



Multiple long-term observations reveal a strategy for soil pH-dependent fertilization and fungal communities in support of agricultural production



Qi Ning^{a,b,1}, Lin Chen^{a,1}, Zhongjun Jia^{a,b}, Congzhi Zhang^a, Donghao Ma^a, Fang Li^c, Jiabao Zhang^{a,b,*}, Daming Li^d, Xiaori Han^e, Zejiang Cai^f, Shaomin Huang^g, Wenzhao Liu^h, Bo Zhuⁱ, Yan Li^j

^a Fengqiu Experimental Station of National Ecosystem Research Network of China, State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

^b University of Chinese Academy of Sciences, Beijing 100049, China

^c College of Resources and Environment, Henan Agricultural University, Zhengzhou 450002, China

^d Jiangxi Institute of Red Soil, Jinxian 331717, China

^e College of Land and Environment, Shenyang Agricultural University, Shenyang 110866, China

^f Qiyang Agro-ecosystem of National Field Experimental Station, Chinese Academy of Agricultural Sciences, Qiyang 426182, China

^g Institute of Plant Nutrition and Environmental Resources Science, Henan Academy of Agricultural Sciences, Zhengzhou 450002, China

^h Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Yangling 712100, China

ⁱ Institute of Mountain Hazards and Environment, Chinese Academy of Sciences, Chengdu 610041, China

^j Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China

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ABSTRACT

Agricultural fertilization plays a crucial role in crop production, and the fungal communities catalyze transformation of soil nutrients in support of crop production. However, it remains controversial about the optimal strategy for fertilizer inputs and the adaptive mechanisms of fungal communities across China. By using seven long-term field fertilization experiments in China, we analyzed crop yields, soil properties and fungal communities in soils that were treated for > 25 years with no fertilizer (control), inorganic fertilizers (NPK) and organic-inorganic fertilizers (NPKM). Long-term NPK resulted in significant acidification up to a decline by 1.20 pH units, while NPKM prevented acidification and increased pH up to 6.39 in three acidic soils with pH < 5.70. NPKM increased crop yields by 1.19–8.72 folds in acidic soils, being significantly higher than NPK. Specific saprotroph *Mortierella* and *Pseudaleuria* in acidic soils were exclusively enriched by NPKM. Soil pH was directly related to the abundance of *Mortierella*, and the enrichment of *Mortierella* species further caused a positive direct effect on crop yield. In four alkaline soils with pH > 8.11, both NPK and NPKM led to only marginal decline of soil pH, and NPK and NPKM showed comparable crop yields. Some members of Ascomycota in alkaline soils were both enriched by NPKM and NPK. Soil available P and C:N ratio, rather than pH, directly or indirectly affect crop yield in alkaline soils. High crop yield can be achieved by the sole use of inorganic fertilizers in alkaline soils, but acidic soil productivity should be maintained by organic amendment to counteract acidification by inorganic fertilization. Our study advances a mechanistic understanding for optimizing fertilization strategies towards sustainable agriculture under increasingly intensified fertilizer inputs.

1. Introduction

The discovery of the Harber-Bosch process for chemical nitrogen (N) synthesis has revolutionized agriculture and has enabled global population to grow from 1.6 billion in 1900 to 7.5 billion today (Smil, 1999).

It is estimated that synthetic inorganic fertilizers have increased crop production by more than 55 % (Li et al., 2009), which is equivalent to approximately 1.57 billion tons of grain yield in 2016 (FAO, 2016). As global population increases to 8.5–10 billion by 2050, the application of inorganic N and phosphorus (P) fertilizers will increase by at least 50 %

* Corresponding author at: State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, 210008, China.

E-mail address: jbzhang@issas.ac.cn (J. Zhang).

¹ These authors contributed equally to this work.

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to guarantee food security (Springmann et al., 2018). However, excessive application of inorganic fertilizers results in severe environmental problems including soil acidification (Guo et al., 2010), loss of belowground biodiversity (Zhou et al., 2016), and eutrophication in waterbodies (Tilman et al., 2002). These factors suggest current rates of inorganic fertilizer application may not be sustainable (Tilman et al., 2002). But these observations represent a general trend derived from large-scale extensive surveys. The site-specific response to intensified inorganic fertilization has received little attention, and whether China should substitute some or all of the inorganic fertilizer application with manure, an organic fertilizer which is often considered green and environmentally friendly, remains a contentious topic (Liu et al., 2010; Miao et al., 2011).

The primary concern for the overuse of inorganic fertilizers is soil acidification due to proton release from oxidation of ammonium-based fertilizers (Chadwick and Chorover, 2001; Guo et al., 2010). Previous studies have found that the combination of inorganic-organic fertilizers can effectively alleviate soil acidification, prevent soil nutrient depletion, increase microbial diversity, stimulate the growth of specific keystone species and, therefore, enhance agro-ecosystem sustainability (Miao et al., 2011; Francioli et al., 2016; Wei et al., 2016). Organic manure could significantly constrain soil acidification by the protonation of organic anions from soil organic matter (SOM) and the subsequent release of effective exchangeable base cations (Shi et al., 2019). Our previous study in an alkaline soil showed that 23 years of inorganic fertilization increased agricultural production substantially, and improved soil nutrient status and physical structure, with a small decline in pH (Xin et al., 2016). It may be that large amounts of base cations played an important role in buffering against soil acidification that was induced by inorganic N fertilizer application (Tian and Niu, 2015). Recently, a nationwide survey with 4060 soil samples across China indicated that soil organic carbon (SOC) increased by about 15 % during 1980–2011, and SOC stocks were positively correlated with N fertilizer inputs (Zhao et al., 2018). These findings indicate that in some case the sole use of inorganic fertilizers can maintain both productivity and sustainability of agro-ecosystems in the long run. We still lack sufficient understanding of the relationships between fertilization regimes and agricultural production. The influence of soil acidification by inorganic fertilization on agricultural production often occurs in acidic soils (Zhang et al., 2009; Guo et al., 2010; Schroder et al., 2011), but it remains uncertain whether fertilization-induced soil acidification occurs in alkaline soils.

Soil microorganisms are mainly decomposers in agro-ecosystems, but the role that fungi play in this process is distinct from that played by bacteria (Meidute et al., 2008). Compared to bacteria, fungi have a wider range in C:N and growth optimum pH value (Strickland and Rousk, 2010). The greater importance of soil fungi is their ability in decomposing recalcitrant (e.g. lignin) and easily degradable (e.g. cellulose and hemicellulose) organic materials, whereas bacteria mainly degrade labile fraction (de Boer et al., 2005; Strickland and Rousk, 2010; Chen et al., 2014). In addition, fungal hyphae involved in nutrient acquisition, and transport nutrients from organics to soils, which play vital roles in nutrient cycling (Frey et al., 2003; Chen et al., 2014; Borrell et al., 2017). Soil acidification probably affects the transformation and availability of nutrients by changing the composition of the soil fungal community which indirectly affects agricultural production (Kemmitt et al., 2006). However, little information exists regarding the responses of fungal communities in acidic and alkaline soils to organic and inorganic fertilization. It is important to create stable soil microbial communities through long-term field managements because soil microbes take a long period to respond to the changes of habitat conditions (Lentendu et al., 2014). Earlier studies have reported the significance of long-term fertilization regimes for assessing the soil microbiome (Borjesson et al., 2012; Lentendu et al., 2014; Francioli et al., 2016; Chen et al., 2019), but these researches did not provide systematic evaluation on the relationships among fertilization regimes,

fungal communities and agricultural production based on multiple long-term observations.

In the present study, we selected seven representative cultivated upland soils from long-term field fertilization experiments which have been treated for more than 25 years with organic and inorganic fertilizers in major grain-producing areas across China. We categorized these soils into acidic and alkaline groups, analyzed the variations in soil properties and crop yields, and compared the soil fungal communities using amplicon sequencing of fungal internal transcribed spacer (ITS) genes. We hypothesized that: (i) long-term sole use of inorganic fertilizers can maintain both productivity and sustainability of alkaline soils; (ii) distinct fertilization sensitive fungal taxa inhabit in acidic and alkaline soils, and play critical roles in maintaining soil productivity. Our study advances the mechanistic understanding for optimizing fertilization strategies and improving the assembly of fungal communities in support of agricultural sustainability.

2. Materials and methods

2.1. Experiment description and soil sampling

Seven long-term fertilization field experiments (located in Jinxian, Qiyang, Shenyang, Zhengzhou, Yanting, Changwu, and Fukang) were established in the 1980s in China. These sites represented the most important grain-producing areas in China, and only field treatments with more than 25 years were selected for analysis in this study (Fig. 1). Annual mean temperature was 6.6–18.1 °C, and annual mean rainfall was 160–1417 mm, with the minimum and maximum temperature and precipitation in Fukang and Qiyang, respectively. The soils are classified as Haplustoll, Hapludult, Entisol, Paleudalf, and Inceptisol in the USA soil taxonomy. Cropping systems at these sites included maize monoculture, wheat monoculture, maize-wheat rotation, and maize-soybean rotation. More detailed information on the amounts of fertilizers at all field sites is available in the supporting information (Table S1). We selected three fertilization treatments at each site: unfertilized control, inorganic fertilization (NPK) and combined organic-inorganic fertilization (NPKM). Each treatment included three replicated rectangular plots in a randomized block design. Soil samples were collected after crop harvested in 2015. In each plot, ten cores (diameter 5 cm, depth 20 cm) of bulk soils were collected randomly and mixed as one sample. Soils passed a 2-mm sieve and were homogenized. One part of soil samples was air-dried to measure basal soil properties, and the other was frozen at –20 °C for molecular analyses.

2.2. Measurement of basal soil properties

Soil pH was determined in a soil:distilled water mixture (1:2.5; CO₂ removed) with a pH probe (METTLER-TOLEDO, Zurich, Switzerland). SOC was measured by titrimetry after dichromate oxidation. Total N was digested with a catalyst and H₂SO₄ and analyzed by Kjeldahl. Total P was determined by molybdenum-blue colorimetry. Total potassium (K) was measured by flame photometry after soil digestion with NaOH melt-flame photometry. Available P was determined by sodium bicarbonate extraction-colorimetry. Available K was determined by ammonium acetate extraction-flame photometry.

2.3. ITS gene amplification and sequencing

Soil DNA was extracted using a FastDNA Spin Kit (MP Biomedicals, Santa Ana, CA, USA) as instructed, and then dissolved in 50 µl Tris-EDTA buffer. DNA concentration was measured using a spectrophotometer, and DNA integrity was verified on 1 % agarose gel. The fungal ITS1 region was amplified using the primer pair ITS1F and ITS2 (Ghannoum et al., 2010). Each reaction mix consists of 1 µl (30 ng) of DNA, 4 µl (1 µM) of each the forward/reverse primer, 25 µl of the PCR Master Mix, and 16 µl of ddH₂O. The PCR amplification was conducted

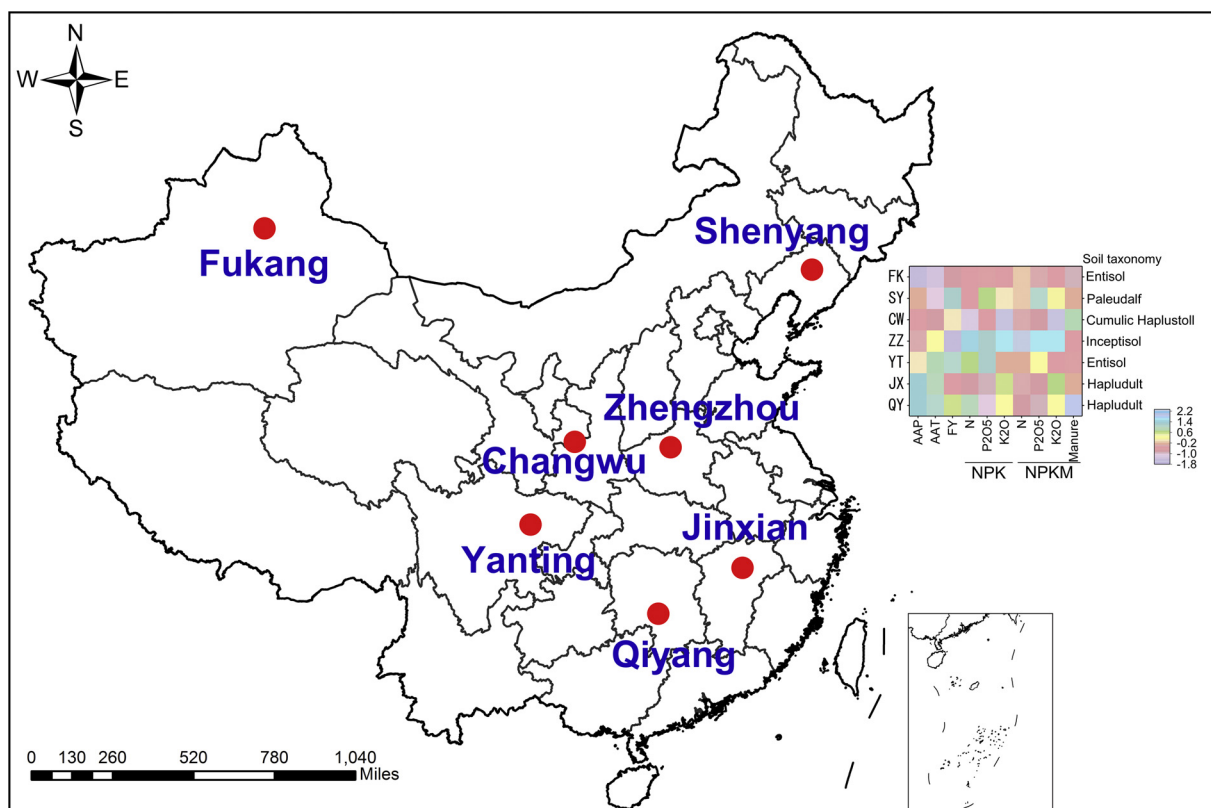


Fig. 1. The geographic location and agricultural management of seven long-term fertilization experimental sites across China. The heatmap shows the key aspects about agricultural regimes and meteorological conditions. The abbreviation at the left side refers to the site where long-term field fertilization was conducted including Jinxian (JX), Qiyang (QY), Shenyang (SY), Zhengzhou (ZZ), Yanting (YT), Changwu (CW), and Fukang (FK). The field fertilization treatments were conducted in replicated plots that received no fertilizer (control), inorganic fertilizer (NPK), and inorganic fertilizer plus organic manure (NPKM). The meteorological information contains average annual precipitation (AAP), average annual temperature (AAT), and fertilization years (FY).

at 98 °C for 3 min, then 30 cycles (98 °C for 45 s, 55 °C for 45 s, and 72 °C for 45 s), with a final extension at 72 °C for 7 min. The PCR products were purified using AMPure XPbeads (AGENCOURT), and the library was quantified by real-time quantitative PCR. The sequencing of paired-end 250 bp was conducted on an Illumina MiSeq instrument (Illumina, San Diego, CA, USA). The sequencing data were deposited in the NCBI Sequence Read Archive with accession number [SRP127749](https://www.ncbi.nlm.nih.gov/seqread/submit/).

The raw sequence data were processed using the Qualitative Insights into Microbial Ecology (QIIME) pipeline (Caporaso et al., 2010). Poor-quality sequences with length less than 200 bp and quality score less than 20 were discarded, the chimera was removed using UCHIME algorithm (Edgar et al., 2011). The remaining sequences were assigned to operational taxonomic units (OTUs) with a 97 % similarity threshold using UCLUST (Edgar, 2010). Taxonomy was assigned using the UCLUST consensus taxonomic assigner algorithm and the UNITE fungal database (Abarenkov et al., 2010). All singletons and no-fungal OTUs were removed and each sample was rarefied to 42,000 sequences for the fungal diversity analysis. The functional group (guild) of the OTUs was predicted using FUNGuild (<https://github.com/UMNFuN/FUNGuild>) (Nguyen et al., 2016).

2.4. Statistical analysis

The means of soil properties and crop yields between treatments at each site were compared using one-way ANOVA with least significant difference (LSD) test. Two-way ANOVA with site as random factor and fertilization as fixed factor was used to test the significance of different sites and fertilizer regimes on soil properties. One-way and two-way ANOVA were performed in SPSS 19.0. Non-metric multidimensional scaling (NMDS) analyses was performed based on Bray-Curtis distances

(Bray and Curtis, 1957). Canonical correspondence analysis (CCA) was used to visualize the associations between fungal communities and soil properties. Permutational multivariate analysis of variance (PERMANOVA) was calculated to test the significant effect of factors. Mantel test (permutation = 999) was conducted to analyze the correlations between soil properties and fungal community compositions. Differential abundance analysis (DAA) was performed to calculate the differential abundance of the OTUs under each fertilization treatment as compared with control. The *P* values were adjusted by false discovery rate using the Benjamini-Hochberg approach (Benjamini and Hochberg, 1995). “Enriched” OTUs were identified with \log_2 fold change > 1.0 and adjusted *P* value < 0.1 (Whitman et al., 2016; Li et al., 2017).

Structure equation modeling (SEM) was conducted to examine the direct or indirect effects of soil properties, fungal community characteristics and specific taxa on crop yields. Two separate models were built for acidic soils and alkaline soils. The fungal community composition was represented with the first scale scores of NMDS. All data were log-transformed, and fitted to the model by the maximum likelihood estimation method. The fitness of the model was evaluated via a non-significant chi-square test ($P > 0.05$), low χ^2/df (< 2), high goodness-of-fit index (GFI > 0.9), and low root square mean errors of approximation (RMSEA < 0.05) (Byrne, 2010; Grace et al., 2010; Jasey et al., 2013). SEM analysis was performed using AMOS 24.0 (AMOS IBM, USA).

3. Results

3.1. Effects of long-term fertilization on crop yields and soil properties

For the alkaline soils at Zhengzhou, Yanting, Changwu, and Fukang

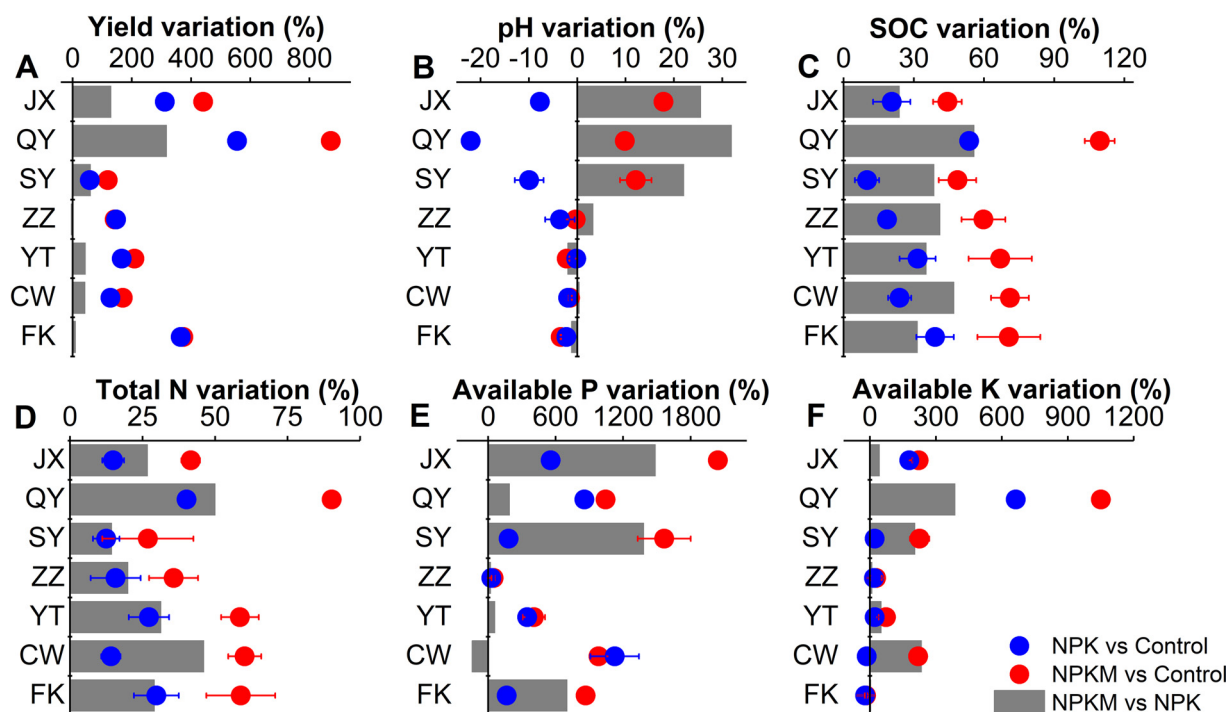


Fig. 2. Responses of soil properties and crop yield to inorganic fertilization (NPK) and combined organic-inorganic fertilization (NPKM) at Jinxian (JX), Qiyang (QY), Shenyang (SY), Zhengzhou (ZZ), Yanting (YT), Changwu (CW) and Fukang (FK). Panels (A), (B), (C), (D), (E) and (F) show the variations of crop yield, soil pH, soil organic C (SOC), total N, available P and available K under unfertilized control, NPK and NPKM fertilization at seven sites, respectively. NPK or NPKM vs Control represents the net effect of NPK or NPKM relative to unfertilized control, which is calculated as (NPK - Control)/Control or (NPKM - Control)/Control, respectively. NPKM vs NPK represents the variation of NPKM compared to NPK, calculated as (NPKM - Control)/Control - (NPK - Control)/Control.

(pH > 8.11) compared with the control, NPK fertilization increased crop yield by 1.47, 1.66, 1.27, and 3.65 folds, respectively, and NPK and NPKM fertilization showed similar crop yields. In contrast, for the acidic soils in Jinxian, Qiyang, and Shenyang (pH < 5.70) compared with the control, NPK fertilization alone increased crop yield by 3.11, 5.55, and 0.58 folds, respectively, and NPKM fertilization increased yield by 4.41, 8.72, and 1.19 folds, respectively (Fig. 2A and Table S2).

The pH values of alkaline soils from four long-term field experiments in Zhengzhou, Yanting, Changwu, and Fukang were 8.11, 8.34, 8.36, and 9.08, respectively. Long-term NPK application only lowered the soil pH slightly, with the greatest decline of 0.29 units. Intriguingly, more than 25 years of NPKM fertilization also decreased soil pH, with a maximum decline of 0.31 units, and the effects of long-term fertilization treatment on soil pH was not significantly in alkaline soils (Fig. 2B, Table 1 and Table S2). The pH values of acidic soils from three long-term field experiments in Jinxian, Qiyang, and Shenyang were 5.10, 5.46, and 5.70, respectively. Chronic enrichment of inorganic fertilizers led to a significant decrease ($P < 0.05$) in soil pH to 4.71, 4.25, and 5.14, respectively, with the greatest decline in pH of 1.21 units in Qiyang. NPKM fertilization effectively reversed the trend in soil acidification, and significantly increased ($P < 0.05$) soil pH for those same sites to 6.01, 5.99, and 6.39, respectively. Compared to NPK, NPKM increased soil pH in Jinxian, Qiyang, and Shenyang by 1.30, 1.74, and 1.26 units, respectively (Fig. 2B and Table S2). Therefore, alkaline and acidic soils exhibited different responses in soil pH to organic and inorganic fertilization.

For both acidic and alkaline soils, NPK and NPKM fertilization, but especially NPKM fertilization, increased most soil nutrients significantly (i.e. SOC, total N, available P, and available K) (Fig. 2C-F, Table 1, and Table S2). Compared with the control, NPKM fertilization in Qiyang increased SOC, total N, and available K to a great degree, by 1.1, 0.9, and 10.5 folds, respectively, and NPKM fertilization in Jinxian increased available P by 20.4 folds. Notably, compared with NPK, NPKM fertilization increased available P significantly by 0.20–4.90 folds in

acidic soils and by 0.13–2.66 folds in alkaline soils.

3.2. Effects of long-term fertilization on soil fungal communities and trophic modes

The fungal community structure showed a distinct separation between acidic soils and alkaline soils by NMDS axis1. In particular, soil fungal communities differed significantly between NPKM and NPK treatments in acidic soils, but relatively similar in alkaline soils (Fig. 3A). PERMANOVA results confirmed that fertilization treatment and sampling site significantly affect soil fungal community structure (Table S3). Further, the seven edaphic factors (i.e. pH, SOC, total N, total P, total K, available P, and available K) explained 22.4 % of the variation in composition of the soil fungal communities (Fig. 3B). A mantel test showed strong correlations between these factors and community composition ($P = 0.001$, Table S4). Among these factors, soil pH was the determinant of community variation ($r = 0.587$, Fig. 3B).

At the class level, Sordariomycetes, Dothideomycetes, and unclassified Ascomycota and Zygomycota dominated the fungal communities, which accounted for the relative abundance of 50.0–85.5 % and 51.8–94.4 % in acidic and alkaline soils, respectively (Fig. 3C). Based on the major feeding habits of fungi, three trophic modes were defined, i.e. pathotroph, symbiotroph, and saprotroph. Saprophytic fungi was the main defined group across all soil samples, accounted for the relative abundance of 28.0–77.2 %. In acidic soils, long-term inorganic-organic fertilization increased the relative abundance of saprotroph by 43.7–84.5 %, but decreased the relative abundance of pathotroph by 34.3–53.0 %. For alkaline soils, the differences of trophic modes were minor between NPK and NPKM fertilization in Yanting and Fukang, but NPK and NPKM fertilization decreased saprophytic fungi in Zhengzhou and Changwu, respectively (Fig. S1).

Table 1

Two-way ANOVA revealing the effects of fertilization, site and their interaction on soil properties in acidic and alkaline soils.

		Acidic soil			Alkaline soil		
		SS	F	Sig.	SS	F	Sig.
pH	Fertilization	9.3	32.1	0.003	0.2	3.4	0.104
	Site	1.5	5.0	0.081	4.0	42.4	< 0.001
	Fertilization*Site	0.6	13.4	< 0.001	0.2	2.9	0.030
	Residuals	0.2			0.3		
	Total	11.5			4.7		
Soil organic C	Fertilization	140.6	11.2	0.023	104.0	75.3	< 0.001
	Site	34.6	2.8	0.177	67.2	32.4	< 0.001
	Fertilization*Site	25.1	20.5	< 0.001	4.2	2.0	0.105
	Residuals	5.5			8.3		
	Total	205.8			183.6		
Total N	Fertilization	1.1	7.1	0.049	0.9	19.4	0.002
	Site	0.6	3.7	0.122	1.4	21.0	0.001
	Fertilization*Site	0.3	15.3	< 0.001	0.1	8.3	< 0.001
	Residuals	0.1			0.1		
	Total	2.0			2.4		
Total P	Fertilization	6.6	10.0	0.028	1.2	7.7	0.022
	Site	2.5	3.8	0.119	1.2	5.2	0.041
	Fertilization*Site	1.3	83.2	< 0.001	0.5	6.12	0.001
	Residuals	0.1			0.3		
	Total	10.6			3.2		
Total K	Fertilization	0.6	0.1	0.937	0.6	1.2	0.352
	Site	394.7	44.3	0.002	97.1	137.8	< 0.001
	Fertilization*Site	17.8	6.3	0.002	1.4	0.8	0.581
	Residuals	12.7			7.1		
	Total	425.8			106.1		
Available P	Fertilization	3735.0	16.0	0.012	3790.4	3.5	0.098
	Site	173.9	0.8	0.52	3691.0	2.3	0.179
	Fertilization*Site	465.7	22.2	< 0.001	3236.4	25.1	< 0.001
	Residuals	94.6			515.0		
	Total	4469.2			11232.8		
Available K	Fertilization	278478.8	8.1	< 0.001	120558.5	1.4	0.319
	Site	45413.2	1.3	0.360	245095.4	1.9	0.233
	Fertilization*Site	68526.6	36.4	< 0.001	260175.8	38.7	< 0.001
	Residuals	8484.6			26880.6		
	Total	400903.2			652710.3		

The significance (sig.) was calculated based on sum of squares (SS).

3.3. Specific fungal taxa enriched by long-term fertilization

Compared with NPK fertilization, the increased relative abundance of enriched phylotypes under NPKM was 53.1–85.1 % in acidic soils, which was higher than the 0.5–29.4 % increases in relative abundance in alkaline soils (Fig. S2). Compared with NPK, NPKM fertilization increased the relative abundance of Pezizomycetes dramatically by 6.5 %, 33.8 %, and 22.3 % in acidic soils in Jinxian, Qiyang, and Shenyang, respectively, although relative abundance only increased by 0.6–1.2 % in alkaline soils. Moreover, compared with the control, the abundance of Tremellomycetes also increased in acidic soils with NPKM fertilization, and their relative abundance in soils in Jinxian, Qiyang, and Shenyang increased by 1.7 %, 6.0 %, and 2.0 %, respectively (Fig. S2).

To better characterize the enriched OTUs by long-term fertilization at the genus level, unclassified fungi were removed and the top five OTUs with the greatest increase in relative abundance were chosen for downstream analysis. Compared with NPK, NPKM significantly enhanced the relative abundance of *Mortierella* and *Pseudaleuria* in acidic soils, and the proportions of total enriched relative abundance were 20.6–59.3 % and 13.1–39.1 %, respectively (Fig. 4A). Regarding functional group (guild), OTUs assigned to *Mortierella* was identified as endophyte-soil saprotroph-undefined saprotroph, and *Pseudaleuria* was identified as undefined saprotroph (Dataset S1). Compared with the control, both NPK and NPKM fertilization significantly increased unclassified Ascomycota in alkaline soils, and the increased abundance under NPK and NPKM treatments accounted for 5.5–69.1 % and 21.9–42.0 % of the total increases in abundance, respectively (Fig. 4B and C). Moreover, NPKM had a more abundant *Mortierella* than control

in alkaline soils, which accounted for 8.0–28.9 % of total increase abundance.

3.4. Linking crop yields to soil properties and fungal communities

The structure equation modeling adequately fitted the data assessing causal relationships among soil properties, fungal communities and crop yields in acidic soils and alkaline soils (Fig. 5). In acidic soils, C:N ratio showed a negative direct influence on the genus *Pseudaleuria*, soil pH had a positive direct effect ($P < 0.05$) on the genus *Mortierella*. Notably, the abundance of *Mortierella* directly altered by soil pH showed a positive direct influence ($P < 0.05$, path coefficient = 0.36) on crop yield (Fig. 5A). A positive direct effect from soil available P was also observed on crop yield in acidic soils. The SEM model for acidic soils explained 76 % of the variation in yield, 20 % and 81 % in fungal diversity and community composition, 45 % and 84 % in *Mortierella* and *Pseudaleuria*, respectively. In alkaline soils, available P and C:N ratio had positive direct effects on crop yield. Soil pH was negatively related with the abundance of unclassified Ascomycota. C:N ratio, rather than pH or available P, directly induced changes in fungal diversity, and further caused positive effects on crop yield in alkaline soils ($P < 0.001$, path coefficient = 0.51) (Fig. 5B). The model explained 72 % of the variation in yield, and it explained 44 %, 23 % and 32 % of fungal diversity, community composition and unclassified Ascomycota, respectively.

4. Discussion

Our results challenge the conventional recognition that inorganic

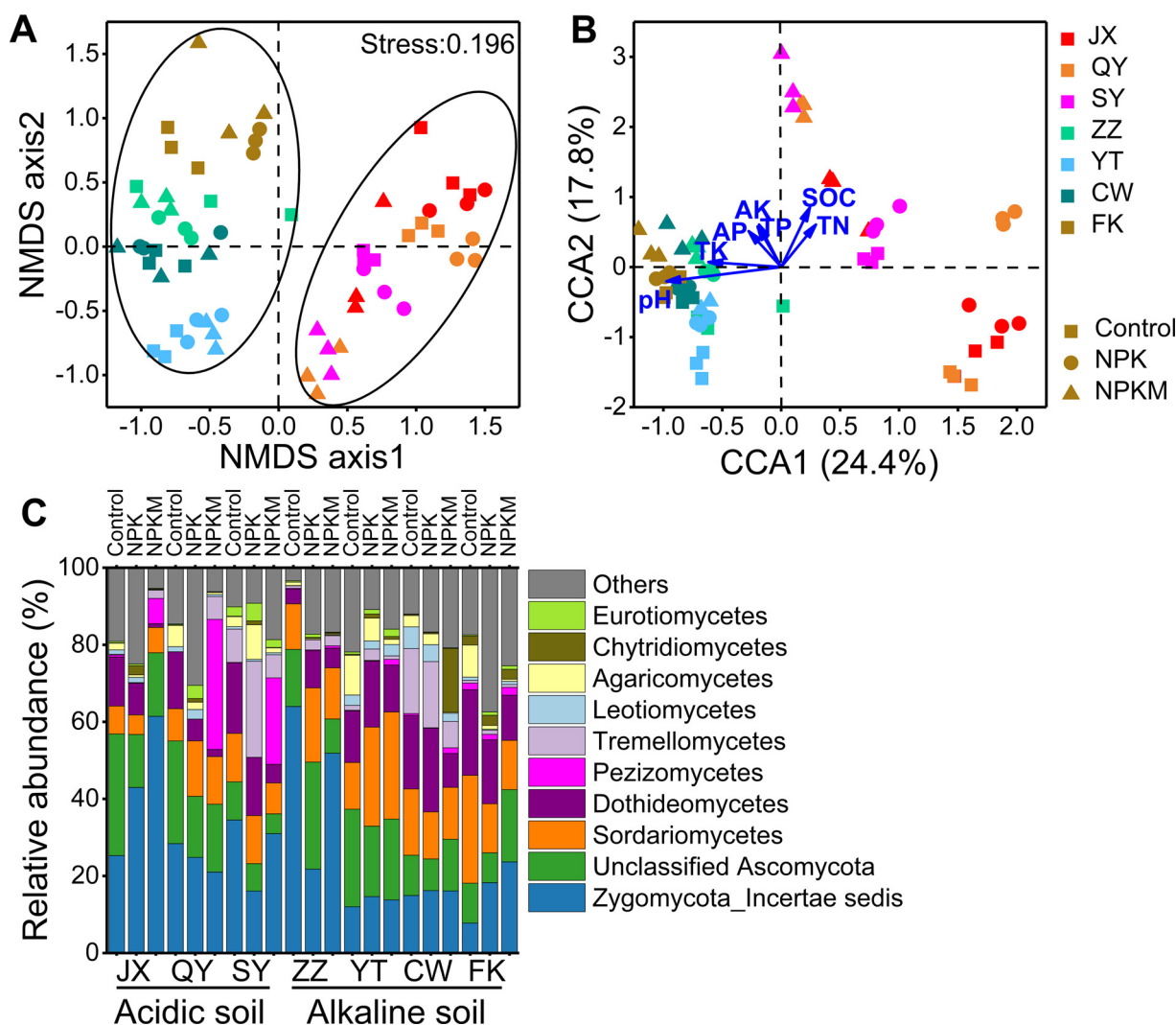


Fig. 3. Responses of fungal community composition and structure to inorganic fertilization (NPK) and combined organic-inorganic fertilization (NPKM) at seven sites. Non-metric multidimensional scaling (NMDS) plot based on Bray-Curtis distance shows variation in the fungal community composition between samples (A). Canonical correlation analysis (CCA) based on Bray-Curtis distance relates fungal community structure to edaphic factors (B). Relative abundance of major fungal classes (C). All other designations are the same as those in Fig. 2.

fertilizer application leads to soil acidification and thus undermines future crop production in the long term. We revealed an initial soil pH-dependent fertilization strategy and fungal microbiome assembly to support agricultural production, and we demonstrated that continuous appropriate use of inorganic fertilizers without organic amendments is still a promising fertilization strategy in Chinese alkaline soils.

4.1. Divergent responses of soil pH and crop yields to long-term fertilization in acidic and alkaline soils

Continuous enrichment of inorganic fertilizers significantly acidified acidic soils, with a maximum decrease of 1.21 pH units, and soil acidification was alleviated with the addition of organic amendments (Fig. 2B and Table S2). In acidic soils, long-term inorganic fertilization depleted exchangeable calcium and magnesium ions and increased exchangeable hydrogen and aluminum ions, and thus declined soil pH (Tian and Niu, 2015). Moreover, because a large number of base cations were removed by crop harvest, soils needed to generate an equal quantity of protons to keep the conservation of charge, which resulted in acidification (Guo et al., 2010). When combined with organic manure, the increase in base cations to soils helped neutralize soil acidity, and the protonation of organic anions to form neutral

molecules was the main mechanism to resist acidification (Shi et al., 2019). Although repeated use of inorganic fertilizers increased crop yields, the addition of organic fertilizers further improved crop production in acidic soils (Fig. 2A and Table S2). We speculate that soil acidification caused by inorganic fertilization is the main limitation to further increases in crop yield. As previous study reported, low pH damaged the photosynthetic electron transport chain, increased the production of reactive oxygen species, reduced the absorption of nutrients and water, and thus decreased CO₂ assimilation in leaves (Long et al., 2017). When soil pH values were < 5, aluminum ions are released and produced aluminum toxicity for plants, which inhibited root cell expansion, elongation and division, and affected the absorption of mineral nutrients by plant roots (Kochian et al., 2004). These impacts suppressed the formation of plant photosynthetic products, and consequently restricted the increase in crop yield. The results from SEM further confirmed that soil pH indirectly affected crop yield in acidic soils (Fig. 5A). Moreover, a combination of organic-inorganic fertilizers improved soil fertility further by facilitating the buildup of SOM (Francioli et al., 2016).

In contrast to acidic soils, pH of alkaline soils showed a different response to fertilization. Inorganic fertilization had a very small effect on alkaline soil pH and maintained high crop productivity that was

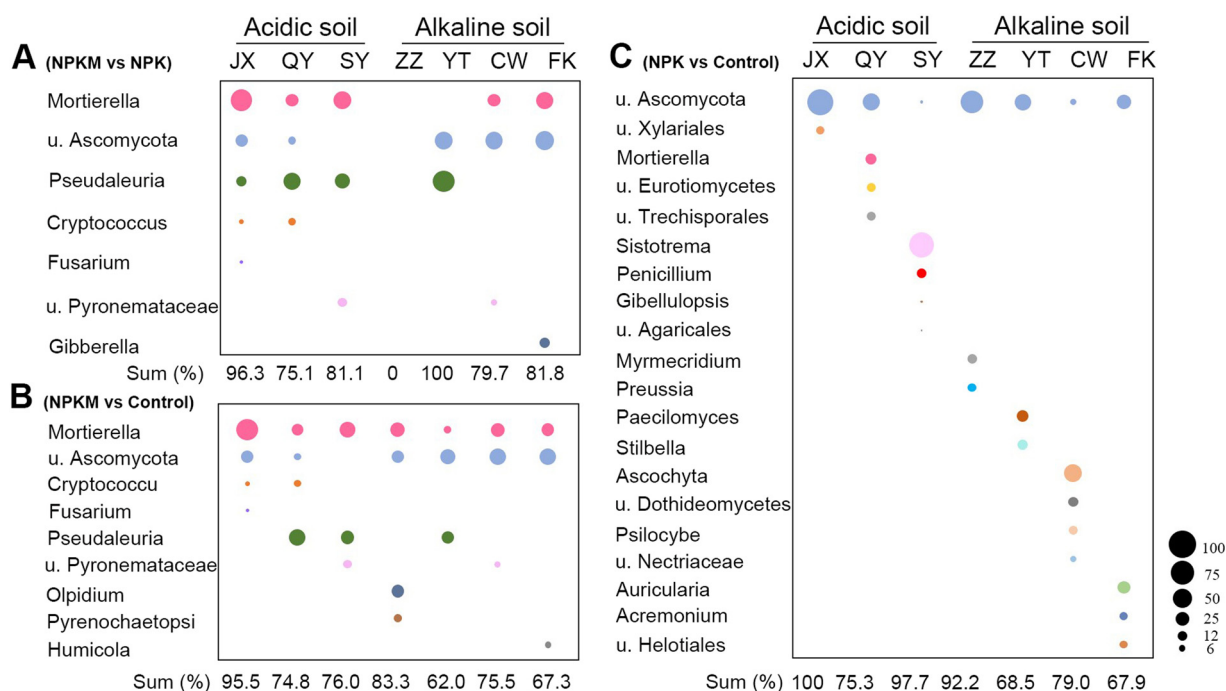


Fig. 4. Bubble plots showed the top 5 increased OTUs in genus level after removing unclassified fungi. The size of bubble indicates the proportion of increased relative abundance of each fungus in all increased relative abundance of enriched OTUs. (A) refers to NPKM vs NPK, (B) refers to NPKM vs Control, (C) refers to NPK vs Control. All other designations are the same as those in Fig. 2.

comparable with that of organic-inorganic fertilization. Moreover, inorganic fertilization increased soil nutrient contents significantly, especially for SOC and total N (Fig. 2, Table 1 and Table S2). Similar results showed that long-term application of inorganic fertilizers improved crop yield and soil fertility in alkaline soils (Xin et al., 2016; Wang et al., 2018). The mechanism for a slight decline in soil pH may be related to the high initial pH values of alkaline soils. Generally, soils with higher initial pH values have greater pH buffering capacity. Large amounts of base cations of calcium, magnesium, sodium, and K ions in alkaline soils buffer against soil acidification that was induced by application of inorganic N fertilizer (Tian and Niu, 2015). The protons caused by N fertilizers are not enough to break the threshold values of alkaline soils and cannot produce aluminum toxicity (Chadwick and Chorover, 2001). On the other hand, a high crop yield after fertilization brought greater returns of organic materials (e.g. root residues and exudates) to the soils (Zhao et al., 2018). Greater amounts of organic matter had greater cation exchange capacity, which increased their buffering against acidification (Tian and Niu, 2015). Furthermore, the accumulation of organic materials improved SOC content. In our results, long-term fertilization increased SOC and total N, C:N ratio and available P showed positive direct effects on crop yield, but the increment in crop yield with addition of organic manure was comparable with the sole use of inorganic fertilizers (Fig. 2 and Fig. 5B). It is noteworthy that there are 99.13 million hectares of saline-alkaline soils in China. If organic fertilizers were not applied to alkaline soils, it is estimated that 31.8 billion dollars would be saved annually. Taken together, we recommend that the balanced use of inorganic fertilizers is the optimal fertilization strategy in alkaline soils.

4.2. Responses of soil fungal communities and specific taxa to long-term fertilization

NMDS and CCA results indicated a pH-based ecological coherence in the community structure of soil fungi (Fig. 3A, B). The results from SEM further confirmed that soil pH caused a direct effect on fungal community composition (Fig. 5). Our findings are in line with previous

reports that soil pH was the determinant of the fungal community structure (Francioli et al., 2016; Zhou et al., 2016). At each site, fungal communities clearly differed between inorganic and organic-inorganic fertilization in acidic soils, but community distances were relatively closer in alkaline soils between inorganic and organic-inorganic fertilization. We speculate that this was due to the notable differences in soil pH between inorganic and organic-inorganic fertilization in acidic soils. However, the variation in pH values between soils fertilized with inorganic or organic-inorganic fertilizers were less than 0.26 units in alkaline soils, which resulted in smaller variations in the fungal community structures in alkaline soils relative to acidic soils.

Organic-inorganic fertilization resulted in more abundant Pezizomycetes and Tremellomycetes than inorganic fertilization in the three acidic soils, and a higher abundance of saprotrophic fungi in organic-inorganic fertilized soil than inorganic fertilization (Fig. 3C, and Figs. S1, S2). An in-depth analysis showed that the genera *Mortierella* (endophyte-soil saprotroph-undefined saprotroph) and *Pseudaleuria* (undefined saprotroph) were substantially enriched in the organic-inorganic fertilization treatment of acidic soils compared with the inorganic fertilization treatment (Fig. 4 and Dataset S1). This was consistent with SEM result that alleviating soil acidification resulted in the increase of abundance of *Mortierella* (Fig. 5A). C:N ratio was negatively correlated with the relative abundance of *Pseudaleuria*, which is in agreement with previous study that some specific fungal groups showed negative relationships with C:N ratio (Ni et al., 2018). Many members of Pezizomycetes are primarily saprobic, and some were identified as ectomycorrhizal symbionts (Hansen et al., 2013). *Pseudaleuria* is the most abundant species of Pyronemataceae, which is the most heterogeneous family of Pezizomycetes. Pyronemataceae was symbionts with roots and obtain carbohydrates from plants, and in turn is beneficial for plants to uptake nutrients (Hansen et al., 2013). *Pseudaleuria* sp. was found to be dominant in healthy soils, and it had a negative correlation with the disease severity index of pea (*Pisum sativum* L.) roots (Xu et al., 2012). Tremellomycetes, which belongs to the phylum Basidiomycota, is abundant in black soils with a high SOM content, and it is implicated in the decomposition of organic material (Liu et al., 2015). The SEM

A $\chi^2/df = 0.95$, $P = 0.46$, GFI = 0.952, RMSEA = 0.000

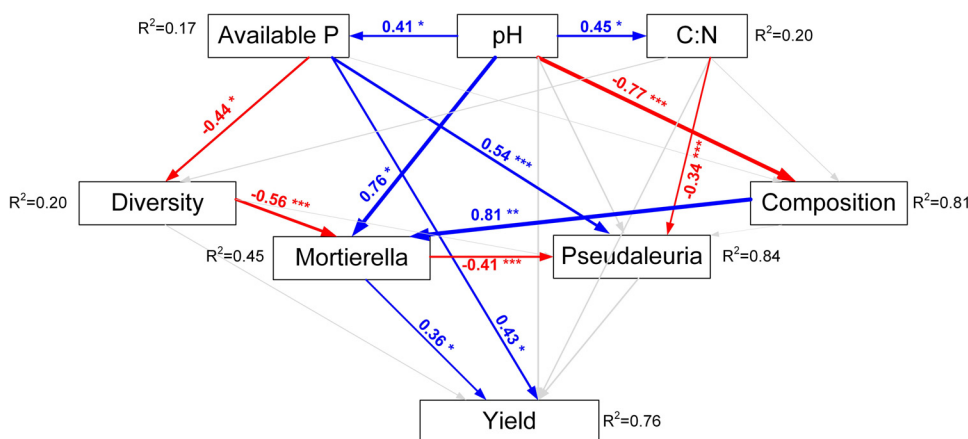
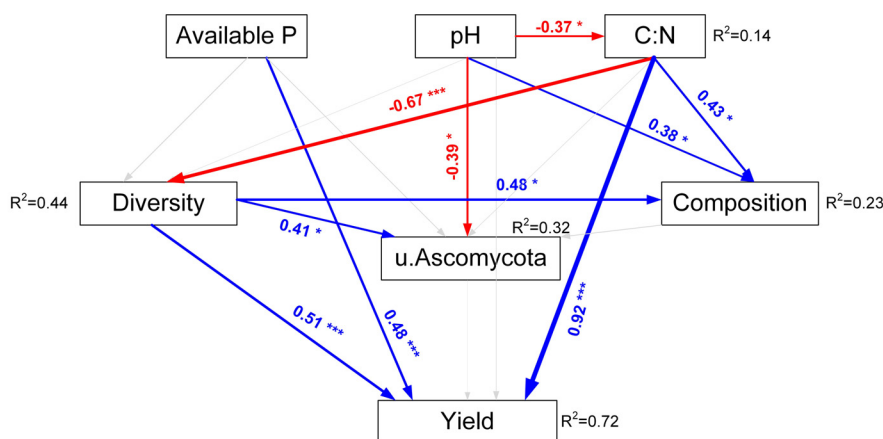


Fig. 5. Structural equation models based on the effects of soil properties (pH, available P, and C:N ratio), fungal diversity and community composition, and several specific fungal taxa on crop yield in acid soils (A) and alkaline soils (B). Blue and red arrows indicate significant positive and negative relationships, respectively. Gray arrows indicate nonsignificant relationships. Numbers at arrows are standardized path coefficients. The width of arrows indicates the strength of the relationships. R^2 indicates the proportion of variance explained by the model. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$ (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

B $\chi^2/df = 0.69$, $P = 0.60$, GFI = 0.979, RMSEA = 0.000



model also suggested that the enrichment of *Mortierella* species caused a positive direct effect on crop yield (Fig. 5A). Recently, several studies have reported that *Mortierella* was beneficial for crop growth and health (Borrell et al., 2017; Li et al., 2018). The growth of *Mortierella* species is affected by the amount and balance of C and N, and lipid synthesis is an important aspect of the metabolism of this species (Koike et al., 2001). Some *Mortierella* species could degrade hemicellulose and chitin (Young-Ju et al., 2008). Recent evidence revealed that *Mortierella* sp. have ability to release P from different soils and to promote the uptake of P by plants (Osorio and Habte, 2015). Available P content showed a larger variation in acidic soils than in alkaline soils under organic-inorganic treatment (Fig. 2E), which may be due to acidic soils having higher abundance of *Mortierella* than alkaline soils. Moreover, some *Mortierella* species improve soil health by degrading a range of toxic compounds and producing antibiotics to against some plant pathogens (Tagawa et al., 2010; Francioli et al., 2016; Li et al., 2018). Therefore, in acidic soils, soil acidification directly regulated fungal community composition and specific fungal taxa (e.g. *Mortierella*), and thus indirectly affected crop yield.

In alkaline soils, C:N ratio directly altered fungal diversity and community composition (Fig. 5B). Exogenous organics are decomposed and transformed by microorganisms after being applied to the soil, some specific microbes obtained C sources in this process and simultaneously acquired N sources from inorganic fertilizers. These microbes grew rapidly and proliferated under appropriate C:N ratio (Li et al., 2017). High C:N ratio may restrain the growth of some microbial

groups, and thus decreased fungal diversity (Sinsabaugh et al., 2013; Ni et al., 2018). The past researches indicated that increasing microbial diversity could promote crop production (Wagg et al., 2011; Tautges et al., 2016). Our study verified this point since the fungal diversity was directly linked with crop yield. Compared to unfertilized control, members of Ascomycota were significantly enriched in both inorganic and organic-inorganic fertilization treatments (Fig. 4B and C). Ascomycota is the dominant phylum of fungi in agricultural ecosystems, and most members of Ascomycota are saprotrophic decomposers of organic materials and therefore, they play significant roles in nutrients and carbon cycling (Francioli et al., 2016; Yu et al., 2018). Some species of Ascomycetes form a symbiotic relationship with plant roots, which is benefit for the uptake of mineral nutrients by plants (Semenova et al., 2015). In return, plant provides metabolic energy for fungi in the form of photosynthetic products. Moreover, organic-inorganic fertilization significantly enriched *Mortierella* in alkaline soils compared with control soils, but no such results were observed in long-term inorganic fertilization soils.

In conclusion, our systematic investigation summarized optimal fertilization strategies for China's upland cultivated soils based on initial soil pH and fungal community characteristics. In part, soil pH indirectly affected crop yields by altering fungal community characteristics in acidic soils. Thus, organic fertilizers are essential to alleviate soil acidification, and thereby to achieve high crop yield in acidic soils. However, soil available P and C:N ratio, rather than pH, directly or indirectly influenced crop yield, and thus crop production can be

maintained by only inorganic fertilization in alkaline soils. It would save significant manpower and material resources if organic fertilizers are not applied to alkaline soils. These findings are of great significance in selecting appropriate fertilization strategies and for offering some guidance for related policy-making. Our study showed that *Mortierella* species were abundant under long-term organic fertilization, in both acidic and alkaline soils. In the future, our work is to isolate single *Mortierella* strains from soils, and further explore the mechanisms of effects of *Mortierella* strains on soil fertility and crop growth.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.agee.2020.106837>.

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