, respectively, extracted with 0.5 M K_2SO_4 (Li et al. 2015). Each soil sample was analyzed three times in all procedures.

Soil DNA extraction and PCR amplification

Total DNA was extracted from triplicated soil samples (each 0.5 g dry weight) using the E.Z.N.A.® Soil Fungi DNA Kit (Omega Bio-tek, Norcross, GA), following the manufacturer's instructions. The fungal *ITS* gene was amplified by PCR using the primers ITS1F (5′-barcode-CTTGGTCATTTAGAGGAAGTAA-3′) and 2043R (5′-GCTGCGTTCTTCATCGATGC-3′), where the barcode is an eight-base sequence unique to each sample (M. Wang et al. 2014; Zhang et al. 2016). The PCR reaction system contained 4 μL 5 × FastPfu Buffer, 2 μL 2.5 mM dNTPs, 0.8 μL each primer (5 μM), 0.4 μL FastPfu Polymerase, 0.2 μL BSA, and 10 ng template DNA (Sheik et al. 2012), and a PCR reaction system (TransGen AP221–02: TransStart FastPfu DNA Polymerase, 20 μL). The amplification was performed in an ABI GeneAmp 9700 thermocycler (ABI, Carlsbad, CA), under the following profile: 95 °C for 3 min; 33 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s; and a final extension at 72 °C for 10 min.

Illumina MiSeq sequencing

Soil fungal taxa were detected using the high-throughput sequencing platform MiSeq PE250 (Illumina, San Diego, CA) following the manufacturer's instructions. Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA), according to the manufacturer's instructions, and then quantified using QuantiFluor-ST (Promega, Madison, WI). Purified amplicons were pooled at equimolar concentrations and paired-end sequenced (2 × 300) on the MiSeq platform according to standard protocols. Each replicate was analyzed three times by high-throughput sequencing, and the median value of the three measurements was selected to represent the value obtained for each replicate.

Data analysis

Raw sequence data were optimized before analysis. Raw fastq files were demultiplexed and quality-filtered using QIIME (version 1.17). Reads 200 bp in length were truncated at any site with an average quality score < 20 over a 50 bp sliding window, discarding truncated reads shorter than 50 bp. Exact barcode matching was used, allowing a two-nucleotide

mismatch in primer matching. Reads containing ambiguous characters were removed. Only sequences that overlapped by more than 10 bp were assembled according to their overlap sequence. Reads that could not be assembled were discarded.

To identify soil fungi and quantify community diversity, the operational taxonomic units (OTUs) obtained were compared to the UNITE database (Release 7.1, last updated: 2016–08-04; http://unite.ut.ee/index.php) and defined by clustering using USEARCH (version 7.1) with a 97% similarity cutoff (Kõljalg et al. 2013). Chimeric sequences were identified and removed using UCHIME. A Bayesian algorithm was used to analyze OTU sequences with a confidence threshold of 0.7 in comparisons to the Ribosomal Database Project (RDP) (Release 11.1). We used Mothur (version v.1.30.1) to perform rarefaction analysis and rarefaction curves were produced in R (Sheik et al. 2012). Several α-diversity indexes were calculated (Ace: http://www.mothur.org/ wiki/Ace, Chao: http://www.mothur.org/wiki/Chao, Shannon: http://www.mothur.org/wiki/Shannon, and Simpson: http://www.mothur.org/wiki/Simpson) to describe the richness and diversity of OTUs at the different sampling sites using Mothur (Schloss et al. 2011). This also allowed comparing the composition of fungal communities among sites, hence quantifying the effect of reclamation on community composition. Based on a diversity distance matrix, a dendrogram was constructed using cluster analysis and the unweighted pair-group method with arithmetic means (UPGMA). This dendrogram indicated the similarity of the fungal community composition between different sites at the OTU level. The relative abundance of ITS gene sequences for dominant genera (relative abundance >1% in at least one site) was quantified to illustrate differences in fungal communities across the three sites (Sheik et al. 2012). This method provides a qualitative comparison of OTU richness between different samples, although low sequence diversity can prevent the accurate estimation of OTU richness and its quantification. To analyze the key environmental factors that influence soil fungal community composition, a redundancy analysis (RDA) was performed in R using the Vegan package, considering normalized OTU abundance and environmental chemical data (Sheik et al. 2012; Zhang et al. 2016). Soil physicochemical properties, microbial biomass, and α-diversity indexes were analyzed using one-way analysis of variance (ANOVA) setting the significance limit to $P < 0.05$. Least significant difference or Tamhane's T2 procedures were used to determine significant differences between each group of dew intensity. Standard deviation was calculated as the square root of variance as it is a measure of the dispersion of a set of data from its mean. The association between soil physicochemical properties and fungal community diversity was analyzed using Pearson correlations or the BIOENV analysis within the Vegan package of R (Rousk et al. 2010). The SPSS software (SPSS Statistics 22 for WINDOWS; IBM, Armonk, NY) was used to calculate Pearson correlation coefficients, independent *t*-tests and to perform one-way ANOVA.

Results

Soil physicochemical properties and microbial biomass

Soil physicochemical properties and soil microbial biomass determine soil fertility, which can be used to characterize soil productivity and ecosystem stability. Reclamation had a significant impact on marsh soil physicochemical properties and microbial biomass ($P < 0.05$) (Table 2). Soil samples from the marsh site (S1) had relatively high mean moisture content, which was 44.44% higher than that of the paddy site (S2) and 108.17% higher than that of the farmland site (S3). The mean pH value in the marsh site was lower than in the reclaimed sites (Table 2). Soil TC, TN, DOC, AN, and SOC in the marsh site were 2.47, 1.35, 4.92, 1.73, and 2.50 fold higher than in the paddy site, and 2.26, 1.06, 2.41, 1.21, and 2.28 fold higher than in the corn site, respectively. The C/N ratio—a key factor influencing soil fungal communities—was 83.16% higher in the marsh site than in the paddy site, and 114.34% higher in marsh site than in the corn site, on average (Table 2). Soil BMC and BMN were 5.65-fold and 3.63-fold higher in the marsh than in the paddy site, and 3.96-fold and 2.66-fold higher in the marsh than in the corn site, respectively.

Sequencing results and OTU analysis

The MiSeq high-throughput sequencing followed by optimization yielded 75,881,872 ITS reads, organized into 287,116 sequences with an average length of 264.5 bp. Sequences between 200 bp and 356 bp accounted for 99.98% of the total number of sequences.

Rarefaction curves were constructed by plotting the number of OTUs against the number of clones (reads) (Fig. 1). All curves showed a decreasing rate of OTU detection, indicating that clone analysis evaluated almost the full extent of taxonomic diversity at the species level. The average coverage of the clone library was 99.85%.

The number of fungal OTUs can be visualized in Fig. 2. Among the 1997 fungal OTUs found across the three sites, 1241 OTUs were found in S1, 614 OTUs in S2, and 817 OTUs in S3 (Fig. 2a). The number of OTUs shared among all sites (153), between each pair of sites (132 shared by S1 and S2, 155 shared by S1 and S3, and 82 shared by S2 and S3), and unique to each site (851 to S1, 247 to S2, and 377 to S3) are evidenced in the Venn diagram (Fig. 2b). Most OTUs, i.e., 1475, were exclusive to one site, while 369 OTUs were shared by two sites, and 153 were shared among all sites (Fig. 2c).

Table 2 Soil physicochemical properties and microbial biomass at the different sample sites (mean ± SE). Different lower case letters denote significant differences between habitats at $P < 0.05$. The maximum values in each column are denoted by "a"

Sites	Mc (%)	pH	TC (g /kg)	TN (g/kg)	DOC (mg/kg)	AN (mg/kg)	SOC (g/kg)	C/N	BMC (mg/kg)	BMN (mg/kg)
S1	56.55 ± 7.56 a	5.40 ± 0.04 c	52.52 ± 5.99 a	3.31 ± 0.34 a	344.63 ± 19.44 a	436.79 ± 23.32 a	51.37 ± 5.78 a	15.99 ± 2.19 a	1862.44 ± 93.73 a	157.26 ± 16.45 a
S2	39.15 ± 2.30 b	5.94 ± 0.06 a	21.29 ± 1.78 b	2.45 ± 0.32 b	70.08 ± 19.15 c	253.02 ± 12.91 c	20.57 ± 1.60 b	8.73 ± 0.92 b	329.51 ± 72.98 c	39.71 ± 1.89 c
S3	27.16 ± 7.78 c	5.77 ± 0.08 b	23.29 ± 1.55 b	3.13 ± 0.24 ab	143.01 ± 17.56 b	359.96 ± 32.86 b	22.57 ± 2.08 b	7.46 ± 0.65 c	513.35 ± 99.54 b	59.19 ± 10.73 b

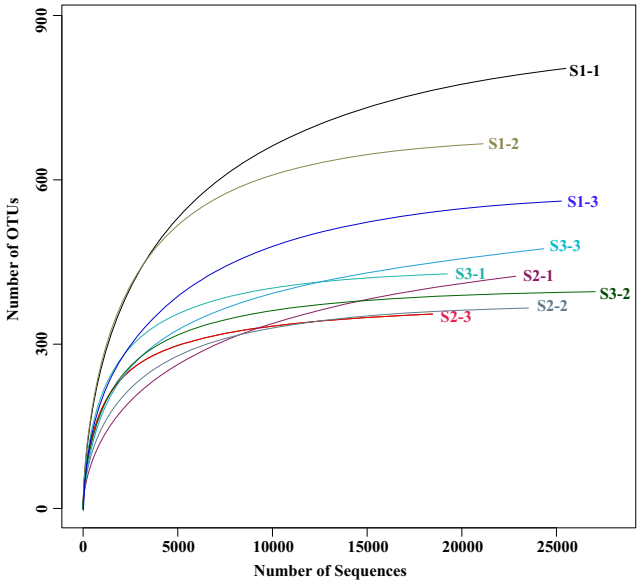

Fig. 1 Rarefaction curves showing the extent of operational taxonomic units (OTUs) (97% similarity level) detection at the different sites. Each curve represents one replicate and is shown in a different *color*. Samples codes comprise the letter "S" plus two numbers: the first indicates the sampling site (S1, S2, and S3) and the second number indicates the quadrat from which the soil sample was taken

Fungal α-diversity across sites

Several α-diversity indexes were calculated to describe the richness and diversity of soil fungi OTUs at the different sites (Table 3). The independent *t*-tests used to explore the differences between the three habitats indicated that the richness of the soil fungal community in S1 was significantly higher than that in S2 and S3 ($P < 0.05$) (Table 3). However, diversity was not significantly different between S1 and S3 and, although richness and diversity were higher in S3 than in S2, this difference was not significant. In general, fungal richness and diversity showed a decreased trend from S1 to S3 to S2.

Fungal community composition across sites

The sequences obtained were assigned to six identified phyla and to some unclassified phyla (Fig. 3). Sequence abundance at the phylum level showed that members of the Ascomycota (53.29%) were the most frequently identified in all soil samples. The next most frequent were Basidiomycota (17.79%); members of Zygomycota (4.49%), Glomeromycota (0.89%), Rozellomycota (0.54%), and Chytridiomycota (0.68%) represented relatively small proportions of the soil fungal communities. We also found that Basidiomycota were relatively less abundant in farmland than in marshland and paddy soils. Some fungal phyla showed variation in relative abundance at different habitats. For example, the relative abundance of Zygomycota, Glomeromycota, and Chytridiomycota was significantly higher in S3, indicating that soil fungal communities

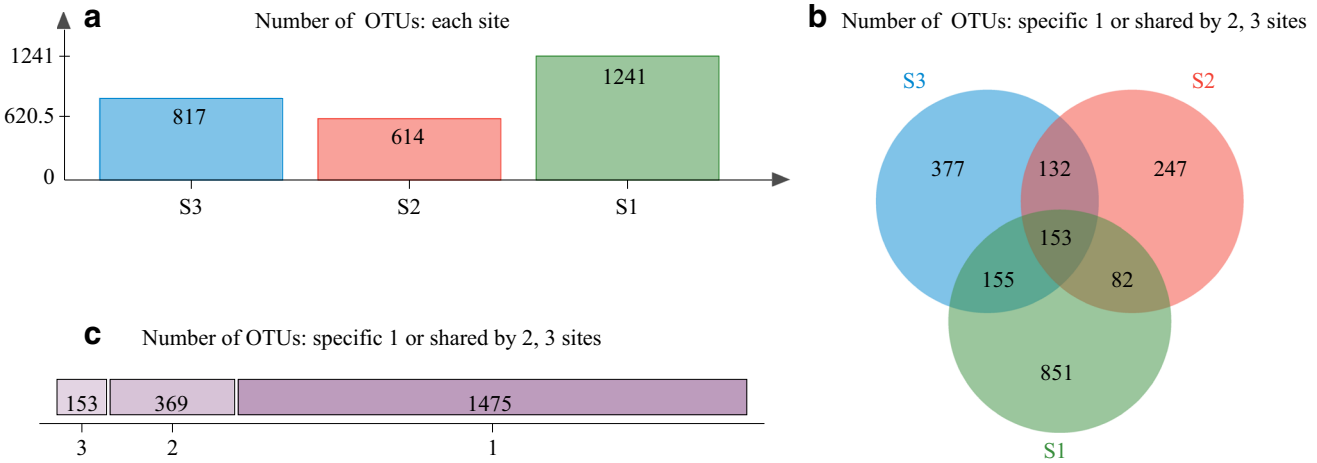

Fig. 2 Number of soil fungi OTUs in each site, specific to a site, or shared by two or three sites. The number of OTUs within each site was calculated after merging the sequences obtained from the three replicates from each site, which were analyzed in triplicate with high-throughput sequencing after PCR. The median value of the three values obtained in the triplicate analysis is that indicated as the value of the replicate. *S1* Pristine marshland, *S2* paddy field, *S3* corn and soybean farmland

showed different trends and structure after the reclamation. One-way ANOVA evidenced that Basidiomycota ($P < 0.05$), Zygomycota ($P < 0.05$), Glomeromycota ($P < 0.05$), and Chytridiomycota ($P < 0.05$) differed significantly between pristine marshland and farmland soils, while Ascomycota and Rozellomycota did not.

The UPGMA dendrogram based on the diversity distance matrix showed that the communities in the nine soil samples clustered roughly into three groups (Fig. 3), each comprising the three soil samples from each site. A clear separation between samples from the S1 site (uncontaminated) and S2 and S3 sites (reclaimed lands) is evidenced, as the later sites are clustered apart from S1 samples. This separation seems to indicate an association between the disturbance caused by large-scale reclamation and the similarity of fungal community composition.

We analyzed the main genera of the fungal community by cluster analysis (relative abundance of fungi above 1.5% in at least one site), and a heatmap was produced based on the clustering results (Fig. 4). Differences in fungal community structure among sites are evidenced in the four clusters obtained. Clusters 1 and 2 mainly included *Tetracladium*, *Hymenoscyphus*, *Lecanicillium*, *Cistella*, *Cladophialophora*, *Monographella*, *Talaromyces*, *Neobulgaria*, *Venturia*, *Sonoraphlyctis*, *Podospora*, *Leotiomycetes*, and *Mortierella* species. The relative abundance of these fungi was highest in S3 and relatively low in S1 and S2. Cluster 3 mainly included *Blumeria*, *Aspergillus*, *Tomentella*, *Peziza*, *Meliniomyces*, *Lachnum*, *Alternaria*, *Hypholoma*, and *Ceratobasidium* species. The relative abundance of these fungi was highest in S1, and relatively low in S2 and S3. Cluster 4 mainly included *Zopfiella*, *Psilocybe*, *Pyrenochaetopsis*, *Fusarium*, *Mrakia*, *Athelia*, *Mrakiella*, *Dimorphospora*, *Guehomyces*, and *Pseudeurotium* species. The relative abundance of most fungi was highest in S2, and only a few had highest relative abundance in S1 and S3. These results showed that variations in the relative abundance of the fungal community were complex.

As shown in Fig. 4, changes in fungal communities were also found at the genera level. Nine dominant genera (relative abundance above 1.5% in at least one site) and many unclassified genera showed significant variations between marsh and reclaimed sites. The relative abundance of *Blumeria*, *Tomentella*, *Peziza*, and *Hypholoma* decreased gradually from marsh to reclaimed sites. On the contrary, the relative abundance of *Zopfiella*, *Mrakia*, and *Fusarium* increased gradually from marsh to reclaimed sites.

Table 3 α-Diversity indexes for each sampling site. Different lower case letters denote significant differences between habitat types at $P < 0.05$. The maximum values in each column are denoted by "a". α-Diversity indexes were calculated at the OTU level. Different lower case letters denote significant differences between habitats at $P < 0.05$

Site	Ace	Chao	Shannon	Simpson
S1	692.67 ± 185.51 a	686.33 ± 181.53 a	4.48 ± 0.15 a	0.0323 ± 0.0031 b
S2	355.33 ± 20.50 b	338.33 ± 38.50 b	2.96 ± 0.53 b	0.1139 ± 0.0531 a
S3	400.67 ± 62.43 b	397.33 ± 63.12 b	3.72 ± 0.48 ab	0.0911 ± 0.0359 ab

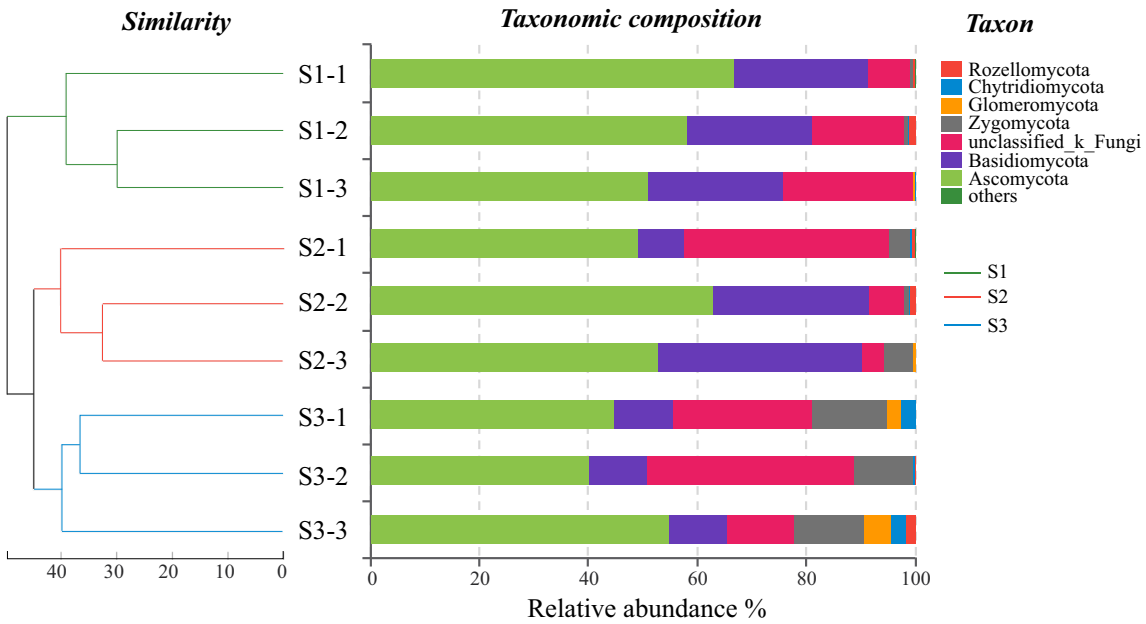

Fig. 3 Phyla identified in the soil fungal communities at pristine marshland (S1), paddy field (S2), and corn and soybean farmland (S3) sites

Environmental determinants of soil fungal community

Pearson's correlation coefficients (Table 4) showed that Mc, pH, TC, AN, SOC, and C/N were significantly correlated with the values obtained for ACE, Chao, Shannon, and Simpson indexes ($P < 0.05$, $N = 9$), whereas DOC was significantly correlated only with the values of ACE and Chao ($P < 0.01$, $N = 9$). These observations indicate that environmental features and fungal diversities differed between pristine marshland and cultivated land. Higher Mc, TC, AN, SOC, DOC, and C/N levels as well as higher soil fungal diversity and richness were observed in pristine marshland than in cultivated land.

The RDA evidenced a clear separation between S1 and S2–S3 (Fig. 5), corroborating the previous analyses. Much of the variance in soil fungal communities among the different sites was associated with habitat type and environmental variables. Axes 1 and 2 explained 93.92% of the variation in soil fungal community, indicating that soil fungal communities varied with differing pH, Mc, TC, TN, DOC, AN, and SOC levels. In particular, the community structure of Ascomycota, Basidiomycota, and Zygomycota was significantly affected by the soil physicochemical properties. Basidiomycota perform better in environments with higher water content and higher pH, while the opposite is better for Zygomycota; Ascomycota perform better in environments with high contents of soil nutrients. The Glomeromycota, Rozellomycota, and Chytridiomycota communities were not significantly affected by these environmental factors. The BIOENV analysis in R using the Vegan package showed that pH, Mc, DOC, and SOC had a significant influence on soil fungal community composition, in the following order: pH > Mc > SOC > TC > AN.

Discussion

Soil fungi are the main group of soil microorganisms, but, in comparison to other organisms, the study of soil fungal communities lags behind. Thus, soil fungi have been compared to "Earth's dark matter" (Jansson and Prosser 2013) as more needs to be known about fungal communities. In the present study, high-throughput sequencing data provided considerable insight into fungal communities in marsh, paddy, and farmland ecosystem. Thus, using this methodology to identify fungal taxa can elucidate on the status and function of soil fungal communities in marsh soils, and quantify the effects of environmental changes on soil fungal communities.

It is known that changes in soil fungal community structure across different habitats are indicative of environmental changes that affect survival conditions for fungi species and imposed selection pressure on the fungal community (Li et al. 2015). In the present study, a clear separation between the fungal community from the marsh site and those developed in reclaimed lands (paddy and farmland) was evidenced in terms of taxa composition and relative abundance. Soil fungal communities within the three habitats were assigned to six phyla. Basidiomycota was the most frequently identified in marsh and paddy soils, while Glomeromycota, Rozellomycota, Zygomycota, and Chytridiomycota were more frequent in farmland soil. Therefore, by identifying the fungal community grown in a soil sample we can determine from which habitat it came from. For example, we can deduce that a soil sample did not come from marshland when the relative abundances of Glomeromycota, Zygomycota, and Chytridiomycota are relatively high. Differences in soil fungal communities between sites were even more obvious at the genus level. *Blumeria*, *Tomentella*, *Peziza*,

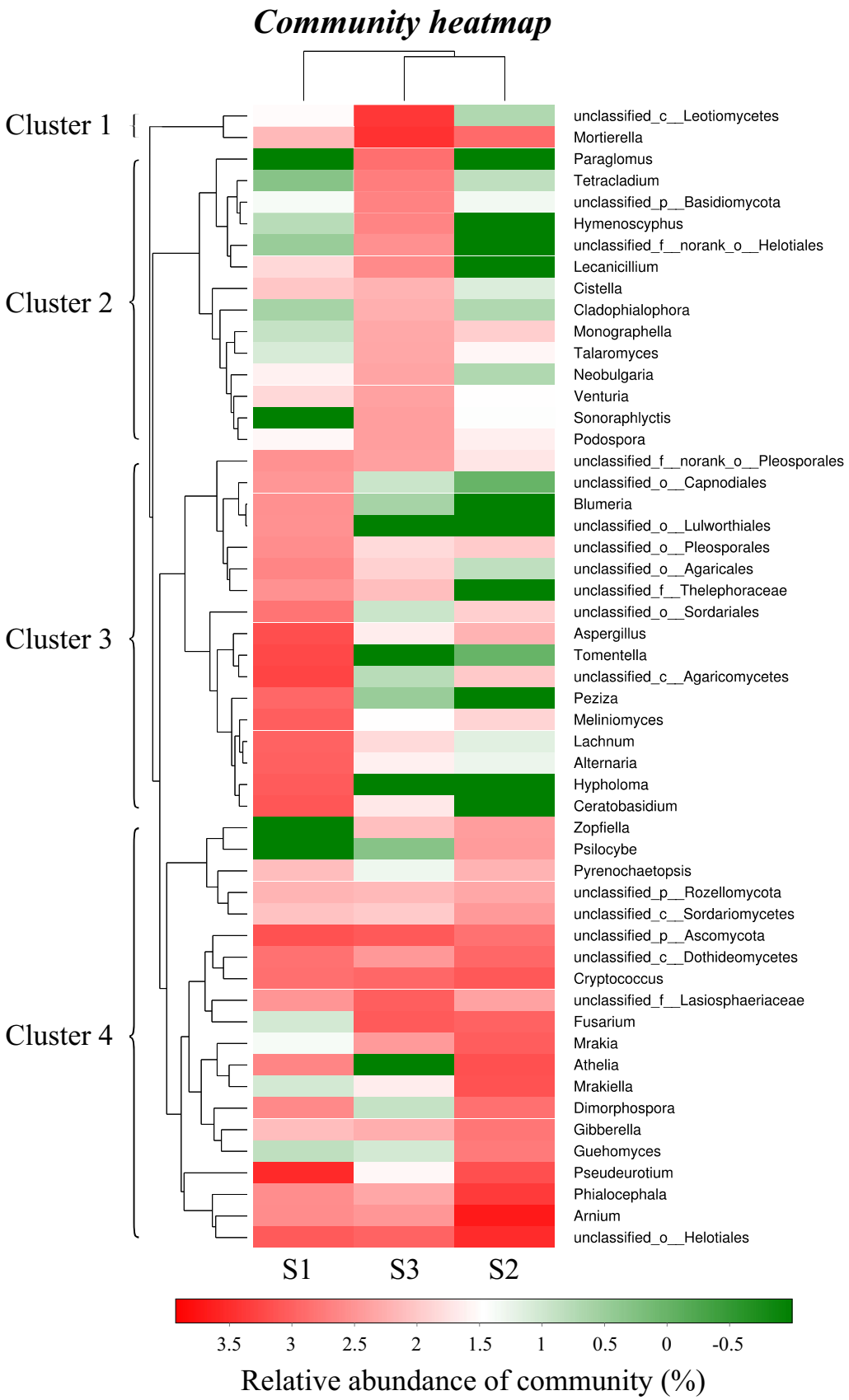

Fig. 4 Heatmap generated from the relative abundance of ITS sequences from soil fungi

Table 4 Pearson's correlation coefficients between soil properties and fungal diversity indices

Diversity index	Mc	pH	TC	TN	DOC	AN	SOC	C/N
ACE	0.641**	−0.721**	0.732**	0.351	0.541*	0.585*	0.733**	0.791**
Chao	0.638**	−0.734**	0.744**	0.389	0.566*	0.603**	0.742**	0.745**
Shannon	0.457*	−0.598*	0.561*	0.296	0.327	0.652**	0.513*	0.596*
Simpson	−0.466*	0.577*	−0.593*	−0.357	−0.331	−0.648**	−0.567*	−0.601*

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

Meliniomyces, *Lachnum*, *Alternaria*, *Hypholoma*, and *Ceratobasidium* were more frequent in marsh soil and reduced (or even absent) in farmland soil. *Hymenoscyphus*, *Lecanicillium*, *Fusarium*, *Mrakia*, *Athelia*, *Mrakiella*, *Dimorphospora*, *Guehomyces*, *Pseudeurotium*, and other fungal genera were present in marsh soil but showed a relative increase in farmland soil. These comparisons showed that the characteristics of the different habitats have an important influence on the structure of soil fungal communities. The reclamation process changes the original habitat of the soil fungal community and destroys its ecological balance. These changes lead to an increase or decrease in the populations of particular soil fungi (Ríšková et al. 2016). At the same time, new taxa are introduced to the reclaimed land, providing suitable habitat conditions for some fungal genera.

Analysis of the stability of soil fungal communities using diversity indexes is a very effective method (Staddon et al. 1997). In our study, there was a decline in the diversity and richness of soil fungi OTUs after large-scale reclamation. Richness and diversity of soil fungal communities was significantly different between the pristine marshland and reclaimed land ($P < 0.05$), except between marsh and farmland sites. This is due to the heavy use of pesticides and fertilizers, which decreased the diversity and richness of the soil fungal community in the marsh reclamation process, leading to soil degradation compared to the primitive wetland, as soil fungal diversity is usually at a low level in degraded wetlands. Our results agree with those of Lin et al. (2012). The latter authors used 454-pyrosequencing to analyze fungal communities and found that the diversity and richness of arbuscular mycorrhizal fungi significantly decreased under large-scale balanced soil fertilization, such as nitrogen-phosphorus-potassium. Results from both studies indicate that when the soil environment is disturbed by human activity, fungal diversity and richness usually decrease. Our previous study also found that the reclamation process promoted an increase in the diversity of the soil bacterial community (Xu et al. 2016). Therefore, marsh soil fungal communities seem to be more vulnerable and sensitive than marsh soil bacterial communities when disturbed by external factors. The present study also showed that the Shannon

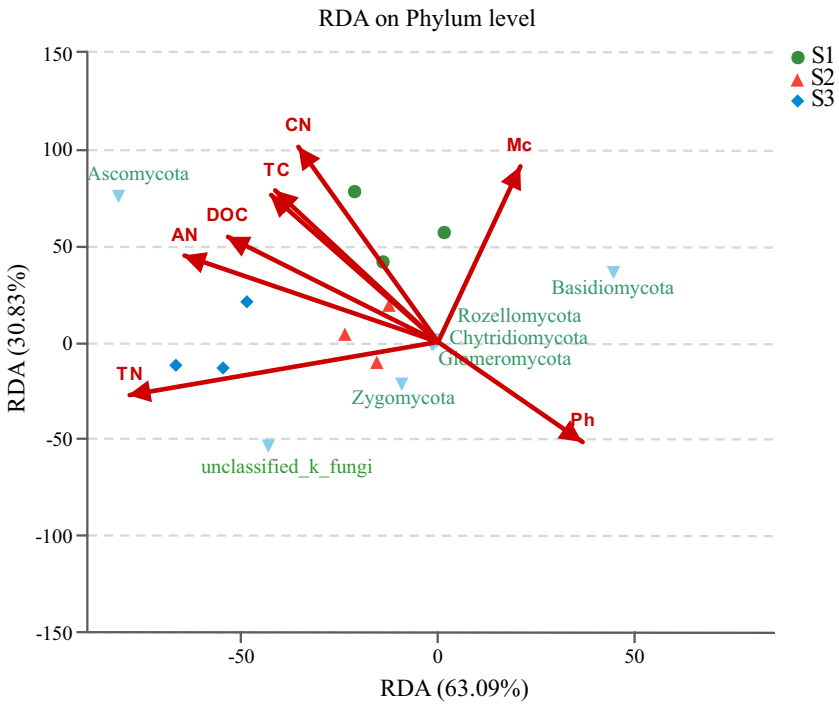

Fig. 5 Redundancy analysis (RDA) of soil fungal community in relation to environmental factors. Soil samples: *green circles* S1, *red triangles* S2, *blue diamonds* S3; *blue triangles* phyla, *arrows* indicate the influence of environmental factors. The angle between sites and environmental factors indicates the direction of the correlation between them (*acute angle* positive correlation, *obtuse angle* negative correlation, *right angle* no correlation). The vertical projection was drawn from different samples, and the closer the projection point the higher the influence of the environmental factor on the sample

index (4.16) of soil fungi in the pristine wetland was higher than in farmland (3.35). On the contrary, Chen et al. (2012) reported that the Shannon index for soil fungi in the wetland of Sanjiang was 3.23, rising slightly to 3.39 when nitrogen fertilizer was added. Thus, the reason leading to the decline of soil fungi community diversity seems to be the long-term reclamation process rather than a moderate excess of soil nutrients. Our research also found that diversity and richness calculated for different taxonomic levels have different trends. In farmland, diversity showed an increasing trend at the phylum level and a decreasing trend at the species level. Therefore, we should pay particular attention to the taxonomic level when studying soil microorganism diversity and richness.

Two principal routes alter soil ecosystems. First, nutrients are added from litter, by the eluviation of soluble matter from the ground, or by decomposition processes of root exudates. These three processes directly provide soluble C, N, and other nutrients to the soil, and indirectly affect the soil microbial community. Second, changes to the quantity and activity of soil microbes can alter the physicochemical characteristics and biological properties of the soil (Li et al. 2009). In the present study, we found that the composition, diversity, and richness of fungal communities are closely related to changes in soil pH, and water, C, and N content, as fungal communities displayed marked changes after land reclamation. This is mainly due to the process of land reclamation significantly reducing soil water content, TC, TN, TOC, SOC, and C/N ratio, and significantly increasing soil pH. Consequently, some fungal community constituents are lost due to interactions within the soil-plant-microorganism system. As the diversity and richness of soil fungal communities decreases by the conversion of marsh to farmland, the reduction of soil C, N, and C/N ratio seems to prevent the growth of the soil fungal community. In addition, changes in the natural forms of soil nutrients can inhibit the use of C and N by soil fungi. This also explains why different forms of C and N might be key limiting factors for soil fungal diversity in wetlands. Birkhofer et al. (2012) showed that soil yeast community composition and abundance in different land use types in Germany were significantly related to soil properties, including pH, N content, and C/N ratio. However, in contrast to other fungal groups, yeasts were highly abundant in the forest soils of the Schorfheide region, which has a low soil pH (3.2) and high soil moisture. Our results were similar to those of Birkhofer et al. (2012) as the diversity of fungal communities in marsh soil was higher than in farmland soil. This suggests that many fungi grow better in environments with relatively low pH and relatively high soil moisture. In addition, the humid summer climate and the high soil moisture content in the Sanjiang Plain might stimulate an increase in the diversity of soil fungi in marsh soil. Thus, soil water content of the marshland might be a key controlling factor for soil fungal communities. The large number of fungi in marsh soil indicates a complex soil fungal community structure. It is therefore vital to understand how to sustain soil fungal communities in order to maintain the balance of soil ecosystems and promote the health of cultivated lands (Xiao et al. 2014).

Conclusions

Overall, our results demonstrated that soil nutrients and soil microbial biomass decreased in different degrees after large-scale reclamation, and that the marsh soil has been degraded in the Sanjiang Plain due to reclamation processes. The heatmap analysis of soil fungal community showed different trends after marshland was reclaimed into paddy field or dry farmland. However, the fungal community structure is more similar between dry farmland and paddy field. RDA for individual soils showed that large-scale reclamation fundamentally restructured soil fungal community composition, soil physicochemical properties, and aboveground vegetation in the Sanjiang Plain marshland. Overall, Mc, pH, DOC, and SOC had a significant influence on soil fungal community composition in the following order: pH > Mc > SOC > TC > AN.

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