Soil fungal community assembly processes under long-term fertilization

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Summary

Understanding the processes that regulate communities of microorganisms is a key issue and focus in microbial ecology. Although fungi play a critical role in soil biogeochemical cycling, their community assembly processes remain largely unknown, especially in agricultural soils. In this study, we investigated the relative importance of five community assembly processes: variable selection, homogeneous selection, homogeneous dispersal, dispersal limitation, and undominated process on soil fungal communities under long-term (28 years, 1990-2018) fertilization management consisting of 12 different treatments in triplicate field plots. Using Illumina MiSeq sequencing of the 18S rRNA eukaryotic gene, we observed that fungal communities in manure treatments were all structured primarily by homogeneous dispersal, while the communities in chemical fertilizer treatments were structured primarily by homogeneous dispersal and undominated process. Soil calcium played an important role in shaping soil fungal community, while soil organic matter concentrations showed a considerable impact on the soil fungal phylogenetic community composition. Overall, our results suggest that fertilization management should be considered as a key factor driving microbial community assembly processes in farmed soils.

Keywords: fungi, assembly process, agricultural soils, soil organic matter.

Introduction

The impact of long-term fertilization on soil bacterial communities has been extensively addressed (Sun et al, 2015; Feng et al, 2018a; Wang et al, 2018). Yet fungal communities, which are essential for terrestrial biogeochemical cycling, have received disproportionally less research attention, even though this group of organisms is recognized as a key contributor to soil fertility (van der Wal et al, 2013). To date, only studies have investigated the diversity and community composition and structure of soil fungi in different habitats (Tedersoo et al, 2014; Smith et al, 2018). For example, using high-throughput metabarcoding (e.g., Seppey et al. 2017) investigated the taxonomic and functional diversity of soil fungi in forests, meadows, and croplands in Switzerland. In Arctic tundra soils, researchers found that the soil fungi could be strongly influenced by various factors, including soil pH, carbon/nitrogen (C/N) ratio, and moisture content (Shi et al, 2015). In short, although the impact of long-term fertilization on soil fungal communities has been studied previously (Sun et al, 2016; Fierer, 2017; Yao et al, 2018), the underlying processes that regulate such soil fungal communities in soils under long-term fertilization remain poorly understood.

Understanding how communities of co-occurring microorganisms are regulated is a major goal of microbial ecology (Dini-Andreote et al, 2015; Shi et al, 2018). Two

dominant themes permeate the study of ecological community dynamics: (1) stochastic processes mainly caused by dispersal or random changes in the community (Hubbell and Borda-De-Agua, 2004), and (2) deterministic processes, mainly driven by abiotic or biotic factors, or both (Tokeshi, 1990). The key issue is the quantification of the relative importance of the two processes in controlling community processes (Hubbell, 2001). Data obtained from microbiological community dynamics could facilitate the understanding of their functions in different ecosystems. Although the processes that govern the microbial community processes remain poorly understood, according to Vellend's (2010) conceptual synthesis, ecological selection (referring to environmental conditions as deterministic processes), organismal dispersal, ecological drift and speciation (referring to stochastic processes) influence ecological communities greatly. In that respect, research to date has disentangled microbial community regulation into five plausible scenarios (Stegen et al., 2015): variable selection (VS), wherein the selective environment is highly spatially heterogeneous (Dini-Andreote et al., 2015; Stegen et al., 2015; Vellend, 2010); homogeneous selection (HS), more prevalent in spatially homogeneous environment; homogeneous dispersal (HD), arising from high rates of dispersal between the communities; dispersal limitation (DL) that is caused by spatial isolation or ecological drift (Whitaker et al, 2003; Zhou et al, 2008); and finally, the

undominated (UD) cases, in which no single process governs local community dynamics (Dini-Andreote et al, 2015; Stegen et al, 2015; Feng et al, 2018b). Recently, for example, Feng and colleagues investigated the relative role of these five processes for shaping the soil bacterial community in regional-scale nutrient and organic matter addition experiments (Feng et al, 2018b). The work offered insights on how to adjust the degrees of change in environmental variables that could influence microbial community assembly. Therefore, investigating the processes controlling soil fungal communities could broaden our understanding of microbial ecology, as so far done in various habitats (Verbruggen et al, 2012; Nemergut et al, 2013; Smith et al, 2018). For example, Dumbrell et al. (2010) evaluated the relative contributions of niche and neutral processes in regulating the arbuscular mycorrhizal fungal community along a soil pH gradient. The characterization of the variance in fungal communities from adjacent fruit, soil and bark material in New Zealand (Morrison-Whittle and Goddard, 2015) revealed the microenvironment and helped to explain at least four times more of the community variation compared to the variation explained by spatial distance. However, the studies predominantly focused on fungal dynamics based on the stochastic versus deterministic processes perspective, which could be extremely broad. The five key approaches of assessing community-level dynamics above; therefore, could facilitate a better understanding of the functional role of fungi in

natural and semi-natural ecosystems.

Due to its vital role in soil nutrient cycling and sustainable crop productivity (van der Heijden et al, 1998; van der Putten & Wim, 2017), disentangling the process underpinning microbial community assembly is an urgent task. Here, a red soil known for its low productivity and widely distribution in the south of China (Xun et al, 2016b) was selected for study; the prevalent management approach for promoting its productivity is through chemical and organic fertilization, and leaving the land fallow (Xun et al, 2016a). In this way, long-term fertilization was found to strongly influence the communities of soil prokaryotes (Wang et al, 2017), but its influence on soil fungal communities remains unclear. Using the Illumina MiSeq platform, we surveyed 18S rRNA genes of 36 soil core samples at the Red Soil Experimental Station of the Chinese Academy of Agricultural Sciences. Previous studies have reported dramatic changes in soil characteristics such as soil pH, soil organic matter (SOM), and total nitrogen following 28 years of differential fertilization management (Xun et al, 2016a). Therefore, in the present study, we hypothesized that the relative importance of the five processes for the community assembly of soil fungi depended on the fertilization strategy employed.

Method and Materials

Site description

The long-term fertilization experiment was established in 1990 at the Red Soil Experimental Station of the Chinese Academy of Agriculture Sciences, Qiyang, Hunan Province (111°53'E, 26°45'N). The soil type is Ferralic Cambisol, developed from Quaternary red clay. The experimental site consists of 12 different fertilization treatments, one plot for each treatment (20 m × 10 m, Figure S1): (i) control (CK); no fertilizer application; (ii) chemical nitrogen (N); (iii) a nitrogen-phosphorus combination chemical fertilizer (NP); (iv) a nitrogen-potassium combination chemical fertilizer (NK); (v) a phosphorus-potassium combination chemical fertilizer (PK); (vi) a balanced nitrogen-potassium-phosphorus combination chemical fertilizer (NPK); (vii) a combination of swine manure and chemical nitrogen-phosphorus-potassium fertilizer (NPKM); (viii) a combination of 1.5-times swine manure and chemical nitrogen-phosphorus-potassium fertilizer (1.5NPKM); (ix) a combination of straw and nitrogen-phosphorus-potassium chemical fertilizer (NPKS); and, (x) a combination of swine manure and chemical nitrogen-phosphorus-potassium fertilizer with crop rotation (NPKMR); (xi) swine manure (M); and (xii) fallow.

Soil sampling

To survey the soil fungal communities, we collected a total of 36 soil samples from the 12 treatments (Figure S1) on June 13, 2018. Within each treatment (10 m × 5 m),

we sampled three plots (1 m ×1 m) ca. 5 m apart from which we collected five soil cores per plot to a depth of 0–15 cm; the latter were then combined to form one composite sample and each was stored in an icebox. All soil samples were transported in a cooler with ice (4°C) to the laboratory, where they were sieved through a 2-mm mesh and divided into two subsamples: ca. 60% was stored at 4°C for the physical and chemical analyses, while the other 40% was stored at -20°C for their DNA extractions.

Soil biogeochemical analysis

Soil pH was quantified with a fresh soil to water ratio of 1:5 using a pH meter (Thermo Orion-868, Waltham, MA, USA). Total soil nitrogen content was determined by combustion (2400 II CHNS/0 Elemental l Analyzer, Perkin-Elmer, Boston, USA). After digestion using hydrofluoric acid (HF) and perchloric acid ($HClO_4$) digestion, total potassium (TK) and total phosphorus (TP) were determined using flame photometry (FP640, INASA, Shanghai, China) and the molybdenum blue method, respectively. Using 1 M ammonium acetate extracts, available potassium (AK) was also determined by flame photometry. The available phosphorus (AP) was extracted using 0.5 M sodium bicarbonate ($NaHCO_3$) and quantified following the molybdenum blue method. To measure organic matter, the potassium dichromate oxidation titration

method was used. The respective total contents of iron (Fe), magnesium (Mg), calcium (Ca) and aluminum (Al) in soil were measured using an inductively coupled plasma atomic spectrometer with an (ICP-AES Optima 8000, Perkin-Elmer, Waltham, MA, USA), while a high-performance liquid chromatography inductively coupled plasma mass spectrometer (HPLC-ICP-MS (7700X, Agilent, Waltham, MA, USA) was used to measure chromium(Cr), nickel (Ni), copper (Cu), zinc (Zn), molybdenum (Mo), cadmium (Cd), lead (Pb) and manganese (Mn) contents.

DNA extraction

To extract the total DNA from soils, a Power Soil DNA kit was used (MO BIO, Carlsbad, USA) followed by Nano Drop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) to quantify the DNA concentrations of samples. The extracted DNA was diluted to approximately 25 ng/µl with distilled water and stored at -20°C until use. 18S rRNA genes (fungi-specific primers) were amplified using a primer set (817F, 5'-TTAGCATGGAATAATRRAATAGGA-3'; 1196R, 5'-TCTGGACCTGGTGAGTTTCC-3') that was combined with adapter sequences and barcode sequences (Borneman et al, 2000). PCR amplifications were performed under the following conditions: 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and an extension at 72°C for 30 s, with a final extension made at

72°C for 10 min; each sample was amplified in triplicate in a 50-µl reaction. Then, the PCR products from each sample were pooled together and purified using a PCR purification kit (Qiagen, Hilden, Germany), and their respective concentrations were measured using a Nano-Drop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Finally, all PCR products were combined together in equimolar amounts and run on the Illumina MiSeq platform (Manufacturer, City, State, Country) (Caporaso et al, 2012).

Data analysis

Using QIIME v1.9.1 (Caporaso et al, 2010), raw data reads were processed and analyzed following the workflow at http://nbvieweripythonorg/github/biocore/qiime/blob/191/examples/ipynb/illumina_overview_tutorialipynb (Caporaso et al., 2011). Briefly, sequences were qualified (max value of 0.5) and clustered according to a minima 97% shared identity. The taxonomy of each phylotype was identified using the Silva132 release database (Quast et al, 2013; Yilmaz et al, 2014). Finally, we obtained between 25,650 and 47846 high-quality eukaryotic sequences per sample, and 98.5% were classified into 22,577 distinct OTUs. To rarify all data sets for each sample to the same extent, 25,000 sequences were randomly selected.

Statistical analysis

To quantify phylogenetic diversity, the Faith index was calculated (Faith, 1992). Both the Shannon (Shannon, 1948) and Chao1 (Chao and Shen, 2003) indices were also calculated at the OTU level using QIIME v1.9.1. Observed species were based on the number of phylotypes encountered. Correlations between each diversity index and soil factors, box plots and significance testing were all conducted in IBM SPSS Statistics 20 (IBM Corp., Armonk, NY, USA). Based on the OTU_25,000 table, sample dissimilarities were calculated using the Bray-Curtis dissimilarity metric (Bray and Curtis, 1957). Based on the dissimilarity matrix, nonmetric multidimensional scaling (NMDS) ordinations were generated using the vegan tool in R (R Development Core Team). Mantel tests were applied to the Bray-Curtis distance for dissimilarity, ß-nearest taxon index (ß-NTI) and soil variables using the 'vegan' package (Oskansen et al., 2019) in the R platform (R Development Core Team). Bray Curtis can reflect the community composition (the presence/absence and abundance data) differences between sites, while beta-NTI can quantify phylogenetic turnover in community composition between sites and phylogenetic distances. To evaluate the cumulative effect of each soil factor on fungal community composition, distance-based multivariate analysis for a linear model using forward selection (DISTLM) was applied based on the OTU_25,000 table and soil factors (Anderson et

al. 2004).

Process analysis

To quantify the community assembly processes, the combined method of ß-NTI and Bray-Curtis-based Raup-Crick metrics (RC_bray) was used, according to Stegen et al (Stegen et al, 2013; Stegen et al, 2015). The ß-NTI measures the deviation of the ß-mean nearest taxon distance (β-MNTD) from the β-MNTD of the null model, and this was calculated in *Phylocom* v42 (Webb et al, 2008). Before calculating ß-NTI, a phylogenetic signal analysis was first required, since the traits regulating the processes of community assembly should be phylogenetically conserved (Stegen et al, 2012). To test for such a phylogenetic signal, the relationship between phylogenetic distance of pairwise OTUs and their corresponding environmental differences was evaluated using 'mantelcorrelog' (Stegen et al, 2012), for which the 'cophenetic' function in the 'picante' package (R 314) was used to calculate the phylogenetic distances. The Euclidean distance of each soil variable of pairwise OTUs was calculated, and then the abundance-weighted mean value obtained for each environment. If a significant relationship occurs over a very short phylogenetic distance, this would suggest the phylogenetic signal is also statistically significant. With respect to the data set of the present study, values of | ß-NTI | > 2 indicate a

community that is dominated by deterministic processes (Stegen et al, 2012). The definitions of variable selection (VS) and homogeneous selection (HS) can be found in Table S1. Conversely, when | ß-NTI | < 2, it indicates that stochastic processes are operating in the community, in the form of dispersal limitation (DL), homogenous dispersal (HD) and undominated process (UD) (Stegen et al, 2015; Feng et al, 2018b; Tripathi et al, 2018). Table S1 describes how to distinguish the three scenarios. In the present study, the fungal OTUs with relative higher abundances (i.e., > 0001%) were selected (5000 OTU_S in our study) to calculate the ß-NTI and RC_{bray} values according to Shi et al (2018) (also see Feng et al, 2018b).

Results

Fungal communities in red soil

Using the Illumina next generation sequencing platform, we obtained 1,438,809 quality sequences from the 36 soil samples of a long-term fertilization experiment of these, 22,577 were identified at 97% similarity, being mostly fungi (~ 95%), followed by other microbial eukaryotes such as SAR (3.5%) and Amoebozoa (0.30%)—as operational taxonomic units (OTUs) (Table S2). At the phyla/class level, the Ascomycota, Basidiomycota, Glomeromycotina, Mucoromycotina, and Mortierellomycotina were dominant, accounting for more than 90% of all sequences

(Figure 1, Table S2). The Ascomycota was the most commonly found phylum among the treatments, occurring at higher abundance in the manure treatments soils (**Figure S5**). Basidiomycota, however, was found at higher abundance in the control and chemical fertilizer treatments **(Figure S5)**. Glomeromycotina was most abundant in the CK, N, NK, and PK treatments (**Figure S5,** Table S2).

Fungal alpha diversity in the red soil

The Shannon index and phylogenetic diversity revealed similar patterns across the 12 treatments, with higher values obtained in CK, PK, NPKS, and F and lower corresponding values in NP, NK, NPKM and 1.5NPKM (Figure S2). Upon closer examination, the diversity-deficient chemical fertilizer treatments of N, NP, and NK had lower index values than that found for PK, while the four diversity indices in NPK and NPKS were also lower when compared with those of PK. However, the diversity indices in the manure treatments had values similar to those in chemical fertilizer treatments, with CK, fallow, and PK treatments displaying the highest values.

To uncover which factors influenced the alpha diversity indices, correlations were performed. Among the soil variables, TK showed significantly positive relationships with phylogenetic diversity and the Shannon index, while OM was negatively

correlated with the two indices above. (Table S3). In particular, for most of the soil factors, such as TN, TP, OM, and AP, were negatively correlated with Shannon values (Table S3, Figure S3).

Soil variables' effects on fungal communities across the treatments

Non-metric multi-dimensional scaling plots (NMDS) revealed that comparisons of soil fungal communities could be distinctly separated by the fertilization treatments based on the Bray-Curtis distance metric (Figure 2A). In particular, those treatments that included manure fertilizer tended to cluster together. The ordination plot clear indicates that soil fungal communities at the study site were distributed along the soil Ca gradient. Yet when considering the variation in phylogenetic community composition (ß-NTI), these scatter plots did not cluster according to treatment and were more inclined to distribute along the OM gradient (Figure 2B). Importantly, the above effects of soil Ca and OM upon the soil fungal community composition and ß-NTI were corroborated by the results of the Mantel tests (Table 1), and the multivariate analysis indicated that, among all variables, it was soil Ca that contributed most (26%) to shaping the soil fungal community (Table 2). Apart from these factors, a few other variables, namely TN, TP, pH and some micronutrients, also showed significant associations with the soil fungal community dissimilarity.

Relative role of the five processes in soil fungal community assembly

Bray Curtis values differed significantly among the 12 fertilizer treatments. (Figure S4A). For example, Bray Curtis values were three-fold lower in the NPKM treatment compared to CK treatment. Afterwards, to determine which processes predominated in the soil fungal community in each treatment, ß-NTI values were calculated and found to be higher in CK, N, NPKMR, and F than in the other treatments (Figure S4B). The results indicated that only in CK did deterministic processes prevail, with VS accounting for 66.67% of the community-level variation (Figure 3; Table S4). In contrast, in all the other 11 treatments stochastic processes were more prominent, namely HD and UD (Figure 3). The results suggested that long-term fertilization could alter the processes that control soil fungal community assembly.

Discussion

Long-term fertilization can alter soil fungal assembly processes

Our study provides evidence that long-term fertilization could alter the processes underpinning soil fungal community assembly. Whether it is chemical only, or a combination of chemical and manure fertilizers, the communities in those treated soils

all featured stochastic processes as most prominent. Although variable selection dominated the treatment without fertilizer, it reflected the strong effect of environmental selection. Treatments containing manure fertilizer were all governed by homogeneous dispersal, while both homogeneous dispersal and undominated processes prevailed in the chemical fertilizer treatments. Additionally, our results revealed the considerable influence of soil organic matter (OM) on the eukaryotic microbial community process. In addition, key soil factors such as Ca and TN were significantly correlated with community composition. Therefore, our results highlight the strong effects of long-term fertilization on soil fungal community structure and assembly.

Usually, homogeneous dispersal is observed at rather small scales with higher community similarity (Stegen et al. 2015). Under the HD process, the effect of environmental selection was weak and higher microbial exchange rates were observed (Caruso et al. 2011). In the present study, the addition of manure could enrich soil nutrients and minimize the effect of the variable selection, which could be a reason why the HD process was predominant in the manure-adding treatment. Undominated dispersal reflects a moderate rate of dispersal, which means a very weak selection effect. High dispersal would lead to homogenous dispersal and low dispersal would

result in dispersal limitation (Stegen et al. 2015). Therefore, under the undominated process scenario, neither dispersal nor selection could cause the differences between the communities. In the present study, treatment with chemical fertilizer treatment drove the soil fungal community assembly toward HD and UD processes. The results suggested different effects of chemical fertilizer, and chemical fertilizer combined with manure on the soil fungal community assembly. Both management approaches could weaken the variable selection effect, while the chemical fertilizer approach only also moderated the rate of dispersal (decreased the microbial turnover rate).

The influence of soil organic matter on fungal phylogenetic community composition assemblages.

When disentangling the mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession, other researchers found that ß-NTI values decreased with the stage of succession in year but increased with SOM (Dini-Andreote et al, 2015), which suggested that shifts in SOM could lead to the modification of assembly processes. In the present study, we found that SOM influenced soil fungal community assembly at our site greatly (Table 1). High soil OM content in manure fertilizer treatments had low ß-NTI values, while chemical fertilizer treatments which had low SOM concentrations had high ß-NTI values

(Figure S4 and Table S5), which may explain why we observed more HD processes in the manure fertilizer treatments than in the chemical fertilizer treatments in the farmed red soils in the present study. The results indicated that soil fungal community assembly processes might be mediated by shifts in SOM content.

Soil Ca is a deterministic factor influencing trends in soil fungal community composition.

In the present study, soil Ca shaped soil fungal community composition at the local scale in the plots. In Chang Bai mountain soil, the patterns of soil fungal community were closely correlated with soil pH (Shen et al, 2014). Usually, for bacterial communities, soil pH, C:N ratio, and soil moisture are frequently reported as the key factors shaping the community structure (Fierer, 2017; Delgado-Baquerizo et al, 2018). Here, in our survey work, we observed strong effects of soil Ca on soil fungal communities. In North China Plain soils, Shi et al. (2018) observed that soil bacterial community was significantly correlated with soil Ca. In addition, soil pH has been reported to be a key factor driving the soil bacterial community composition in various habitats (Delgado-Baquerizo et al. 2018). Furthermore, soil pH could be greatly influenced by the soil Ca greatly. Our study also found a strong correlation between soil pH and Ca. Few studies have examined the relationship between soil

microbial community and environmental factors (Shi et al. 2018). Therefore, the influence of soil Ca on soil microbial communities could be overlooked. Our findings suggest that more soil characteristics should be tested when investigating the influence of environmental factors on soil microbial diversity and community assembly.

Conclusions

In conclusion, long-term chemical fertilization resulted in homogeneous dispersal (HD) and undominated (UD) processes and a combination of chemical fertilizer and manure mainly fostered HD processes. Our results highlight the role of fertilization in weakening the variable selection effect, and the varying impacts of chemical and manure fertilizer on fungal community assembly. In addition, soil Ca played an important role in the shaping of soil fungal communities while soil organic matter strongly influenced the soil phylogenetic community composition in agricultural red soil. Our results provide novel insights that could facilitate the better understanding of the dynamics driving soil fungal community assembly in agricultural systems.

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Data availability statement

All data is available. The soil fungal dataset has been deposited in the European Nucleotide Archive under accession number: PRJEB34565.

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Table 1 Mantel test results for relationships between soil eukaryotic Bray_Curtis distance, βNTI and soil factors.

Mantel Test	Bray_Curtis		BetaNTI	
	r	p	r	p
Cr	0.00	0.513	-0.02	0.581
Ni	**0.18**	0.001	0.19	0.011
Cu	**0.57**	0.001	**0.20**	0.002
Zn	**0.58**	0.001	**0.21**	0.002
Mo	**0.12**	0.019	0.04	0.295
Cd	**0.42**	0.001	0.11	0.088
Pb	**0.22**	0.001	-0.02	0.559
Fe	**0.16**	0.002	0.01	0.423
Mn	**0.64**	0.001	**0.17**	0.001
Mg	**0.19**	0.001	-0.01	0.496
Ca	**0.72**	0.001	**0.15**	0.004
Al	0.05	0.166	-0.05	0.731
pH	**0.57**	0.001	**0.10**	0.027
TN	**0.72**	0.001	**0.22**	0.001
TP	**0.62**	0.001	**0.14**	0.014
TK	**0.22**	0.001	0.00	0.457
OM	**0.60**	0.001	**0.23**	0.001
AP	**0.46**	0.001	**0.18**	0.009
AK	**0.34**	0.001	-0.01	0.512

Abbreviations of variables: OM, organic matter (g/kg); TN, total nitrogen (g/kg); TP, total phosphorous (g/kg); TK, total potassium (g/kg); AP, available phosphorous (mg/kg); AK, available potassium (mg/kg); Al, aluminum (mg/g); Mg, magnesium (mg/g); Ca, calcium (mg/g); Fe, iron (mg/g); Mn, manganese (mg/g); Cd, cadmium (ug/g); Zn, zinc (ug/g); Pb, plumbum (ug/g); and Cu, copper (ug/g).

Table 2 Distance-based multivariate analysis of the effects of soil biogeochemical properties on the eukaryotic communities across the all soils.

Variable	SS(Trace)	pseudo-F	P	Proportion	Cumulative
Ca	19266.86	11.84	0.001	**0.26**	0.26
Cu	6683.29	4.53	0.001	**0.09**	0.35
TN	5770.67	4.31	0.001	**0.08**	0.43
TP	3088.37	2.41	0.001	**0.04**	0.47
TK	3501.34	2.89	0.001	**0.05**	0.51
AK	2556.67	2.20	0.001	**0.03**	0.55
Mn	2109.16	1.87	0.001	**0.03**	0.58
pH	1782.72	1.61	0.001	**0.02**	0.60
Fe	1756.45	1.63	0.001	**0.02**	0.62
Mo	1292.31	1.21	0.115	0.02	0.64
Zn	1248.12	1.17	0.164	0.02	0.66
AP	1231.81	1.17	0.194	0.02	0.67
Cd	1367.93	1.31	0.058	0.02	0.69
Pb	1230.93	1.19	0.185	0.02	0.71
OM	1132.05	1.10	0.347	0.02	0.72
Al	1074.79	1.05	0.423	0.01	0.74
Ni	1073.78	1.05	0.438	0.01	0.75
Mg	1029.21	1.01	0.515	0.01	0.77
Cr	940.61	0.91	0.646	0.01	0.78

Abbreviations of variables: OM, organic matter (g/kg); TN, total nitrogen (g/kg); TP, total phosphorous (g/kg); TK, total potassium (g/kg); AP, available phosphorous (mg/kg); AK, available potassium (mg/kg); Al, aluminum (mg/g); Mg, magnesium (mg/g); Ca, calcium (mg/g); Fe, iron (mg/g); Mn, manganese (mg/g); Cd, cadmium (ug/g); Zn, zinc (ug/g); Pb, plumbum (ug/g); and Cu, copper (ug/g).

SS indicates sum of squares

Pseudo-F indicates pseudo F statistic

Proportion indicates proportion of community variance that the variable accounts for

cumulative indicates the cumulative effect of each soil factor on fungal community composition.

FIGURE CAPTIONS

Figure 1 Relative abundances of the eukaryotic soil microbial groups and dominant fungal phyla in soils grouped by fertilization treatment. For abbreviations: CK: control N: chemical nitrogen, NP: a nitrogen-phosphorus combination chemical fertilizer, NK: a nitrogen-potassium combination chemical fertilizer, PK: a phosphorus-potassium combination chemical fertilizer, NPK: a balanced nitrogen-potassium-phosphorus combination chemical fertilizer, NPKM: a combination of swine manure and chemical nitrogen-phosphorus-potassium fertilizer, 1.5NPKM: a combination of 1.5 times swine manure and chemical nitrogen-phosphorus-potassium fertilizer, NPKS: a combination of straw and nitrogen-phosphorus-potassium chemical fertilizer, NPKMR: a combination of swine manure and chemical nitrogen-phosphorus-potassium fertilizer with crop rotation, M: swine manure and F: fallow.

Figure 2 Fungal community compositional structure in soils as indicated by non-metric multi-dimensional (NMDS) scaling plots of the Bray-Curtis distance (A) and ß-NTI between samples (B). Samples are colour-coded according to the soil Ca and OM gradients. For abbreviations see Fig 1 OM: organic matter.

Figure 3 The relative contributions (%) of the five community assembly processes. For abbreviations see Fig 1. HS means homogeneous selection, DL means dispersal

limitation, HD means homogenizing dispersal, UD means Undominated process, VS means variable selection.

Figure S1 (A) Location of the site. (B) Aerial view of the experiment site. (C) The soil-sampled quadrat sets.

Figure S2 Alpha diversity indices for sequences rarefied to 25 000 sequences per sample, displayed as boxplots showing the median by treatment. Boxplots with same letter indicate no significance between the treatments, as detected by Duncan's test.

Figure S3 Relationships between soil fungal Shannon index and soil TN (total nitrogen), TP (total phosphorus), OM (organic matter), and AP (available phosphorus).

Figure S4 Soil eukaryotic Bray_Curtis and BetaNTI displayed as boxplots showing the median by treatment. Boxplots with same letter indicate no significance between the treatments, as detected by Duncan's test.

Figure S5 Dominate soil eukaryotic groups and fungal phyla or class displayed as boxplots showing the median by treatment. Boxplots with same letter indicate no significance between the treatments, as detected by Duncan's test.

Table S1 The values to distinguish the five scenarios.

Table S2 Relative abundance of the eukaryotic soil microbial groups and fungal phyla in soils grouped by treatment.

Table S3 Relationships between soil eukaryotic alpha diversity indices and soil factors. Values in bold indicate those with statistical significance. For abbreviations, please see Table 1. Relationships were calculated based on Pearson.

Table S4 The relative contribution (%) of five scenarios in each fertilization treatment.

Table S5 Soil physiochemical characteristics among all sampling sites.