

## PRIMARY RESEARCH ARTICLE

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**Abundant fungi adapt to broader environmental gradients than rare fungi in agricultural fields**

**Running title:** Environmental adaptation of agro-soil fungi

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# Abundant fungi adapt to broader environmental gradients than rare fungi in agricultural fields

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## Abstract

Soil communities are intricately linked to ecosystem functioning, and a predictive understanding of how communities assemble in response to environmental change is of great ecological importance. Little is known about the assembly processes governing abundant and rare fungal communities across agro-ecosystems, particularly with regard to their environmental adaptation. By considering abundant and rare taxa, we tested the environmental thresholds and phylogenetic signals for ecological preferences of fungal communities across complex environmental gradients to reflect their environmental adaptation, and explored the factors influencing their assembly based on the large-scale soil survey in agricultural fields across eastern China. We found that the abundant taxa exhibited remarkably broader response thresholds and stronger phylogenetic signals for the ecological preferences across environmental gradients compared to the rare taxa. Neutral processes played a key role in shaping the abundant sub-community compared to the rare sub-community. Null model analysis revealed that the abundant sub-community was less clustered phylogenetically and governed primarily by dispersal limitation, while homogeneous selection was the major assembly process in the rare sub-community. Soil available sulfur was the major factor mediating the balance between stochastic and deterministic processes of both the abundant and rare sub-communities, as indicated by an increase in stochasticity with higher available sulfur concentration. Based on macro-ecological spatial scale datasets, our study revealed the potential broader environmental adaptation of abundant fungal taxa compared to rare fungal taxa, and identified the factors mediating their distinct community assembly processes in agricultural fields. These results contribute to our understanding of the mechanisms underlying the generation and maintenance of fungal diversity in response to global environmental change.

## KEYWORDS

Rare fungi, Environmental thresholds, Phylogenetic distribution, Community assembly, Biogeography, Stochastic processes, Deterministic processes, Agricultural ecosystems

## 1 | INTRODUCTION

Revealing the fundamental mechanisms for maintaining and generating species diversity is

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critical to determine the links between community stability and ecosystem function (Hanson *et al.*, 2012; Meyer *et al.*, 2018). Microorganisms typically present a skewed abundance distribution in a local community, because relatively few dominant species co-occur with a high number of low-abundance species, referred to as the “rare biosphere” (Jia *et al.*, 2018). Prior studies indicated that abundant and rare microbial taxa often exhibited different distribution patterns and functional traits (Pedrós-Alió, 2012; Wu *et al.*, 2017). Therefore, distinguishing between abundant and rare microbial taxa in terms of their biogeography and community assembly is useful for understanding microbially-driven ecosystem processes and functions.

Biogeographic studies of abundant and rare microbial taxa have explored bacterial communities in diverse environments (Jiao *et al.*, 2017a; Mo *et al.*, 2018). Different from bacteria with unicellular growth, filamentous fungi are characterized by hyphal growth and hence a larger body size (Powell *et al.*, 2015). The hyphal filaments of fungi are interconnected to form a network, which facilitates the perception of and response to environment change at the micrometer scale, and contributes to the sharing of resources and coordination of activities across heterogeneous environments (Chen *et al.*, 2004; Peay *et al.*, 2016). Fungi play a fundamental ecological role in driving soil nutrient cycling, mediating plant mineral nutrition, and alleviating nutrient limitation in other organisms (Hug *et al.*, 2016). Agricultural fields are typical human-managed terrestrial ecosystems that make a crucial contribution to global food security (Mondiale, 2008). High physiological and morphological plasticity of fungi could improve soil nutrient availability and crop nutrient uptake across environmental gradients (Lilleskov *et al.*, 2011). However, little is known about the biogeography of abundant and rare taxa or the mechanisms controlling rare taxa in the soil fungal community across agro-ecosystems at large scales.

Environmental filtering is a key determinant of species distribution and abundance (Kivlin *et al.*, 2014). The abundance of a given microbial species is the result of a balance between its growth and extinction rates (Pedrós-Alió, 2012). Abundant and rare bacterial taxa exhibit distinct responses to the ongoing environmental change (Jiao *et al.*, 2017b; Jiao *et al.*, 2019b). The environmental thresholds of ectomycorrhizal fungi across European forests have been evaluated by the accumulated change-point values of all individual species in a

community (van der Linde *et al.*, 2018). Available environmental thresholds seldom integrate the occurrence, abundance, and directionality of fungal responses at the species level, and very few studies are based on large-scale standardized datasets of natural sites (van der Linde *et al.*, 2018). By considering the interactions of land use and environmental gradients, a prior study found that agriculture could erase climate constraints on soil nematode communities across a large spatial scale (Li *et al.*, 2019). Moreover, microbial responses to changing environmental conditions appeared to be phylogenetically conserved and not distributed randomly across the tree of life (Martiny *et al.*, 2015). For example, bacterial responses to soil nitrogen addition and warming exhibited strong phylogenetic conservation (Oliverio *et al.*, 2017; Isobe *et al.*, 2019); the decisive roles of pH in the terrestrial bacterial biogeographic distribution (Bahram *et al.*, 2018; Delgado-Baquerizo *et al.*, 2018) appeared to be associated with the deep phylogenetic conservation of the response traits for pH adaptation (Martiny *et al.*, 2015). Therefore, revealing the phylogenetic distributions of microbial response traits offers predictions for the microbial biogeographic patterns and their evolutionary adaptation in response to the ongoing environmental changing, and ultimately for the alteration the biodiversity-driven ecosystem functioning (Maherali and Klironomos, 2007; Martiny *et al.*, 2015; Goberna and Verdu, 2018). However, the response thresholds and phylogenetic distribution of fungal communities to environmental change, particularly rare or abundant taxa, have not been reported in agricultural fields. This lack of knowledge hampers our ability to predict the responses of agricultural ecosystems to global environmental change, and to mitigate climate change impacts.

A key issue in microbial ecology is quantifying the relative contributions of deterministic processes (e.g., homogeneous selection) and stochastic processes (e.g., dispersal limitation) to microbial community assembly (Stegen *et al.*, 2012; Dini-Andreote *et al.*, 2015). Abundant taxa have been found to be limited by dispersion more than rare taxa in the surface layer of the northwestern Pacific Ocean (Wu *et al.*, 2017) and in inland freshwater ecosystems in China (Liu *et al.*, 2015). Reversely, other studies have shown that rare taxa are limited by dispersion more than abundant taxa in oil-contaminated soils (Jiao *et al.*, 2017a) and subtropical bays (Mo *et al.*, 2018). The balance between stochastic and deterministic assembly processes is mediated by environmental factors (Dini-Andreote *et al.*, 2015; Feng *et al.*, 2018;

Tripathi *et al.*, 2018; Zhang *et al.*, 2019). For example, the variation in soil organic matter could alter the relative influences of different assembly processes in shaping soil bacterial communities (Dini-Andreote *et al.*, 2015; Feng *et al.*, 2018). In addition, extreme soil pH would lead to deterministic assembly of soil bacterial communities, while shifts in soil pH toward neutral conditions would contribute to stochasticity during the pedogenic processes (Tripathi *et al.*, 2018). It is still unclear whether similar environmental factors regulate the dominance of deterministic or stochastic processes in the community assembly of abundant and rare fungi in agricultural soils.

In the present study, we aimed to (I) evaluate the potential environmental thresholds and phylogenetic distributions of abundant and rare fungi across complex environmental gradients in agro-ecosystems; and (II) uncover the major factors influencing the assembly of rare and abundant fungal sub-communities. We addressed these questions using the high-throughput sequencing datasets of soil fungi along with 23 environmental variables in adjacent pairs of maize (water-unsaturated) and rice (water-saturated) cultivated fields across eastern China. We estimated the environmental conditions “preferred” by each taxon (e.g. ecological preferences) to reflect their potential response traits to environmental changes (Treseder *et al.*, 2014; Oliverio *et al.*, 2017). Given the low competition capacity and growth rate of rare taxa (Barberan *et al.*, 2014; Jousset *et al.*, 2017), we hypothesized that (I) rare fungal taxa exhibited narrower environmental thresholds and weaker phylogenetic signals of ecological preferred traits than abundant fungal taxa; and (II) the assembly processes governing abundant and rare fungal sub-communities were influenced by distinct environmental factors. Our results could help predict the responses of soil fungi to global change, and understand the generation and maintenance of fungal diversity in agricultural ecosystems.

## 2 | MATERIALS AND METHODS

### 2.1 | Data collection

Soil samples were collected from fields under long-term maize and rice cultivation during the planting season (July–September 2017), as described in Jiao *et al.* (2019a). Two-hundred and forty two soil samples were collected from 127 agricultural fields throughout eastern China.

There were 115 paired sites, five maize-only, and seven rice-only sites, yielding 120 maize and 122 rice soil samples. This sampling strategy allowed us to explore whether water-unsaturated (maize fields) and -saturated (rice fields) agro-ecosystems exhibited similar trends.

Fungal diversity was analyzed using high-throughput sequencing of the internal transcribed spacer (ITS1) region with the primer pair ITS5-1737F (GGAAGTAAAAGTCGTAACAAGG) and ITS2-2043R (GCTGCGTTCTTCATCGATGC) on a HiSeq2500 platform (Illumina Inc., San Diego, USA). The acquired sequences were filtered for quality according to Caporaso *et al.* (2011). Sequences were assigned to their corresponding samples according to the barcode sequence and then quality-trimmed with an average Phred quality score cut-off of 20. Any chimeric sequences were removed with the USEARCH tool based on the UCHIME algorithm (Edgar *et al.*, 2011). Sequences were clustered into operational taxonomic units (OTUs) using a 97% similarity cut-off. The representative sequences were classified within the UNITE database and the International Nucleotide Sequence Databases (release 7) (Nilsson *et al.*, 2018). The affiliations of the OTUs to ecological guilds were determined in FUNGuild (Nguyen *et al.*, 2016).

Edaphic variables, including pH, cation exchange capacity (CEC), organic matter (OM), dissolved organic carbon (DOC), total nitrogen (TN), available nitrogen (AN), nitrate-nitrogen ( $\text{NO}_3^-$ ), ammonium-nitrogen ( $\text{NH}_4^+$ ), total phosphorus (TP), available phosphorus (AP), total potassium (TK), available potassium (AK), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), total iron (TFe), available iron (AFe), total sulfur (TS), available sulfur (AS), and soil particle size distribution (clay, silt, and sand), were measured using standard analytical methods (Bao, 2000; Ma *et al.*, 2016; Shi *et al.*, 2018). Mean annual temperature (MAT) and mean annual precipitation (MAP) data were obtained from the WorldClim database (<https://www.worldclim.org>).

## 2.2 | Data analysis

To avoid random effects on the identification of rare taxa, OTUs that contained less than 20 reads were discarded. Data analyses were performed using a randomly selected subset of 29,635 sequences from each sample to standardize the sequencing effort across samples. Here, OTUs with relative abundances above 0.1% of the total sequences were defined as “abundant”

taxa, those with relative abundances below 0.01% were defined as “rare” taxa, and those with relative abundances between 0.01% and 0.1% were “intermediate” OTUs (Liu *et al.*, 2015; Jiao *et al.*, 2017a).

The spatial distributions of the relative abundances of abundant and rare taxa were estimated using a kriging interpolation method and cross-validated using the “autoKrig.cv” function in the “automap” R package (Hiemstra *et al.*, 2009). A Pearson correlation analysis was performed to extract predicted values for selected soil samples and correlate them with the values observed at corresponding sites. The associations between the relative abundances of fungal taxa and environmental variables were evaluated using a random forest analysis (Archer, 2013; Delgado-Baquerizo *et al.*, 2016; Trivedi *et al.*, 2016), with environmental variables serving as predictors for the relative abundances of abundant and rare taxa.

To evaluate the phylogenetic clustering of abundant and rare taxa, the standardized effect size measure of the mean nearest taxon distance (SES.MNTD) was calculated using the null model “taxa.labels” (999 randomization) in the “ses.mntd” function of the “picante” R package (Kembel *et al.*, 2010). Beta mean nearest taxon distance ( $\beta$ MNTD) metric was used to evaluate pairwise phylogenetic turnover between communities using the ‘comdistnt’ function (abundance.weighted = TRUE). The distance-decay relationship (DDR) was calculated as the slope of an ordinary least-squares regression between geographic distance and phylogenetic community similarity ( $1 - \beta$ MNTD metric).

Threshold values of abundant and rare taxa in response to each environmental variable were calculated using threshold indicator taxa analysis (TITAN2) (Baker and King, 2010). The sums of indicator taxa scores for fungal OTUs were used to determine lower and upper thresholds of change in the abundant and rare sub-communities based on the environmental variables. In addition, the phylogenetic signals were estimated for each sub-community and the major phyla to test whether the environmental preference of a fungal OTU was related to the phylogeny, reflecting the degree of phylogenetic conservatism in response to complex environmental gradients (Martiny *et al.*, 2015). Firstly, to obtain the potential trait information, we identified the ecological preferences for each OTU via the Spearman’s correlations between relative abundances of rare or abundant OTUs and environmental variables according to Oliverio *et al.* (2017). For example, the OTUs positively or negatively

correlated with MAT were identify as “warm-preferred” or “cold-preferred”; the OTUs positively or negatively correlated with soil pH were identify as “alkaline-preferred” or “acid-preferred”. Next, we applied two approaches (Blomberg’s K and D-test of Fritz and Purvis) to measure the phylogenetic signals for the taxa’ environmental preferences (positive or negative) (Goberna and Verdu, 2016; Oliverio et al., 2017; Isobe et al., 2019). Blomberg’s K was calculated using the ‘multiPhylosignal’ function in the “picante” R package (Kembel et al., 2010). The K value describes how well a taxon is correlated with phylogeny as expected in a Brownian motion-based metric of the strength of phylogenetic signal (Blomberg *et al.*, 2003). K values closer to zero indicate a random or convergent pattern of evolution, while K values greater than 1 indicate strong phylogenetic signals and conservatism of traits. The D-test of Fritz and Purvis was performed using the ‘phylo.D’ function in the “caper” R package (Orme *et al.*, 2012). This phylogenetic dispersion (D) value, developed by Fritz and Purvis, compares the observed sister-clade differences in the trait against those with the random phylogenetic pattern (Fritz and Purvis, 2010). For comparative purposes with Blomberg’s K statistic, we transformed the D value into  $-D+1$  (Goberna and Verdu, 2016). The evolution of a trait (I) does not show a significant signal when  $-D+1 = 0$ , (II) is more conserved than expected than expected by chance when  $-D+1 > 0$ .

The assembly processes of abundant and rare sub-communities were evaluated using a null model analysis (Stegen et al., 2012; Stegen *et al.*, 2013; Dini-Andreote et al., 2015). This method can be used to detect community assembly mechanisms by estimating the standard deviation of the observed ecological patterns compared to the randomly shuffled ecological patterns produced by null models (Chase, 2010; Chase and Myers, 2011). If the observed ecological patterns are not statistically different from null expectations, the community dynamics are largely considered stochastic with respect to the processes excluded; otherwise, they are regarded as being deterministic. Here, the null model analysis was performed using a framework described by Stegen et al. (2013) to classify community pairs into underlying drivers of deterministic processes (e.g., homogeneous selection and variable selection) or stochastic processes (e.g., dispersal limitation, homogeneous dispersal and “non-dominant”). Briefly, variations in phylogenetic and taxonomic diversity were measured using This article is protected by copyright. All rights reserved

null-model-based phylogenetic and taxonomic  $\beta$ -diversity metrics, namely the  $\beta$ -nearest taxon index ( $\beta$ NTI) and the Bray–Curtis-based Raup–Crick ( $RC_{Bray}$ ).  $|\beta$ NTI| > 2 indicates the dominance of deterministic processes, with significantly less (i.e., homogeneous selection;  $\beta$ NTI < -2) or more (i.e., variable selection;  $\beta$ NTI > 2) phylogenetic turnover than expected. When  $|\beta$ NTI| < 2,  $RC_{Bray} < -0.95$  and  $RC_{Bray} > 0.95$  represent the relative influences of homogeneous dispersal and dispersal limitation, respectively, and  $|RC_{Bray}| < 0.95$  indicate the influence of the “non-dominant” fraction (Zhou and Ning, 2017; Tripathi et al., 2018).

We then explored the major factors that influenced the assembly processes of abundant and rare sub-communities. Variations in community assembly processes along the gradients of the derived environmental variables were assessed using the mantel tests comparing  $\beta$ NTI values with the Euclidean distance matrices of each of the variables. Partial mantel test was used to assess the relationship between phylogenetic turnover and the derived environmental variables after controlling for spatial or other environmental variables. The statistical significance of such comparisons was determined using 999 permutations and the analyses were performed using the “mantel” function of the “ecodist” package in R (Goslee and Urban, 2007). To determine the contribution of neutral processes, a neutral community model was fitted to the abundant and rare taxa by predicting their relationship between the frequency of taxonomic occurrence and the abundance (Sloan *et al.*, 2006).  $R^2$  indicates the fit to the neutral model.

### 3 | RESULTS

#### 3.1 | General distribution patterns of abundant and rare taxa

After quality filtering and the removal of chimeric sequences, 7,171,667 high-quality sequences were clustered into 11,244 OTUs. As expected, a high proportion of the OTUs were identified as rare taxa (mean = 68.2%), but they only accounted for 3.6% of the average relative abundance in each sample (Supplementary Figure S1a). Conversely, a very low proportion of the OTUs (mean = 8.6%) were identified as abundant taxa, which accounted for 85.3% of the average relative abundance in each sample. Abundance–occupancy relationships revealed that the rare taxa had stronger positive correlations than the abundant taxa. Twelve abundant taxa occupied >50% of sites, while none of the rare taxa were present in >50% of

sites (Supplementary **Figure S1b**). Among the taxa that were locally abundant, 15.1% were abundant and 48.1% were still present in the other samples (Supplementary **Figure S1c**). However, among the taxa that were locally rare, 78.9% were not detected, 11.1% were still rare, and almost none (1.9%) were abundant in the other samples (Supplementary **Figure S1C**). The results indicate that the abundant and rare taxa had distinct distribution patterns in agricultural fields.

The abundant sub-community had a higher OTU relative abundance in maize soils than in rice soils, and the rare sub-community exhibited an opposite trend (**Figure 1a**). Spatial distribution analysis by kriging interpolation showed that the abundant taxa had greater OTU relative abundance at high latitudes than at low latitudes, while an opposite trend was observed for the rare taxa. Spearman correlation analysis revealed that the distinct distributions corresponded to the contrasting environmental associations between the abundant and rare sub-communities (**Figure 1b**), indicating their distinct environmental preferences. Crop type explained the variation in the relative abundance of both abundant and rare taxa the most. Regarding functional groups, plant pathogens accounted for higher read numbers than symbiotrophs in the abundant sub-community; however, an opposite trend was observed in the rare sub-community. The functional groups for the abundant and rare sub-communities were driven by different environmental factors (**Figure 1b**). For plant pathogens, AFe and TS accounted for the greatest proportion of variation in both the abundant and rare sub-communities. In addition, the positive effects of MAT and NH<sub>4</sub> were the strongest predictors of the distribution of saprotrophs, while NO<sub>3</sub> and crop type contributed the most toward explaining variation in the relative abundance of symbiotrophs in both the abundant and rare sub-communities.

There was great phylogenetic diversity in both the rare and abundant sub-communities, although the species mostly belonged to Ascomycota, Basidiomycota, and Zygomycota (**Figure 2a and 2c**). The abundant taxa were primarily comprised of *Fusarium*, *Gibberella*, and *Trichoderma* in the phylum Ascomycota, and *Rhizopus* and *Mortierella* in the phylum Zygomycota. Furthermore, the rare taxa comprised primarily of *Fusarium*, *Acremonium*, *Chaetomium* and *Epicoccum* in the phylum Ascomycota. In addition, the abundant taxa had stronger environmental associations than the rare taxa (**Figure 2a**). The mean values of

SES.MNTD were significantly higher for the abundant than the rare taxa, which were all less than zero (Wilcoxon rank-sum test,  $P < 0.001$ , **Figure 2b**).

### 3.2 | Environmental responses of abundant and rare sub-communities

Pearson's correlation coefficients for phylogenetic  $\beta$ -diversity [Beta mean nearest taxon distance ( $\beta$ MNTD) metric] versus geographic distance were highly significant for both the rare ( $r = 0.143$ ,  $P < 0.001$ ) and abundant sub-communities ( $r = 0.160$ ,  $P < 0.001$ ; Supplementary **Figure S2**). In addition, the abundant sub-community exhibited a significantly higher phylogenetic  $\beta$ -diversity than the rare sub-community (Supplementary **Figure S2**). In maize soils, the DDRs for the rare sub-community were not significant, and the phylogenetic  $\beta$ -diversity was higher in the rare sub-community than in the abundant sub-community. However, in rice soils, the rare sub-community had stronger DDRs and lower phylogenetic  $\beta$ -diversity than the abundant sub-community. The influences of environmental variables on the structure of each sub-community were assessed using a distance-based linear model and forward selection procedure (Supplementary **Figure S3**). Crop type, soil pH, MAT, and AS were the most important factors influencing the structures of both the abundant and rare sub-communities (Supplementary **Table S1-S2**).

To determine the environmental thresholds for the abundant and rare sub-communities in response to each of the variables, we evaluated the cumulating z- and z+ change points using threshold indicator taxa analysis (Supplementary **Figure S4-S6**). Notably, the abundant sub-community exhibited a broader range of environmental thresholds than the rare sub-community for almost all the variables, excluding MAT, NO<sub>3</sub>, and AK (**Figure 3a**). In addition, we examined the strength of the relationship between phylogeny and environmental preferences to determine whether ecological traits could be predictive of phylogenetic levels (**Figure 3b** and **3c**). Both Blomberg's K statistics and D-test of Fritz and Purvis revealed that the abundant sub-community exhibited stronger phylogenetic signals for almost all of the variables than the rare sub-community, except for TK (**Figure 3b** and **3c**). This indicates that within the abundant sub-community, closely related species exhibited more similar ecological preferences to the environmental variables. Phylum-level taxonomy validated such observations (Supplementary **Figure S7**). For instance, the abundant taxa of Ascomycota (100%), Basidiomycota (83.3%), Chytridiomycota (94.4%), and Zygomycota (72.2%)

showed stronger and significant phylogenetic signals (Blomberg's K; Supplementary **Figure S7a**) for more than 70% of the 18 variables, except for one phylum, Glomeromycota (44.4%). The D-test of Fritz and Purvis also showed similar trends (Supplementary **Figure S7b**).

### 3.3 | Assembly processes in rare and abundant sub-communities

The relationships between  $\beta$ NTI and major environmental variables were used to estimate changes in the relative influences of deterministic and stochastic assembly processes. Mantel tests results showed that soil AS was the best predictor of assembly processes in both the abundant and rare sub-communities (**Table 1**). The relationships remained significant after controlling for spatial distance and other measured environmental variables (Supplementary **Table S3**). Pairwise comparisons of  $\beta$ NTI values for the abundant and rare sub-communities were significantly and positively correlated with differences in AS (**Figure 4a**), indicating that an increasing divergence of AS led to a shift from homogeneous selection to stochasticity in the assembly of each sub-community. Samples were separated into sub-groups based on the AS and correlated with  $\beta$ NTI. With increasing AS, the relative influence of stochastic assembly increased in the abundant sub-community, while it first decreased and then increased in the rare sub-community (**Figure 4b**, Supplementary **Figures S8**).

By quantifying the deviation of phylogenetic turnover, we observed that stochastic assembly (68.6%) was dominant in the abundant sub-community, while deterministic assembly (86.7%) primarily governed the rare sub-community (**Figure 5**). Homogeneous selection contributed a larger fraction to the assembly of the rare sub-community (86.3%) than the abundant sub-community (29.4%). Dispersal limitation influenced the abundant sub-community (45.9%) more than the rare sub-community (3.1%). The neutral community model explained a larger fraction of variation in the abundant sub-community ( $R^2 = 0.8162$ ) than in the rare sub-community ( $R^2 = 0.2785$ ).

## 4 | DISCUSSION

Our knowledge on fungal biogeography in natural ecosystems has accumulated rapidly over the past few years (Tedersoo *et al.*, 2014; van der Linde *et al.*, 2018). However, few large-scale studies have investigated the assembly processes driving abundant and rare fungal communities across agro-ecosystems, and particularly with regard to their environmental

thresholds. Based on the large-scale survey of agro-soil fungi, the present study provides statistical evidence of potential broader response thresholds and stronger phylogenetic signals for ecological preferred traits of abundant fungal taxa in agricultural fields when compared to rare fungal taxa, indicating the broader environmental adaptation of abundant ones. We also reveal distinct assembly processes governing the abundant and rare sub-communities, which are mediated by similar factors, mainly soil AS concentration.

#### 4.1 | Broader environmental adaptations of abundant fungal taxa

Environmental change mediates the dynamic balance in microbial communities, and in turn influences the associated ecosystem function (Smith *et al.*, 2016). Most studies have focused on the influences of environmental factors on the bacterial community composition of abundant and rare taxa, regardless of their environmental adaptation (Liu *et al.*, 2015; Jiao *et al.*, 2017a; Mo *et al.*, 2018). Here, we provide a more in-depth understanding of whether the environmental adaptation of soil fungi varies between the abundant and rare taxa in agricultural fields. The environmental adaptation was reflected from two aspects: (I) the breadth of environmental thresholds (van der Linde *et al.*, 2018); (II) the strength of phylogenetic signals to ecological preferences (Martiny *et al.*, 2015). The environmental thresholds evaluate the responses and thresholds for community change across environmental gradients (Baker and King, 2010; van der Linde *et al.*, 2018). The ranges of the environmental thresholds could reflect niche breadths of the species, akin to ecological adaptation. For the phylogenetic signals, revealing the phylogenetic conservation for traits relies on microbial ecological preferences could offer predictions for their evolutionary adaptation in response to the ongoing environmental changing (Martiny *et al.*, 2015; Amend *et al.*, 2016).

Firstly, we observed that the abundant fungal taxa exhibited potential broader response thresholds to the environmental variables compared to the rare taxa. This could be explained that the abundant taxa could effectively utilize a broader array of resources than the rare taxa (Jia *et al.*, 2018). Indeed, we observed that abundant taxa were more ubiquitous than the rare taxa across agricultural fields at a regional scale, which was consistent with previous studies (Jiao *et al.*, 2017a; Mo *et al.*, 2018) and could support the above point. Rare fungal taxa were not evenly distributed throughout agricultural soils, and most were present only in a few

samples. It is possible that the rare taxa have low competition capacity and growth rate, and are, therefore, limited in their environmental breadths (Barberan et al., 2014; Jousset et al., 2017). The result of our study, from a novel perspective, lends support to the notions that abundant species occupy a broader niche breadth and are suitably adapted to a broader range of environmental gradients. The environmental thresholds analysis was according to a previous study, estimating the environmental thresholds of ectomycorrhizal fungi across European forests at a large spatial scale (van der Linde et al., 2018). The environmental threshold analysis was based on statistical methods (Baker and King, 2010) and the results might remain controversial for implication to the real world. Therefore, experimental verification is required when using these statistical results in the formation of environmental policy. Despite this, our results based on the large-scale observed datasets still provide statistical evidence for the potential broader environmental thresholds of abundant fungal taxa compared to rare fungal taxa in agricultural ecosystems at a such spatial scale that is—to our knowledge—unprecedented.

Secondly, the abundant taxa displayed stronger phylogenetic signals for the ecological preferences than the rare taxa, via Blomberg's K statistics and D-test of Fritz and Purvis. This indicated that within the abundant sub-community, closely related species exhibited more similar ecological preferences across environmental gradients. The traits relies on ecological preferences of an organism depend on its evolutionary history (Martiny et al., 2015; Amend et al., 2016). For example, one previous study examined the large-scale distributions of soil fungi, and demonstrated that fungal evolutionary histories could be reflected in their large-scale biogeography (Treseder et al., 2014). They found that fungal preferences for higher latitudes and lower precipitation levels were significantly phylogenetically conserved among the younger phyla, while older phyla preferred significantly lower latitudes and warmer, wetter conditions (Treseder et al., 2014). In addition, the response traits of pH and salinity preference for soil microbial communities were found to be relatively deeply conserved (Martiny et al., 2015), corresponding that pH and salinity acted as major factors affecting the microbial biogeographic distributions (Bahram et al., 2018; Delgado-Baquerizo et al., 2018). Therefore, revealing the phylogenetic signal and conservation of traits relies on microbial ecological preferences could offer predictions for their evolutionary adaptation in

response to the ongoing environmental changing (Martiny et al., 2015; Amend et al., 2016). In the present study, the stronger phylogenetic signals for the ecological preferences of abundant taxa suggested their more phylogenetic niche conservatism with respect to the evolutionary history of environmental adaptation; this might also explain their broader environmental breadths and distinct biogeographic patterns compared to the rare taxa. Together, these two aspect results indicated that fungal abundant and rare sub-communities had distinct adaptations to different environmental conditions in agricultural fields, with potential broader ranges for the abundant taxa.

We also found that the rare taxa exhibited higher relative abundance at low latitudes, suggesting their preference of warmer environments. Based on the metabolic theory of ecology, species diversity, metabolic activity, and population growth rates increase exponentially with an increase in temperature (Zhou et al., 2016). This suggests that the rare taxa are more defined by the metabolic theory of ecology, compared to the abundant taxa. Climate warming accelerates temporal turnover and divergent succession of soil microbial communities (Guo et al., 2018; Guo et al., 2019). Higher metabolic rates and intense competition between microorganisms at higher temperatures could result in higher species turnover or new microbial inputs (Liang et al., 2015). This could also explain the uneven distribution of rare microbial taxa. Rare taxa act as a “seed bank” for microorganisms that can become dominant under suitable conditions, which increases functional redundancy and resilience to environmental disturbances (Pedrós-Alió, 2012). In the present study, we observed that rice soils contained more rare fungal taxa than maize soils, suggesting potentially higher resilience of soil fungal communities to environment change in wetland agricultural fields. This result implies that the differences in the distribution of soil fungal communities between wetland and dryland should be taken into consideration when formulating sustainable management plans and baselines for agricultural fields under climate changes.

#### **4.2 | Different assembly processes of abundant and rare fungal sub-communities**

Revealing the mechanisms underlying community assembly is a key topic in microbial ecology (Nemergut et al., 2013). Microbial spatial turnover is potentially controlled by different ecological mechanisms; thus, it is critical to quantify the relative influences of

deterministic and stochastic processes on community assembly (Cline and Zak, 2014; Bahram *et al.*, 2016). In the present study, the null model analysis showed that stochastic processes (e.g., dispersal limitation) were dominant in the abundant fungal sub-community, and deterministic assembly (e.g., homogeneous selection) primarily governed the rare fungal sub-community in agricultural fields (**Figure 5**). Our result supported prior studies reporting that abundant taxa were more limited by dispersion than rare taxa in the northwestern Pacific Ocean (Wu *et al.*, 2017) and in lakes and reservoirs in China (Liu *et al.*, 2015). The most abundant taxa can disperse readily as there are, by definition, many more individuals that can potentially be involved in a dispersal event (Liu *et al.*, 2015). Moreover, significantly lower mean values of SES.MNTD for rare taxa indicate that the rare sub-community is more closely clustered phylogenetically than the abundant sub-community (Fan *et al.*, 2017). A prior study has demonstrated that environmental filtering causes phylogenetic clustering in bacterial communities (Horner-Devine and Bohannan, 2006). Here, our results showed a similar trend that the rare fungal sub-community, more governed by deterministic processes, was more clustered phylogenetically. One point we should concern about is that most of the abundant taxa were locally abundant and present in less than 10% of the sampling sites; this might lead to an overestimation of the influence of dispersal limitation.

In contrast to our results, some studies demonstrated that rare taxa were more limited by dispersion than abundant taxa in oil-contaminated soils (Jiao *et al.*, 2017a) and in subtropical bays (Mo *et al.*, 2018). Such discrepancies could be due to the differences in habitats and geography (Shi *et al.*, 2018). In addition, the inconsistent results might be attributed to the differences between fungi and bacteria in terms of their body size and lifestyle. Soil bacteria are typically characterized by unicellular growth and smaller cell sizes compared to filamentous fungi (Powell *et al.*, 2015). Body size has been shown to influence dispersal ability and spatial aggregation of organisms (Nekola and White, 1999). As soil bacteria and fungi exhibit fine-scale differences in body size (Anderson *et al.*, 2014), their metabolic activity and dispersal potential may influence the adequacy of deterministic or stochastic processes for explaining their community structure (DeLong *et al.*, 2010; Wu *et al.*, 2018). Furthermore, the neutral model explained a large fraction ( $R^2 > 0.8$ ) of abundant fungal taxa, indicating that the abundant fungal sub-community was more influenced by neutral processes. Together, the

results of the null model and neutral model analyses support the notion that stochastic and deterministic processes respectively dominate the assembly of abundant and rare fungal sub-communities in agricultural ecosystems.

#### 4.3 | Available sulfur mediates the assembly processes of agro-soil fungal communities

To gain an advanced mechanistic understanding of microbial ecology, it is essential to reveal the factors affecting the relative influences of stochastic and deterministic assembly processes of microbial communities (Feng et al., 2018; Tripathi et al., 2018). Numerous studies have shown that soil pH, OM content, and salinity are important factors influencing bacterial community assembly processes in various environments (Dini-Andreote et al., 2015; Feng et al., 2018; Tripathi et al., 2018; Zhang et al., 2019). However, little is known about the environmental factors affecting the relative influences of stochastic and deterministic processes that govern the assembly of fungal communities in agricultural soils. Based on the between-community null model analysis, we found  $\beta$ NTI of both abundant and rare communities to be more strongly associated (Mantel and partial Mantel coefficient) with soil AS than with spatial distance or other environmental distances (**Tables 1** and Supplementary **Table S3**). This indicated that soil AS was the major factor mediating the balance between stochastic and deterministic assembly for both abundant and rare fungal sub-communities in agro-ecosystems.

The decisive role of soil AS in mediating the dominance of fungal community assembly processes might be partially explained by the function of sulfur nutrient and physiological characteristics of fungi (Gahan and Schmalenberger, 2014). Sulfur is an essential nutrient for plant and microbial growth (Gahan and Schmalenberger, 2014), fungi are well known for their metabolic versatility and they are involved in the cycling of sulfur apart from other nutrients (Holt *et al.*, 2017; Linder, 2018). Moreover, sulfur could enhance plant defense against soil-borne fungal diseases in plants through the formation of sulfur-containing defense compounds (Rausch and Wachter, 2005). In this case, we speculated that soil sulfur might influence the fungal community assembly via two mechanisms: (I) directly affecting the fungal growth; (II) indirectly interacting with plant to shape the fungal community assembly.

Though soil sulfur is known to affect fungal community composition and diversity (Holt  
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et al., 2017), it has not been clear how soil sulfur mediates fungal community assembly processes in agricultural ecosystem. Our results showed that the relative influence of deterministic processes was greater in low-AS soils, while stochastic community assembly was more influential in high-AS soils. Several mechanisms are likely associated with changes in assembly processes in response to the variation of AS. First, low-AS soils may impose more resource (e.g. sulfur) limitation on fungal taxa, which might allow for less colonization, greater exclusion, and therefore lead to an increased influence of deterministic processes (Feng et al., 2018). Second, high-AS soil introduced more resource to soil fungi, which can improve the ability of microorganisms to disperse, and in turn increase the dominance of stochastic processes (Dini-Andreote et al., 2015). Additionally, the increase in stochasticity could imply that adapted lineages of soil fungi accumulate in high-AS environments, suggesting that weaker niche-based exclusion and increased influx of abundant and rare fungal lineages may dominate high-AS agricultural fields. Our findings reveal the important linkage between environmental variables and community assembly processes, which might influence the microbial diversity and soil ecosystem processes (Maherali and Klironomos, 2007).

#### 4.4 | Testing the robustness of the conclusions

While, PCR dynamics and PCR bias might play an enormous role in the number of reads per taxon for a given fungal community and the proportion of reads for a taxon does not necessarily represent the proportion of abundance of that taxon in that community. In addition, there is a plenty of evidence that fungi are not distributed homogeneously, neither spatially, nor temporally (Pickles *et al.*, 2012). Given these points, we examined the main observations based on presence absence data. In this case, based on presence absence data, the OTUs occurring in more than 80% of all soil samples were defined as “abundant” taxa, those present in less than 20% of all soil samples were defined as “rare” taxa. Most of the observations showed similar trends between presence absence data and abundance data, further supporting the robustness of our conclusions. Firstly, species in both the rare and abundant sub-communities mostly belonged to Ascomycota, Basidiomycota, and Zygomycota, with stronger environmental associations and higher SES.MNTD of the abundant taxa compared to rare taxa (Supplementary **Figure S9**). Secondly, the abundant

sub-community exhibited a broader range of environmental thresholds and stronger phylogenetic signals for most of the variables than the rare sub-community (Supplementary **Figure S10**). Thirdly, soil AS was the best predictor of the assembly processes for rare sub-communities (Mantel tests for  $\beta$ NTI, Supplementary **Table S4**), and the relative influence of stochastic assembly first decreased and then increased with increasing AS (Supplementary **Figure S11**). While no environmental variables significantly correlated with the  $\beta$ NTI of abundant sub-communities, suggesting that the occurring frequency should be considered when defining the abundant fungal taxa. Overall, the additional analyses based on presence absence data indicated that our results and inferences were robust.

#### 4.5 | Summary and outlook

We propose a conceptual paradigm portraying the responses and assembly processes of abundant and rare fungal sub-communities in agricultural ecosystems based on changes in environmental variables (**Fig. 6**). The abundant and rare sub-communities had distinct response thresholds and phylogenetic signals of ecological preferences to different environmental conditions in agricultural fields, with potential broader environmental adaptation of the abundant taxa. Meanwhile, the rare sub-community was more closely clustered phylogenetically than the abundant sub-community. Stochastic processes were dominant in driving the assembly of the abundant sub-community, rather than in the rare sub-community. Soil AS was the major factor mediating the balance between assembly processes of the abundant and rare sub-communities, with an increase in stochastic assembly in higher AS environments. Based on a macro-ecological scale analysis, the present work linked abundant and rare fungal species to environmental adaptation, and revealed the factors mediating community assembly processes. The findings could improve our understanding of the generation and maintenance of fungal diversity, and facilitate the prediction of fungal responses to global change in agricultural ecosystems. Considering the importance of soil fungal communities for ecosystem function, experimental validation of the model should be conducted in a wide range of systems.

#### Conflict of Interest

The authors declare no conflicts of interest.

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## Data Accessibility Statement

The raw sequence data reported in this paper are available in the NCBI Sequence Read Archive under BioProject PRJNA544819.

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## Author contributions

S.J. conceived and designed the study with the help of Y.L.; S.J. performed the experiments, analyzed the data, and drafted the manuscript; Y.L. reviewed the manuscript.

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**Tables****TABLE 1** Mantel tests of environmental variables against the phylogenetic turnover ( $\beta$ -nearest taxon index) of abundant and rare fungal sub-communities in agricultural soils.

	<b>Abundant</b>	<b>Rare</b>
Geographic distance	<b>0.050*</b>	0.021
MAT	0.053	0.013
pH	-0.025	0.012
CEC	<b>0.096**</b>	<b>0.053*</b>
DOC	-0.064	0.013
AN	0.040	0.005
NO <sub>3</sub>	0.017	-0.077
NH <sub>4</sub>	0.042	<b>0.107**</b>
TP	-0.035	0.035
AP	-0.082	-0.123
TK	-0.072	-0.028
AK	0.023	0.012
TS	0.058	0.062
AS	<b>0.179***</b>	<b>0.125**</b>
TFe	0.018	0.029
AFe	-0.073	0.061

Notes. \*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001. MAT, mean annual temperature; CEC, cation exchange capacity; DOC, dissolved organic carbon; AN, available nitrogen; NO<sub>3</sub>, Nitrate-nitrogen; NH<sub>4</sub>, ammonium-nitrogen; TP, total phosphorus; AP, available phosphorus; TK, total potassium; AK, available potassium;; TS, total sulfur; AS, available sulfur; AFe, available iron; and TFe, total iron.

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**FIGURE 1** General distribution patterns of abundant and rare fungal taxa in agricultural soils.

(a) Spatial distributions of the relative abundances of abundant and rare taxa at the operational taxonomic unit (OTU) level were mapped by kriging interpolation. Cross-validation (CV) of the maps was based on Pearson correlation between the predicted and observed values at each sampling site. The Pearson correlation coefficient and *P*-value are shown in the maps. The upper boxplot shows differences in relative abundance between maize and rice soils (\*\*\*,  $P < 0.001$ ; Wilcoxon rank-sum test). The lower boxplot shows differences in relative abundance between maize and rice soils (\*\*\*,  $P < 0.001$ ; Wilcoxon rank-sum test). The boxplot below shows differences in functional groups, and those not sharing a letter are significantly different ( $P < 0.05$ ; multiple comparison with Kruskal-Wallis tests). Pa, plant pathogen; Sa, saprotroph; and Sy, symbiotroph. (b) Environmental associations of the relative abundance of abundant and rare taxa and different functional groups in agricultural soils evaluated by correlation and best random forest model Circle size represents the variable's importance [that is, decrease in the prediction accuracy (estimated with out-of-bag cross-validation)]. Colors represent Spearman correlations. The abbreviations of environmental variables are defined in *Materials and Methods*

**FIGURE 2** Taxonomic and environmental correlations of abundant and rare fungal sub-communities in agricultural soils. (a) Phylogenetic distribution of the top 25 most abundant taxa in the rare and abundant sub-communities and their environmental preferences (e.g., positive or negative). The phylogenetic tree was constructed using the neighbor-joining method. Taxa that could be assigned to genus level are shown as genus, otherwise as OTU ID. (b) Boxplot showing difference in SES.MNTD values between abundant and rare sub-communities (\*\*\*,  $P < 0.001$ ; Wilcoxon rank-sum test). (c) A circos plot showing the taxonomic distribution of abundant and rare taxa at the phylum level. The thickness of each ribbon represents the number of abundant and rare taxa assigned to different phyla. The abbreviations of environmental variables are defined in *Materials and Methods*

**FIGURE 3** Environmental adaptation of abundant and rare fungal taxa in agricultural soils.

(a) Environmental breadth estimated by the threshold values of abundant and rare taxa in

response to environmental variables calculated using threshold indicator taxa analyses. The threshold values were standardized. Phylogenetic signal showing the trait conservatism for environmental preferences of the abundant and rare sub-communities using Blomberg's K (**b**) and D-test of Fritz and Purvis (**c**). The abbreviations of environmental variables are defined in *Materials and Methods*

**FIGURE 4** Relative influences of deterministic and stochastic assembly processes in shaping abundant and rare fungal sub-communities in agricultural soils. **(a)** Relationships between  $\beta$ -nearest taxon index ( $\beta$ NTI) and differences in soil available sulfur (AS) for the abundant and rare sub-communities. Linear regression models (shown as red lines) and associated correlation coefficients are provided on each panel. Horizontal dashed lines indicate the  $\beta$ NTI significance thresholds of +2 and -2. **(b)** Patterns of  $\beta$ NTI across different categories in soil AS for the abundant and rare sub-communities

**FIGURE 5** The fraction of turnover in the assembly of abundant and rare fungal sub-communities governed primarily by deterministic (homogeneous and variable selection) and stochastic (dispersal limitations and homogenizing dispersal) processes, and the fraction that was not dominated by any single processes ("Undominated") in agricultural soils

**FIGURE 6** A conceptual paradigm showing **(a)** environmental adaptation and **(b)** stochastic processes in the assembly of abundant and rare fungal sub-communities under the influence of soil available sulfur in agricultural ecosystems











