Responses of arbuscular mycorrhizal fungi to rice–upland crop rotations in an 8-year paddy ecosystem

Qingfeng Wang[1], Deping Zhou[1], Changbin Chu[1], Zheng Zhao[1], Jing Zhou[2], and Shuhang Wu[1]

[1]Shanghai academy of agricultural sciences
[2]Qufu nomal university

November 22, 2022

Abstract

Arbuscular mycorrhizal fungi (AMF), which can form symbiotic associations with many terrestrial plants, are critical for crop yields and agroecosystem sustainability. In this study, we assessed the influence of rice–upland crop rotations on soil AMF diversity and composition. We also explored the mechanisms of rice–upland crop rotations that affect AMF using trait-based guild methods. We found that rotations of rice with different plants differentially influenced soil AMF. Rice–wheat (RW) and rice–Chinese milk vetch (RV) rotations significantly altered the soil AMF composition, with RW and RV significantly increasing and decreasing AMF diversity, respectively, compared with the rice–fallow (RF) treatment. In addition, RW and RV affected AMF abundance in intra- and extra-radical portions in different ways. For example, both the RW and RV treatments increased AMF spore density, but decreased AMF colonization rate. Different AMF guilds showed different responses to rice–upland crop rotations. The RW treatment increased the rhizophilic guild by 4.9% and decreased the edaphophilic guild by 27.9%, while the RV treatment produced opposite trends. The rhizophilic and edaphophilic guilds were moderated mainly by soil pH, but the former was also significantly influenced by soil available P and the N:P ratio. Structural equation modeling analysis showed that AMF root abundance (colonization rate) was directly and significantly negatively correlated with rice yield under different rotations. Thus, rice–upland crop rotations changed soil AMF diversity, AMF composition, and trait-based guilds in different ways, and rice yield was mainly correlated with AMF colonization rate.

Hosted file

essoar.10510784.1.docx available at https://authorea.com/users/540024/articles/600090-responses-of-arbuscular-mycorrhizal-fungi-to-rice-upland-crop-rotations-in-an-8-year-paddy-ecosystem

Responses of arbuscular mycorrhizal fungi to rice–upland crop rotations in an 8-year paddy ecosystem

Abstract

Arbuscular mycorrhizal fungi (AMF), which can form symbiotic associations with many terrestrial plants, are critical for crop yields and agroecosystem sustainability. In this study, we assessed the influence of rice–upland crop rotations on soil AMF diversity and composition. We also explored the mechanisms of rice–upland crop rotations that affect AMF using trait-based guild methods. We found that rotations of rice with different plants differentially influenced soil AMF. Rice–wheat (RW) and rice–Chinese milk vetch (RV) rotations significantly altered the soil AMF composition, with RW and RV significantly increasing and decreasing AMF diversity, respectively, compared with the rice–fallow (RF) treatment. In addition, RW and RV affected AMF abundance in intra- and extra-radical portions in different ways. For example, both the RW and RV treatments increased AMF spore density, but decreased AMF colonization rate. Different AMF guilds showed different responses to rice–upland crop rotations. The RW treatment increased the rhizophilic guild by 4.9% and decreased the edaphophilic guild by 27.9%, while the RV treatment produced opposite trends. The rhizophilic and edaphophilic guilds were moderated mainly by soil pH, but the former was also significantly influenced by soil available P and the N:P ratio. Structural equation modeling analysis showed that AMF root abundance (colonization rate) was directly and significantly negatively correlated with rice yield under different rotations. Thus, rice–upland crop rotations changed soil AMF diversity, AMF composition, and trait-based guilds in different ways, and rice yield was mainly correlated with AMF colonization rate.

Keywords: Arbuscular mycorrhizal fungi; rice–upland crop rotation; abundance and diversity; above- and below-ground interactions

Abbreviations

AMF: Arbuscular mycorrhizal fungi

RW: Rice–wheat

RV: Rice–Chinese milk vetch

RF: Rice–fallow

OUT: operational taxonomic unit

RDA: redundancy analysis

PCoA: principal coordinates analysis

Paddy rice–upland crop rotations, which frequently shift between wet and dry seasons that result in anaerobic and aerobic conditions, respectively, are the most important cropping systems in southern and eastern Asian countries. (Zhou et al., 2014). In addition, upland crops can be used as green manure to increase soil sustainability by ameliorating the physical properties of the

soil, reducing soil erosion (Zhang et al., 2017), and enhancing soil fertility and nutrient retention (Dennis et al., 2010). Returning green manure to paddies has the potential to reduce insect pests and increase rice production. Zhang et al. (2017) reported that a 31-year paddy rice–upland crop (with the upland crop used as green manure) rotation increased rice yields by 11.9%–15.6%, and shaped the rice rhizospheric microbial composition by increasing the accumulation of beneficial bacteria. Zhou et al. (2020) reported that paddy rice–upland crop rotations increased rice yields by 4.1%–9.6% and improved soil fertility, increased enzyme activities, and stimulated microbial growth. Although paddy rice–upland crop rotations increase rice production and alter the soil microbial composition's structure, the effects of microbial communities, especially the functional microbial communities, on rice production are poorly understood.

Arbuscular mycorrhizal fungi (AMF), which are the most common type of mycorrhizal symbiosis worldwide (van der Heijden et al., 2015), play a prominent role in plant growth (Dueñas et al., 2020). AMF can increase the uptake of soil nutrients, especially phosphorus (P), in exchange for plant-derived carbon (C) (Smith and Smith, 2012). In addition, they regulate plant diversity and microbial communities (Bever et al., 2010; Yang et al., 2014; Poosakkannu et al., 2017; Xu et al., 2018), and they alter soil structures (Rillig and Mummey, 2006; Han et al., 2020). Because AMF are strictly biotrophic, fallow periods are expected to reduce the AMF inoculum's potential to support subsequent plant growth (Hontoria et al., 2019; Elliott et al., 2020). Crop rotations have been reported to enhance root AMF colonization rate in subsequent cash crops (García-González et al., 2016; García-González et al., 2018). However, those studies focused mostly on upland cropping systems (Higo et al., 2013; Hontoria et al., 2019), not wetland and upland cropping rotation systems. In addition, AMF have intra- and extra-radical structures, and different radical structures have contrasting life history strategies (Han et al., 2020). For example, some fungal taxa allocate more biomass to extra-radical structures and less to the colonization of roots, and these AMF are classified in the edaphophilic guild. Others colonize roots heavily with less extra-radical hyphae, and these fungi are classified in the rhizophilic guild (Maherali and Klironomos, 2007; Sikes et al., 2010; Weber et al., 2019). The edaphophilic guild members, such as those in Gigasporaceae, play important roles in increasing plant nutrient uptake, while the rhizophilic guild enhances plant pathogen protection (Weber et al., 2019; Wang et al., 2020). The different rice–upland crop rotations and life history strategies may affect AMF abundance in root and soil in different ways.

Over the past 100 years, chemical fertilizers have been the excessively applied to agricultural lands, resulting in higher levels of soil available nutrients (Zhou et al., 2015). This elevated nutrient availability influences AMF communities. For example, increased soil available P and N reduce AMF richness and diversity (Cheng et al., 2013; Xiang et al., 2014), soil biomass (Qin et al., 2015), and root colonization (Mäder et al., 2000). In addition, different AMF guilds respond differently to fertilization. For example, fertilization inhibits the rhizophilic

guild, but has a neutral effect on the edaphophilic guild (Han et al., 2020). Because plant–AMF symbiosis relies on mutualism or parasitism, increases in soil available nutrients may shift AMF from mutualistic partnerships towards parasitic partnerships (Verbruggen et al., 2010). When soil available P and N are present in sufficient quantities, AMF reduces the P supply and increases the C demand of the plant (Williams et al., 2017). This may reduce the benefits of soil AMF to plants. Plant responses to AMF colonization rate are frequently mixed and lack consistency. For example, AMF colonization rate increases plant nutrient assimilation and growth, or AMF partnerships offer little or no measurable benefits (Ellouze et al., 2015; Sawers et al., 2017; Watts-Williams et al., 2019; Elliott et al., 2020). This suggests that the effects of AMF on plants are likely condition-dependent, such as plant species or soil nutrient concentrations. However, how AMF composition, diversity, and colonization rate effect plant production in paddy rice–upland crop rotations need to be elucidated.

In this study, an 8-year field experiment, consisting of rice–fallow (RF), rice–wheat (RW), and rice–Chinese milk vetch (RV) rotations, was performed with the following main objectives: i) investigating the impacts of paddy rice–upland crop rotations on soil AMF and trait-based guild community structures; and ii) exploring how changes in soil nutrients, AMF communities, and colonization influence rice production. To achieve our goals, we classified AMF families into different guilds on the basis of biomass allocation to intra- and extra-radical portions in accordance with Weber et al. (2019). We hypothesized the following: i) different rice– plant rotations would change soil AMF diversity, composition, and abundance; ii) different rice–plant rotations would change different AMF guilds in different ways; and iii) AMF colonization would decrease rice production, because it consumes more plant-derived C during the 8-year rice–upland crop rotations and because it does not significantly alter the available soil nutrients.

1. MATERIALS AND METHODS

1.1. Experimental site description

Soil samples were collected from a field experimental station in Shanghai City, China (30°53' N, 121°23' E). The soils at this experimental field are classified as Anthrosols according to FAO soil classification. The climate of this region is subtropical, and the average annual temperature and precipitation are 16°C and 1,200 mm, respectively. The long-term field experiment was started in 2010, comprising three rotations: rice–fallow (RF), rice–wheat (RW), and rice–Chinese milk vetch (RV), which were the three main rotations planted in Shanghai. Each rotation had three replications and an area of 7 m × 8 m per replication. To prevent lateral seepage among plots, an impermeable membrane was buried vertically around each plot at a 1.2-m depth. From 2011 to 2018, the total fertilizer applied during each season included urea (200 kg/ha), P_2O_5 (90 kg/ha), and K_2O (225 kg/ha). The urea was applied as basal fertilizer, tillering fertilizer and heading fertilizer at rates of 50%, 30% and 20%, respectively. Aliquots of K_2O were applied as the basal fertilizer or as the heading fertilizer,

whereas the P_2O_5 was all applied as a basal fertilizer. For RW and RV rotations, the wheat and Chinese milk vetch were sown in winter after the rice was harvested, using the customary seeding quantities of 150 kg/ha and 75 kg/ha, respectively. For RW and RV rotations, the fresh green plants were mulched on each plot surface after harvest and ploughed into the soil before the rice was transplanted.

1.2. Sampling and analysis

Ten plant roots were sampled randomly from each replicated plot in late September 2018 before the rice was harvested. Sampling was performed using a spade, and samples were packed into sterile plastic bags and transported to the laboratory immediately. Soil samples were collected using a drill (2.5 cm in diameter) from the plough layer (0–20 cm), which unvegetated soil adjacent to the plants. To minimize within-plot variation, we collected 12 cores per plot (7 m × 8 m) and mixed them uniformly. To further minimize bias, we collected two composite samples per replicate plot, for a total of 18 composite samples. A 2-mm sieve was used to remove plant residues and gravels, and partial samples were stored at −80°C for molecular analyses or air dried at room temperature for chemical analyses.

AMF' abundance indicators included root colonization rate and spore density. The magnified grid line intersection method was used to determine the AMF colonization rate (Koske and Gemma, 1989; McGonigle et al., 1990). The colonization rates were calculated according to the following equation:

The colonization rates (F%) = (the number of root fragments colonized by AMF mycelium / the total number of analyzed root fragments) * 100%

Spore density was determined in accordance with Silva-Flores et al. (2019). Rice yield was determined by weighting the air-dried rice of the entire plot area. About 200 g air-dried rice was stoved to measure the stover yield. Soil pH was measured using a glass-combination electrode with a 2.5: 1 water: soil ratio (Li et al., 2013). Soil electrical conductivity was determined using a conductivity meter at a 5:1 water:soil ratio. Soil organic matter was analyzed in accordance with Strickland and Sollins (1987). Soil available N and available P were determined in accordance with Zhang et al. (2017) and Olsen et al. (1954), respectively. Soil available K was assayed using the procedure described by Helmke and Sparks (1996).

1.3. PCR amplification and barcode pyrosequencing

The selected treatment soil's total DNA was extracted using a Soil DNA Isolation Kit (MOBIO Isolation Inc., Carlsbad, CA, USA) in accordance with the instructions. We extracted six successive soil total DNAs from a replication and mixed them to minimize the DNA extraction bias. The genomic DNA was purified and checked in accordance with Wang et al. (2017).

Nested PCR was used to amplify AMF libraries of the appropriate size for MiSeq with primer sets AML1F/AML2R (Shi et al., 2019) and AMV4/-5NF_AMDGR

(Suzuki et al., 2020). The first-round PCR performed using primer AML1F - AML2R according to the following procedure: 95°C for 3 min; 32 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s; and following a final extension at 72°C for 10 min. The second-round PCR was performed using primer AMV4-5NF_AMDGR, with an 8-mer multiplexing identifier and sequencing adapters at both the forward and reverse terminals, and run for 30 cycles under the same conditions as the first round. For each sample, the second-round PCR products were purified and pooled together in equimolar ratios with other purified samples. The combined sample was sequenced using the Illumina MiSeq platform at the Shanghai Majorbio Bio-pharm Technology Co., Ltd. Raw sequence data have been deposited in the NCBI Sequence Read Archive under accession number PRJNA697883.

1.4. Sequence analysis

QIIME software (1.9.1) (http://qiime.sourceforge.net/) was used to analyze the sequencing data, and the low-quality sequences were trimmed using Fastp (0.19.6) in accordance with the following criteria: (1) reads with a quality score less than 20 over a 50-bp window size were removed; (2) only reads with a more than 10-bp overlap and less than 20% mismatches were selected to form longer sequences; and (3) sequences were assigned to different sample libraries on the basis of the unique barcodes and primers, with less than 0% and 2% mismatches, respectively. The UCHIME software was used to check and remove potential chimeras (Edgar, 2013). The remaining high-quality sequences were clustered with UPARSE (7.0.1090) into OTUs with a dissimilarity of less than 0.03. The longest sequence from each OTU was selected to assign taxonomic data from the UNITE database using RDP Classifier (Bodenhausen et al., 2013). The singletons and non-Glomeromycota sequences were removed. The AMF alpha-diversity indices, including Shannon, Simpson, Chao1 and ACE, were estimated using Mothur software (Yousuf et al., 2012).

1.5. Statistical analyses

The differences in soil properties, AMF colonization rate and density, alpha-diversity and AMF community abundances among samples were analyzed using a one-way analysis of variance. Paired comparisons of treatment means were determined by Duncan's procedure using SPSS (version 24.0) statistical software (SPSS, Chicago, IL, USA). The relationships between alpha-diversity, abundant AMF genera, and soil properties were estimated using Pearson's procedure. A principal coordinates analysis (PCoA) was performed to estimate the beta-diversity based on the Weighted Fast UniFrac distances between samples. An analysis of similarities was calculated with 999 permutations in the R "vegan" package based on Bary–Curtis distances. The correlations between environmental variables (pH, Available P, Available K, Available N, and soil organic matter) and the AMF community composition were determined using a redundancy analysis (RDA).

Structural equation modeling (SEM) was applied to analyze how paddy rice–

upland crop rotations change soil properties, including AMF diversity, colonization rate and composition, and how they influence rice yields. The estimates with $^2/df < 2$, high goodness-of-fit statistics (GFI) indices, and low root-mean squared error of approximation (RMSEA) values were used to determine whether the data fitted the models (Zeng et al., 2016).

2. RESULTS

2.1. Rice–upland crop rotations change soil properties, AMF colonization rate and spore density

After an 8-year rice–upland rotation, the rice yield significantly increased in RV and RW by 2% and 1%, respectively, compared with RF. Soil organic matter slightly increased, although not significantly ($P > 0.05$). Soil pH was significantly ($P < 0.05$) decreased by the RW treatment, but was less effected by the RV treatment, compared with the RF treatment. Soil available P significantly increased from 15.49 to 17.59 in the RW and RV rotations compared with the RF treatment. However, AMF colonization rate significantly decreased in the RW and RV rotations compared with the RF treatment. The AMF spore density decreased in RW, but was not significantly different in RV compared with RF. Other soil properties were not significantly changed (Table 1). The rice yield was significantly negatively correlated with AMF colonization rate ($R = -0.667$, $P < 0.05$) (Table 2).

Table 1 Soil properties, as well as AMF colonization rate and density levels, during rice–upland crop rotations

RWRFRVpH (1: 2.5 H_2O)6.58±0.06b6.86±0.02a6.92±0.04aEC (S cm^{-1})194.13±3.52a174.3±14.22ab168.1±10.8
(%)2.83±0.23a2.91±0.41a2.83±0.05aAvail N (mg kg^{-1})130.97±3.52a130.94±21.03a138.81±10.06aAvial
P (mg kg^{-1})17.59±0.52a12.81±1.03c15.49±1.33bAvail K (mg kg^{-1})143.33±15.28a143.33±15.28a140±10aN:
P ratio7.45±0.05c10.18±0.81a8.97±0.19bSpore density ((g soil)$^{-1}$)35.88±0.99a33.39±1.5b36.16±0.42aColonizat
rate (%)87.62±2.07b100±0a93.33±6.67abYield (t hm^{-2})9.61±0.04a9.45±0.06b9.58±0.10abRice
height (cm)113.13±0.9ab109.33±3.49b114.47±2.14aSoil properties were calcu-

lated for each treatment replicate (n = 6). Data are the means ± standard deviations. Values followed by different letters are significantly different ($P < 0.05$) as assessed by Duncan's test. RF (rice–fallow rotations), RW (rice–wheat rotations), and RV (rice–Chinese milk vetch rotations)

Table 2 Pearson's correlation coefficients of soil properties, rice yield and AMF families and abundance levels

@ >p(- 16) * >p(- 16) * >p(- 16) * >p(- 16) * >p(- 16) * >p(- 16) * >p(- 16) * >p(- 16) * >p(- 16) * @ &

pH

&

EC

&

SOM

&

Avail N

&

Avail P

&

Avail K

&

N: P

&

Yield

Spore density &

-0.271

&

0.404

&

-0.243

&

0.506

&

0.764[*a]

&

-0.195

&

-0.486

&

0.218

Colonization rate &

0.569

&

-0.299

&

0.153

&

0.183

&

-0.642

&

-0.111

&

0.781[*]

&

-0.667[*]

Glomeraceae &

-0.486

&

0.294

&

0.381

&

-0.350

&

-0.155

&

0.283

&

0.002

&

-0.163

Diversisporaceae &

0.755^{*}

&

-0.582

&

-0.229

&

0.305

&

-0.276

&

-0.150

&

0.417

&

-0.086

Paraglomeraceae

&

-0.872^{**b}

&

0.757*

&

-0.179

&

-0.079

&

0.842**

&

-0.120

&

-0.890**

&

0.279

Archaeosporaceae &

0.862**

&

-0.498

&

-0.111

&

0.143

&

-0.618

&

-0.085

&

0.690*

&

-0.140

Acaulosporaceae &

0.004

&

0.044

&

-0.065

&

0.282

&

0.542

&

-0.236

&

-0.414

&

0.602

Bold values are significant at $P < 0.05$.

[a] Significant at the 0.05 level.

[b] Significant at the 0.01 level.

2.2. Rice–upland crop rotations changed the AMF OTU richness and diversity

We obtained 216,382 high-quality sequences from the nine samples, which ranged from 23,052 to 24,847 per sample. The Good's coverage indices of the nine samples were all greater than 99.98%, indicating that the sequence numbers captured in this study were sufficient to evaluate the AMF diversity.

The diversity levels indicated by the Shannon's and Simpson indices significantly changed in response to the 8-year paddy rice–upland crop rotations (Table 3). Shannon's index, which ranged from 1.98 to 2.53, was lower in RV and higher in RW, compared with RF. The ACE and Chao1 indices of AMF richness were not significantly changed.

Table 3 Estimators of AMF diversity and richness during rice–upland crop rotations.

OTUsShannonSimpsonACEChao1RF36±1a2.41±0.02b0.13±0.01b41.31±0.95a40.53±0.65aRV33±1b2.02±0.01

properties were calculated for each treatment replicate (n = 6). Data are the means ± standard deviations. Values followed by different letters are significantly different ($P < 0.05$) as assessed by Duncan's test. RF (rice–fallow rotations), RW (rice–wheat rotations), and r RV (ice–Chinese milk vetch rotations)

The relationships between soil properties and AMF diversity are shown in Table S1. Shannon's index was accompanied by a corresponding decrease in pH (R= −0.879, P < 0.01), but greatly correlated with soil electrical conductivity (R = 0.817, P < 0.01). The Simpson index was accompanied by a corresponding increase in soil pH.

2.3. Rice–upland crop rotations changed the AMF community composition

The analysis of similarities results demonstrated that the AMF community in RF was significantly different from those in RV and RW (R^2 = 0.82 and 0.52, P < 0.01), and the AMF community was also significant different between RV and RW (R^2 = 0.82, P < 0.01) (Table S2). Although the AMF communities significantly differed from each other, we found that the AMF community in RW was more similar to that in RF than the community in RV. The PCoA profile also illustrated that the AMF community in RW clustered more closely with the RF than with the RV community (Fig. 1).

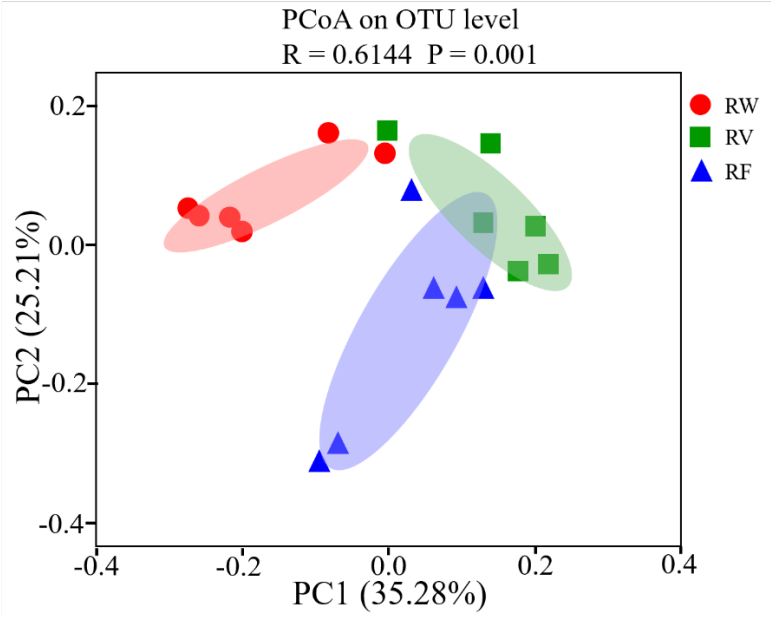

Fig. 1 Principal coordinates analysis (PCoA) of the -diversity in response to rice–upland crop rotations.

The AMF community's composition in the different rotations was further investigated. The AMF sequences were affiliated with five families. Glomeraceae was the dominant family in all the rotations, comprising 79.5%–84.7% of the total OTUs, followed by Diversisporaceae (9.7%–15.7%), Paraglomeraceae (0%–1.1%), Archaeosporaceae (0.1%–1.9%), and Acaulosporaceae (0.2%–1.1%) (Fig. 2). The proportion of the Glomeraceae family in RV was significantly lower than in RF and RW by 6.2% and 5.4%, respectively. However, the most abundant family in RV was Diversisporaceae, being 1.2 and 1.6 times greater than in RF and RW, respectively. The relative abundances of Paraglomeraceae and Acaulosporaceae were greater in RV and RW than in RF, whereas Archaeosporaceae was more abundant in RF and RV than in RW.

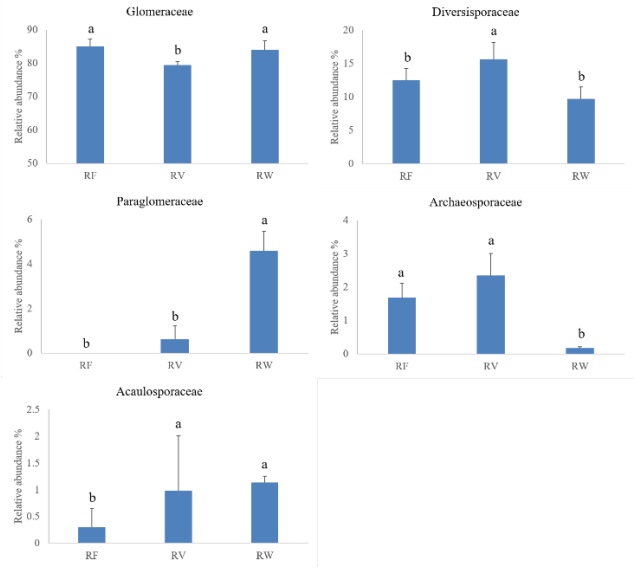

Fig. 2 Relative abundances of arbuscular mycorrhizal fungal families during rice–upland crop rotations. Vertical bars represent the standard deviations (n = 6), and the different letters above the columns denote significant differences ($P < 0.05$, Duncan's test).

The effects of rice–upland crop rotations varied among trait-based AMF guilds (Fig. 3). The RV treatment significantly decreased the rhizophilic guild by 8.5% (Fig. 3A), but increased the edaphophilic guild by 45.5% (Fig. 3B), compared with the RF treatment. However, the RW treatment decreased the edaphophilic guild by 27.9% and increased the rhizophilic guild by 4.9% compared with the RF treatment.

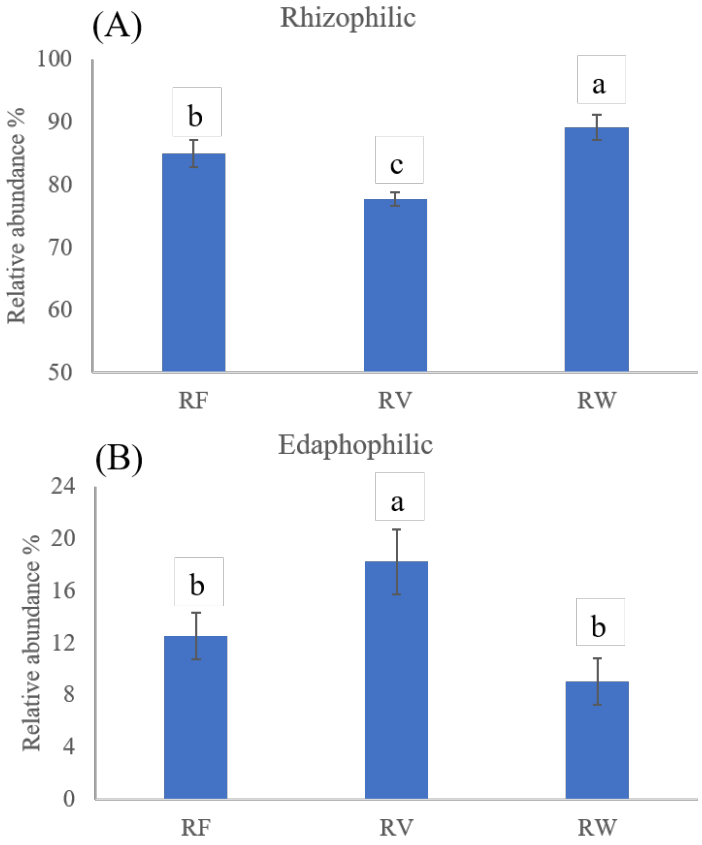

Fig. 3 Effects of rice–upland crop rotations on the relative abundance of (A) rhizophilic and (B) edaphophilic guilds. Vertical bars represent the standard deviations (n = 6), and the different letters above the columns denote significant differences ($P < 0.05$, Duncan's test).

2.4 Factors influencing AMF responses

We analyzed the correlations of soil properties with the AMF community, rhizophilic guild, and edaphophilic guild. All the soil properties explained 54.37%, 88.60%, and 54.34% of the variety in the AMF community, rhizophilic guild, and edaphophilic guild, respectively. Soil pH, available P, and the soil N:P ratio were significantly correlated with the AMF community and rhizophilic guild, while the edaphophilic guild was only significantly correlated with soil pH (Fig. 4). At the family level, the relative abundance of Diversisporaceae, which belong to edaphophilic guild, was significantly correlated only with soil pH. The abundance of the rhizophilic guild family Paraglomeraceae was significantly correlated with soil pH, available P and N:P ratio (Table 2).

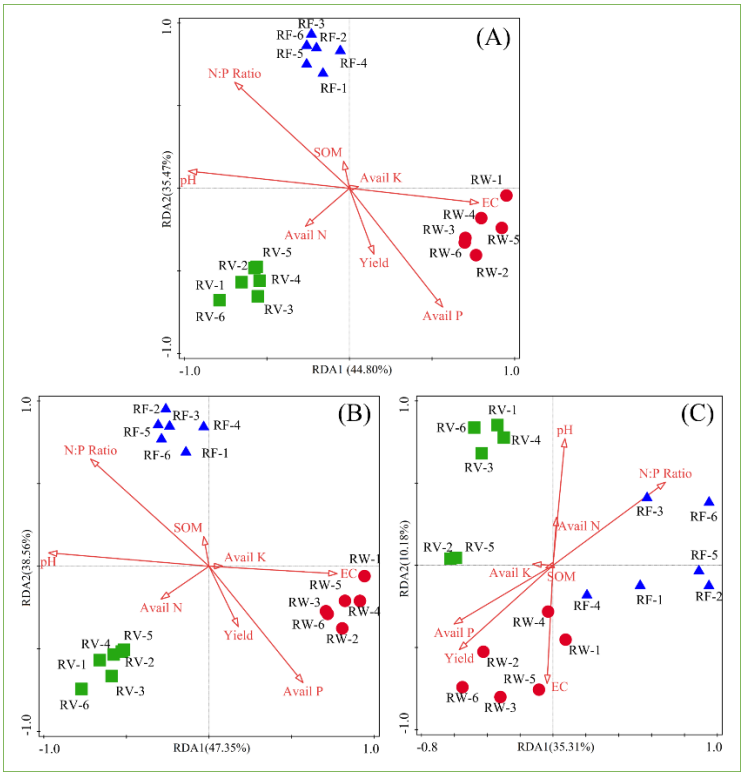

Fig. 4 Redundancy analysis (RDA) of the relationships between soil physiochemical characteristics and AMF communities during rice–upland crop rotations. (A) AMF communities; (B) rhizophilic guild; and (C) edaphophilic guild. Soil factors indicated in red text are available potassium, Avail K; available phosphorus, Avail P; pH; available N, Avail N; soil organic matter, SOM; yield; Avail N:Avail P ratio, N:P ratio; and electrical, EC.

2.5 Ecological relationships between soil properties, AMF community structure and crop production

The integrated responses of the overall AMF composition, soil properties, and rice production were studied using SEM, which elucidated soil properties, AMF community structures and rice production during paddy rice-–upland crop rotations. The model proved a good fit to the data (^{2}/df = 0.495, P = 0.686) and accounted for 60% of the variation in soil available P, 42%, 95% and 81% of the variation in AMF richness, composition and diversity, respectively, and 95% and 60% of the variation in AMF colonization rate and rice production, respectively (Fig. 5). This analysis provided further statistical evidence that AMF colonization rate was the main factor influencing rice production through their significant negative correlation. However, AMF colonization rate was directly or indirectly influenced by paddy rice–upland crop rotations. The soil pH could directly alter AMF colonization rate or indirectly alter it by influencing

AMF community. The soil available P could alter AMF colonization rate by influencing the AMF diversity level.

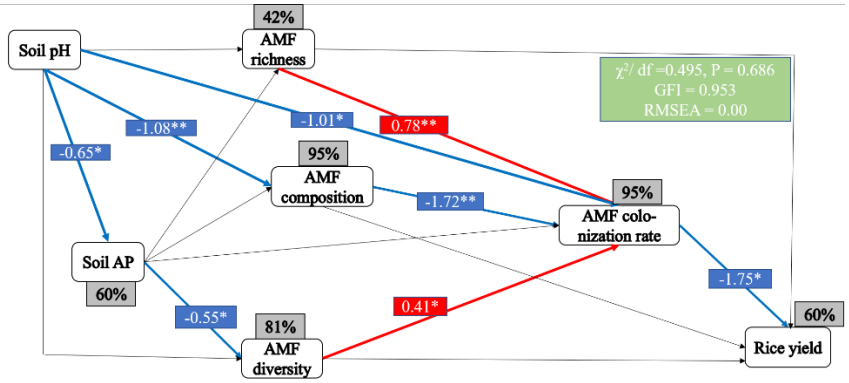

Fig. 5 Structural equation modeling (SEM) of the relationships among soil properties, AMF diversity, composition and colonization, and rice yield during rice–upland crop rotations. The model resulted in a good fit to the data, with $^2/\mathrm{df} = 0.485$, $\mathrm{P} = 0.686$, $\mathrm{RMSEA} = 0.000$ and $\mathrm{GFI} = 0.953$. Red arrows indicate positive correlations, while blue indicate negative correlations ($\mathrm{P} < 0.05$). The numbers are the correlation coefficients. Percentages close to variables indicate the variance accounted for by the model (R2).

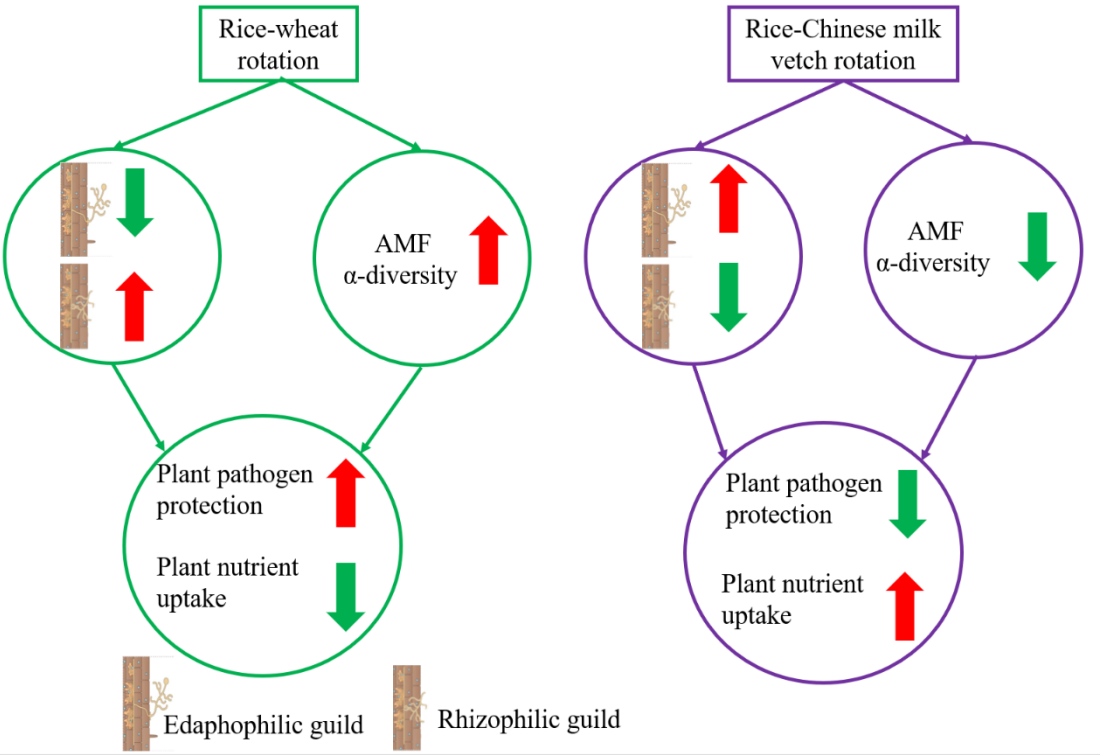

Fig. 6 Impacts of rice–wheat and rice–Chinese milk vetch rotations on soil AMF communities and their potential functions.

3. DISCUSSION

Rice–upland crop rotations are now considered important management practices in sustainable agriculture. These rotations, coupled with the return of the straw to fields, enhance rice production (Zhang et al., 2017; Zhou et al., 2020). Here, RW and RV rotations increased rice yields by 1%–2% compared with the RF treatment when the fertilizer regimes were constant (Table 1). However, the characteristics of the underground microbial communities in response to this practice are largely unclear, especially that of the functional AMF. A 31-year rice–upland crop rotation indicated that soil pH, total K, and rice yield are significantly associated with soil bacterial communities (Zhang et al., 2017). However, the 31-year treatment significantly changed most soil properties, and we cannot determine whether the changes in the microbial community resulted from one of the soil properties or the combination of changes in all the soil properties. By using an 8-year rice–upland crop rotation experiment, we could distinguish which soil property altered the microbial community because most soil properties were not significantly changed (Table 1). In this study, we only found soil pH and available P changed significantly. Soil pH significantly de-

creased in the RW rotation (Table 1), compared with the RF rotation, which was in good agreement with Hao et al. (2019). This may be to the result of straw return and root exudates, such as organic acids, that decrease soil pH. For, example, Mommer et al. (2016) found that *Aspalathus linearis* L. could exudate OH^- and HCO_3^- in its root exudate and change its rhizospheric pH.

In the present study, we found that the AMF Shannon index significantly increased under the RW treatment, which may result from the rice–upland crop rotations significantly changing the soil pH, and the soil pH was significantly correlated with Shannon index (Table S1). In addition, wheat roots exudates, which are mainly composed of sugars, attract a wide range of microbes (Iannucci et al., 2021). The greater diversity may result in a more stable agroecosystem and greater pathogen resistance (Wang et al., 2020). Here, to our surprise, the AMF Shannon index significantly decreased as a result of the 8-year RV treatment, possibly because of great increases in some AMF taxa. For example, OTU21 occupied almost half of the sequence (45%) in RV, but only occupied 24% and 21% of the sequences in RF and RW, respectively (Table S3). A lower AMF diversity has been shown to decrease ecosystem productivity and to increase ecosystem instability (Maček et al., 2011), but it may lead to high-speed element cycling and nutrient acquisition (Fan et al., 2017). This result may indicate that the RV rotation increased the AMF acquisition of nutrients by sacrificing ecosystem stability. In addition, the loss of AMF diversity was directly changed by soil available P, while the AMF community composition was mainly influenced by soil pH (Fig. 5). This result was in agreement with those of Wang et al. (2020), in which the soil pH was the main factor that influenced AMF community but did not significantly correlate with AMF species diversity. A potential reason for this dynamic is that the soil pH decreased the pH sensitive AMF species but increased the neutral species. A higher soil available P may directly change the AMF diversity by reducing the benefits provided by these symbionts and some species that cannot obtain enough C may undergo extirpation. This result indicated that soil pH directly affected AMF composition, while soil available P directly affected AMF diversity.

We found that different rice–upland crop rotations had significant effects on overall AMF abundance (Table 1), which was consistent with another study (Cofré et al., 2017). Unlike the previous study, we analyzed two indicators of AMF abundance: spore density in soil and root colonization rate in host roots. We found that RW and RV rotations increased AMF spore density, but decreased AMF colonization rate (Table 1). This may result from root exudates that provide a more suitable environment for AMF spores in soil, whereas plants may select for special species by changing components of their root exudates (Turner et al., 2013; Bulgarelli et al., 2015). Thus, only the required AMF species were selected. Thus, the RV rotation affected AMF abundance in the soil (spore density) and roots (AMF colonization rate) in different ways.

Because AMF allocate most of their biomasses to plant roots or soil, we grouped them into different guilds, namely the rhizophilic or edaphophilic guild, respec-

tively (Weber et al., 2019). In our study, the relative abundance of rhizophilic guild was lower, while the edaphophilic was higher under RV treatment compared with RF. A previous study showed that those two guilds appear to play a role in reducing root pathogen infection (Sikes et al., 2010) and increasing plant nutrient uptake via their extensive extra-radical mycelium (Sikes et al., 2010; Weber et al., 2019; Han et al., 2020). The decrease of the rhizophilic guild and increase of the edaphophilic guild under RV compared with RF indicated that the rotation between rice and Chinese milk vetch increased rice nutrient uptake, but led to less resistance to pathogen infection. This is consistent with the decreased soil AMF diversity from RV treatment (Table 3), as lower diversity reduces pathogen resistance. However in RW treatment, the oppose trend was observed. The increase of the rhizophilic guild and decrease of the edaphophilic guild under RW compared with RF indicated that the rotation between rice and wheat decreased rice nutrient uptake, but increased pathogen resistance. This result is consistent with a study that found that wheat root exudates could enrich pathogen-resistant microbial species (Fan et al., 2017), and with our result that RW treatment increased soil AMF diversity and pathogen resistance (Table 3). In summary, those results offer evidence that the rotation between rice and Chinese milk vetch increases rice nutrient uptake, but leads to less resistance to pathogen infection, whereas the rotation between rice and wheat decreases rice nutrient uptake, but increases pathogen resistance (Fig. 6).

We found that AMF was significantly affected by soil pH, available P, and the soil N:P ratio (Fig. 4A). These results are in agreement with those of previous studies (Han et al., 2020; Wang et al., 2020). However, when we divided AMF species into two guilds according to their characteristics, we found that the rhizophilic guild was significantly affected by soil pH, available P, and the soil N:P ratio (Fig. 4B). This may be because the rhizophilic guild is considered to colonize roots heavily, and these colonized roots are more sensitive to soil nutrients, especially the soil P level (Vázquez et al., 2020). However, the edaphophilic guild was only affected by soil pH (Fig. 4C). This may be because the edaphophilic guild allocates more biomass to soils and colonizes plant roots less. These features make the edaphophilic guild more sensitive to soil acidification (Zhou et al., 2016), and less affected by soil nutrients, especially available P.

Some studies found that plants could allocate up to 30% of their photosynthate to colonized AMF (Johnson et al., 1997; Johnson, 2010; Wang et al., 2020). The heavy consumption of C resources from plants could be responsible for the negative growth responses to AMF (Graham and Abbott, 2000; Li et al., 2008; Elliott et al., 2020). In our study, we found that AMF colonization rate was negatively correlated with rice yield (Table 2). This is perhaps because croplands usually receive ample fertilizer and are maintained for a long period, such that the soil is not deprived of P (Bakhshandeh et al., 2017; Ercoli et al., 2017; Wang et al., 2017; Han et al., 2020). Therefore, in such P-rich cropland soil, host plants can acquire P from the soil by their roots other than AMF, but plants need to provide more C to the colonized AMF. This may explain

why high AMF colonization rate decreased rice yield in our study. In addition, SEM showed that AMF colonization rate directly decreased rice yield in these rice–plant rotation ecosystems (Fig. 5). This is evidence that higher arbuscular mycorrhizal fungi colonization rate did not increase rice yield in this 8-year rice-upland crop rotation ecosystem.

4 CONCLUSIONS

In summary, we found that RW and RV rotations significantly changed the soil AMF diversity, abundance and composition, but in different ways. The AMF diversity was increased by RW rotations, while decreased by RV rotation. The rhizophilic guild diversity of AMF was increased by RW rotation, which may enhance AMF-related pathogenic resistance. However, the RV treatment decreased increased the AMF edaphophilic guild, and this may increase plant nutrient acquisition. Soil available P and the N:P ratio significantly influence rhizophilic guilds composition, but not edaphophilic guilds composition. Our results also showed that AMF colonization rate directly correlated with rice yield and that higher colonization rate levels decreased rice yields in an 8-year rice–upland crop rotation.

ACKNOWLEDGMENTS

Redacted

DECLARATION OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Bakhshandeh, S., Corneo, P. E., Mariotte, P., Kertesz, M. A., & Dijkstra, F. A. (2017). Effect of crop rotation on mycorrhizal colonization and wheat yield under different fertilizer treatments. *Agriculture, Ecosystems & Environment*, 247, 130-136. https://doi.org/10.1016/j.agee.2017.06.027.

Bever, J. D., Dickie, I. A., Facelli, E., Facelli, J. M., Klironomos, J., Moora, M., Rillig, M. C., Stock, W. D., Tibbett, M., & Zobel, M. (2010). Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology & Evolution*, 25, 468-478. https://doi.org/10.1016/j.tree.2010.05.004.

Bodenhausen, N., Horton, M. W., & Bergelson, J. (2013). Bacterial communities associated with the leaves and the roots of Arabidopsis thaliana. *PLoS One,* 8, e56329. https://doi.org/10.1371/journal.pone.0056329.

Bulgarelli, D., Garrido-Oter, R., Münch, P. C., Weiman, A., Dröge, J., Pan, Y., McHardy, A. C., & Schulze-Lefert, P. (2015). Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell Host & Microbe*. 17, 392-403. https://doi.org/10.1016/j.chom.2015.01.011

Cheng, Y., Ishimoto, K., Kuriyama, Y., Osaki, M., & Ezawa, T. (2013). Ninety-year-, but not single, application of phosphorus fertilizer has a major impact on arbuscular mycorrhizal fungal communities. *Plant and Soil*, 365, 397-407. https://doi.org/10.1007/s11104-012-1398-x.

Dennis, P. G., Miller, A. J., & Hirsch, P. R. (2010). Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? *FEMS Microbiology Ecology*, 72, 313-327. https://doi.org/10.1111/j.15746941.2010.00860.x

Dueñas, J. F., Camenzind, T. , Roy, J., Hempel, S., Homeier, J., Suárez, J. P., & Rillig, M. C. (2020). Moderate phosphorus additions consistently affect community composition of arbuscular mycorrhizal fungi in tropical montane forests in southern Ecuador. *New Phytologist*, 227, 1505-1518. https://doi.org/10.1111/nph.16641.

Edgar, R. C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature methods*, 10, 996-998. https://doi.org/10.1038/nmeth.2604

Elliott, A. J., Daniell, T. J., Cameron, D. D., & Field, K. J. (2020). A commercial arbuscular mycorrhizal inoculum increases root colonization across wheat cultivars but does not increase assimilation of mycorrhiza-acquired nutrients. *Plants People Planet*, 00, 1-12. https://doi.org/10.1002/ppp3.10094

Ellouze, W., Hamel, C., DePauw, R. M., Knox, R. E., Cuthbert, R. D., & Singh, A. K. (2015). Potential to breed for mycorrhizal association in durum wheat. *Canadian Journal of Microbiology*, 62, 263-271. https://doi.org/10.1139/cjm-2014-0598.

Ercoli, L., Schüßler, A., Arduini, I., & Pellegrino, E. (2017). Strong increase of durum wheat iron and zinc content by field-inoculation with arbuscular mycorrhizal fungi at different soil nitrogen availabilities. *Plant and Soil*, 419, 153-167. https://doi.org/10.1007/s11104-017-3319-5.

Fan, K., Cardona, C., Li, Y., Shi, Y., Xiang, X., Shen, C., Wang, H., Gilbert, J. A., & Chu, H. (2017). Rhizosphere-associated bacterial network structure and spatial distribution differ significantly from bulk soil in wheat crop fields. *Soil Biology and Biochemistry*, 113, 275-284. https://doi.org/10.1016/j.soilbio.2017.06.020.

García-González, I., Quemada, M., Gabriel, J. L., Alonso-Ayuso, M., & Hontoria, C. (2018). Legacy of eight-year cover cropping on mycorrhizae, soil, and plants. *Journal of Plant Nutrition and Soil Science*, 181, 818-826. https://doi.org/10.1002/jpln.201700591.

García-González, I., Quemada, M., Gabriel, J. L., & Hontoria, C. (2016). Arbuscular mycorrhizal fungal activity responses to winter cover crops in a sunflower and maize cropping system. *Applied Soil Ecology*, 102, 10-18. https://doi.org/10.1016/j.apsoil.2016.02.006.

Graham, J. H., & Abbott, L. K. (2000). Wheat responses to aggressive and non-aggressive arbuscular mycorrhizal fungi. *Plant and Soil*. 220, 207-218. https://doi.org/10.1023/A:1004709209009.

Han, Y., Feng, J., Han, M., & Zhu, B. (2020). Responses of arbuscular mycorrhizal fungi to nitrogen addition: A meta-analysis. *Global Change Biology*, 26, 7229-7241. https://doi.org/10.1111/gcb.15369.

Hao, M., Hu, H., Liu, Z., Dong, Q., Sun, K., Feng, Y., Li, G., & Ning, T. (2019). Shifts in microbial community and carbon sequestration in farmland soil under long-term conservation tillage and straw returning. *Applied Soil Ecology*, 136, 43-54. https://doi.org/10.1016/j.apsoil.2018.12.016.

Helmke, P. A., & Sparks, D. L. (1996). Lithium, sodium, potassium, rubidium and cesium. In: Sparks, D.L. (Ed.), Methods of Soil Analysis Part 3: Chemical Methods. *Soil Science Society of America Journal*, 551-574.

Higo, M., Isobe, K., Yamaguchi, M., Drijber, R. A., Jeske, E. S., & Ishii, R. (2013). Diversity and vertical distribution of indigenous arbuscular mycorrhizal fungi under two soybean rotational systems. *Biology and Fertility of Soils*, 49, 1085-1096. https://doi.org/10.1007/s00374-013-0807-5.

Hontoria, C., García-González, I., Quemada, M., Roldán, A., & Alguacil, M. M. (2019). The cover crop determines the AMF community composition in soil and in roots of maize after a ten-year continuous crop rotation. *Science of The Total Environment*, 660, 913-922. https://doi.org/10.1016/j.scitotenv.2019.01.095.

Iannucci, A., Canfora, L., Nigro, F., De Vita, P., & Beleggia, R. (2021). Relationships between root morphology, root exudate compounds and rhizosphere microbial community in durum wheat. *Applied Soil Ecology*, 158, 103781. https://doi.org/10.1016/j.apsoil.2020.103781.

Johnson, N. C. (2010). Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. *New Phytologist*, 185, 631. https://doi.org/10.1111/j.1469-8137.2009.03110.x.

Johnson, N. C., Graham, J., & Smith, F. (1997). Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytologist*, 135, 575-585. https://doi.org/10.1046/j.1469-8137.1997.00729.x.

Koske, R., & Gemma, J. (1989). A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research*, 92, 486-488. https://doi.org/10.1016/S0953-7562(89)80195-9.

Li, H., Smith, F. A., Dickson, S., Holloway, R. E., & Smith, S. E. (2008). Plant growth depressions in arbuscular mycorrhizal symbioses: not just caused by carbon drain? *New Phytologist*, 178, 852-862. https://doi.org/10.1111/j.1469-8137.2008.02410.x.

Li, T., Hu, Y. J., Hao, Z. P., Li, H., Wang, Y. S., & Chen, B. D. (2013). First cloning and characterization of two functional aquaporin genes from an

arbuscular mycorrhizal fungus Glomus intraradices. *New Phytologist*, 197, 617. https://doi.org/10.1111/nph.12011.

Maček, I., Dumbrell, A. J., Nelson, M., Fitter, A. H., Vodnik, D., & Helgason, T. (2011). Local adaptation to soil hypoxia determines the structure of an arbuscular mycorrhizal fungal community in roots from natural CO2 springs. *Applied and Environmental Microbiology*, 77, 4770-4777. DOI:10.1128/AEM.00139-11

Mäder, P., Edenhofer, S., Boller, T., Wiemken, A., & Niggli, U. (2000). Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic, biological) and high-input (conventional) farming systems in a crop rotation. *Biology and fertility of Soils*, 31, 150-156. https://doi.org/10.1007/s003740050638.

Maherali, H., & Klironomos, J. N. (2007). Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science*, 316, 1746-1748. DOI: 10.1126/science.1143082

McGonigle, T., Miller, M., Evans, D., Fairchild, G., & Swan, J. (1990). A new method which gives an objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi. *New phytologist*, 115, 495-501.

Mommer, L., Kirkegaard, J., & van Ruijven, J. (2016). Root–root interactions: towards a rhizosphere framework. *Trends in Plant Science*, 21, 209-217. https://doi.org/10.1016/j.tplants.2016.01.009

Noelia Cofré, M., Ferrari, A., Becerra, A., Domínguez, L., Wall, L. G., & Urcelay, C. (2017). Effects of cropping systems under no-till agriculture on arbuscular mycorrhizal fungi in Argentinean Pampas. *Soil Use and Management*, 33, 364-378. https://doi.org/10.1111/sum.12349.

Poosakkannu, A., Nissinen, R., & Kytöviita, M.-M. (2017). Native arbuscular mycorrhizal symbiosis alters foliar bacterial community composition. *Mycorrhiza*. 27, 801-810. https://doi.org/10.1007/s00572-017-0796-6.

Qin, H., Lu, K., Strong, P., Xu, Q., Wu, Q., Xu, Z., Xu, J., & Wang, H. (2015). Long-term fertilizer application effects on the soil, root arbuscular mycorrhizal fungi and community composition in rotation agriculture. *Applied Soil Ecology*, 89, 35-43. https://doi.org/10.1016/j.apsoil.2015.01.008.

Rillig, M. C., & Mummey, D. L. (2006). Mycorrhizas and soil structure. *New Phytologist,* 171, 41-53. https://doi.org/10.1111/j.1469-8137.2006.01750.x.

Sawers, R. J. H., Svane, S. F., Quan, C., Grønlund, M., Wozniak, B., Gebreselassie, M.-N., González-Muñoz, E., Chávez Montes, R. A., Baxter, I., Goudet, J., Jakobsen, I., & Paszkowski, U. (2017). Phosphorus acquisition efficiency in arbuscular mycorrhizal maize is correlated with the abundance of root-external hyphae and the accumulation of transcripts encoding PHT1 phosphate transporters. *New Phytologist*, 214, 632-643. https://doi.org/10.1111/nph.14403.

Shi, Z., Yin, K., Wang, F., Mickan, B. S., Wang, X., Zhou, W., & Li, Y. (2019). Alterations of arbuscular mycorrhizal fungal diversity

in soil with elevation in tropical forests of China. *Diversity*, 11, 181. https://doi.org/10.3390/d11100181.

Sikes, B. A., Powell, J. R., & Rillig, M. C. (2010). Deciphering the relative contributions of multiple functions within plant–microbe symbioses. *Ecology*, 91, 1591-1597. https://doi.org/10.1890/09-1858.1.

Silva-Flores, P., Bueno, C. G., Neira, J., & Palfner, G. (2019). Factors affecting arbuscular mycorrhizal fungi spore density in the Chilean mediterranean-type ecosystem. *Journal of Soil Science and Plant Nutrition*, 19, 42-50. https://doi.org/10.1007/s42729-018-0004-6.

Smith, S. E., & Smith, F. A. (2012). Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. *Mycologia*, 104, 1-13. https://doi.org/10.3852/11-229.

Strickland, M. S., & Rousk, J. (2010). Considering fungal: bacterial dominance in soils Methods, controls, and ecosystem implications. *Soil Biology and Biochemistry*, 42, 1385-1395.

Suzuki, K., Takahashi, K., & Harada, N.(2020). Evaluation of primer pairs for studying arbuscular mycorrhizal fungal community compositions using a MiSeq platform. *Biology and Fertility of Soils*, 56, 853-858. https://doi.org/10.1007/s00374-020-01431-6.

Turner, T. R., Ramakrishnan, K., Walshaw, J., Heavens, D., Alston, M., Swarbreck, D., Osbourn, A., Grant, A., & Poole, P. S. (2013). Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants. *The ISME journal*, 7, 2248-2258. https://doi.org/10.1038/ismej.2013.119.

van der Heijden, M. G. A., Martin, F. M., Selosse, M.-A., & Sanders, I. R. (2015). Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist*, 205, 1406-1423. https://doi.org/10.1111/nph.13288.

Vázquez, E., Benito, M., Espejo, R., & Teutscherova, N. (2020). No-tillage and liming increase the root mycorrhizal colonization, plant biomass and N content of a mixed oat and vetch crop. *Soil and Tillage Research*, 200, 104623. https://doi.org/10.1016/j.still.2020.104623.

Verbruggen, E., Röling, W. F., Gamper, H. A., Kowalchuk, G. A., Verhoef, H. A., & Mg, V. D. H. (2010). Positive effects of organic farming on below-ground mutualists: large-scale comparison of mycorrhizal fungal communities in agricultural soils. *New Phytologist*, 186, 968. https://doi.org/10.1111/j.1469-8137.2010.03230.x.

Wang, Q., Jiang, X., Guan, D., Wei, D., Zhao, B., Ma, M., Chen, S., Li, L., Cao, F., & Li, J. (2017). Long-term fertilization changes bacterial diversity and bacterial communities in the maize rhizosphere of Chinese Mollisols. *Applied Soil Ecology*, 125, 88-96. https://doi.org/10.1016/j.apsoil.2017.12.007

Wang, Q., Ma, M., Jiang, X., Guan, D., & Li, J. (2020). Influence of 37 years of nitrogen and phosphorus fertilization on composition of rhizosphere arbuscular mycorrhizal fungi communities in black soil of northeast China. *Frontiers in Microbiology*, 11, 539669. https://doi.org/10.3389/fmicb.2020.539669.

Watts-Williams, S. J., Cavagnaro, T. R., & Tyerman, S. D. (2019). Variable effects of arbuscular mycorrhizal fungal inoculation on physiological and molecular measures of root and stomatal conductance of diverse Medicago truncatula accessions. *Plant, Cell & Environment*, 42, 285-294. https://doi.org/10.1111/pce.13369.

Weber, S. E., Diez, J. M., Andrews, L. V., Goulden, M. L., Aronson, E. L., & Allen, M. F. (2019). Responses of arbuscular mycorrhizal fungi to multiple coinciding global change drivers. *Fungal Ecology*, 40, 62-71. https://doi.org/10.1016/j.funeco.2018.11.008.

Williams, A., Manoharan, L., Rosenstock, N. P., Olsson, P. A., & Hedlund, K. (2017). Long-term agricultural fertilization alters arbuscular mycorrhizal fungal community composition and barley (Hordeum vulgare) mycorrhizal carbon and phosphorus exchange. *New Phytologist*, 213, 874-885. https://doi.org/10.1111/nph.14196.

Xiang, D., Verbruggen, E., Hu, Y., Veresoglou, S. D., Rillig, M. C., Zhou, W., Xu, T., Li, H., Hao, Z., & Chen, Y. (2014). Land use influences arbuscular mycorrhizal fungal communities in the farming–pastoral ecotone of northern China. *New Phytologist*, 204, 968-978. https://doi.org/10.1111/nph.12961.

Xu, J., Liu, S., Song, S., Guo, H., Tang, J., Yong, J. W. H., Ma, Y., & Chen, X. (2018). Arbuscular mycorrhizal fungi influence decomposition and the associated soil microbial community under different soil phosphorus availability. *Soil Biology and Biochemistry*, 120, 181-190. https://doi.org/10.1016/j.soilbio.2018.02.010.

Yang, G., Liu, N., Lu, W., Wang, S., Kan, H., Zhang, Y., Xu, L., & Chen, Y. (2014). The interaction between arbuscular mycorrhizal fungi and soil phosphorus availability influences plant community productivity and ecosystem stability. *Journal of Ecology*, 102, 1072-1082. https://doi.org/10.1111/1365-2745.12249.

Yousuf, B., Sanadhya, P., Keshri, J., & Jha, B. (2012). Comparative molecular analysis of chemolithoautotrophic bacterial diversity and community structure from coastal saline soils, Gujarat, India. BMC Microbiology, 12, 150. https://doi.org/10.1186/1471-2180-12-150.

Zeng, J., Liu, X., Song, L., Lin, X., Zhang, H., Shen, C., & Chu, H. (2016). Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. *Soil Biology and Biochemistry*, 92, 41-49. https://doi.org/10.1016/j.soilbio.2015.09.018.

Zhang, X., Zhang, R., Gao, J., Wang, X., Fan, F., Ma, X., Yin, H., Zhang, C., Feng, K., & Deng, Y. (2017). Thirty-one years of rice-rice-

green manure rotations shape the rhizosphere microbial community and enrich beneficial bacteria. *Soil Biology and Biochemistry*, 104, 208-217. https://doi.org/10.1016/j.soilbio.2016.10.023.

Zhou, G., Cao, W., Bai, J., Xu, C., Zeng, N., Gao, S., Rees, R. M., & Dou, F. (2020). Co-incorporation of rice straw and leguminous green manure can increase soil available nitrogen (N) and reduce carbon and N losses: An incubation study. *Pedosphere*, 30, 661-670. https://doi.org/10.1016/S1002-0160(19)60845-3

Zhou, J., Guan, D., Zhou, B., Zhao, B., Ma, M., Qin, J., Jiang, X., Chen, S., Cao, F., Shen, D., & Li, J. (2015). Influence of 34-years of fertilization on bacterial communities in an intensively cultivated black soil in northeast China. *Soil Biology and Biochemistry*, 90, 42-51. https://doi.org/10.1016/j.soilbio.2015.07.005.

Zhou, J., Jiang, X., Zhou, B., Zhao, B., Ma, M., Guan, D., Li, J., Chen, S., Cao, F., Shen, D., & Qin, J. (2016). Thirty four years of nitrogen fertilization decreases fungal diversity and alters fungal community composition in black soil in northeast China. *Soil Biology and Biochemistry*, 95, 135-143. https://doi.org/10.1016/j.soilbio.2015.12.012.

Zhou, W., Lv, T.-F., Chen, Y., Westby, A. P., & Ren, W. J. (2014). Soil physicochemical and biological properties of paddy-upland rotation: A Review. *Scientific World journal*, 2014, 856352. https://doi.10.1155/2014/856352.