Long-term nitrogen and phosphorus additions change community diversity of arbuscular mycorrhizal fungi and crop yield are mainly determined by Glomus and Paraglomus in the Loess Plateau

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Research Article

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Abstract

Aims

Arbuscular mycorrhizal fungi (AMF) can form mutualistic symbiosis with more than 80% of plant roots on land, playing an important role in promoting plant growth. Similarly, nitrogen (N) and phosphorus (P) addition, a common fertilization practice in the Loess Plateau, China, can improve crop yield, and further has an important effect on soil microbial community. However, the potential effects of long-term N and P addition on AMF community structure in farmland system in loess arid region are still unclear.

Methods

Based on the Changwu Agricultural Ecology Experimental Station of the Chinese Academy of Sciences, this study set three N and P concentration gradients: low (0 kg hm^{-2}), medium (90 kg hm^{-2}) and high (180 kg hm^{-2}), and carried out a 3 × 3 complete interaction experiment with 9 treatments, which were CK, N_{12}, N_{24}, P_{12}, $N_{12}P_{12}$, $N_{24}P_{12}$, P_{24}, $N_{12}P_{24}$, $N_{24}P_{24}$. In this study, we combined high-throughput sequencing technology with traditional biochemical methods to analyze the composition and structural diversity of AMF community. Besides, correlation analysis between AMF community composition, soil environmental factors and crop yield was also preformed.

Results

Our results showed that AMF community diversity increased significantly under the N and P interaction. The AMF community composition was differentiated by N and P additions across the concentration gradients, driven mostly by the available phosphorus (AP), total phosphorus (TP) and pH as the main environmental factors. Correlation analysis revealed a significant positive correlation between *Glomus* and wheat yield, *Paraglomus* opposite.

Conclusions

Long-term N and P addition not only directly increased the crop yield, but also affected wheat yield indirectly by affecting soil physicochemical properties and AMF community diversity. Rational N and P application could improve the ecological and physiological functions of AMF community, which was of positive significance for improving crop yield and agricultural sustainable development.

Highlights

1. N and P interaction treatment enhanced AMF community structure diversity.

2. pH and AP significantly affect AMF community.

3. Relative abundance of *Glomus* increased due to N and P addition, *Paraglomus* opposite.

4. Long-term N and P addition and AMF community diversity directly affect crop yield.

Introducation

Arbuscular mycorrhizal fungi (AMF), one of the most widely distributed fungi, can form mutualism symbionts with more than 80% of plant roots on land (Treseder and Cross, 2006). Its vast mycelium network links multiple plant roots together to promote nutrient migration and transformation between plants (Cairney and Burke, 1996). AMF can resist drought stress (Augé, 2001), increase soil stability (Garg and Chandel, 2011), improve soil structure (Sharmah and Jha, 2014) ,and increase crop yield (Zhang et al., 2019). In addition, it can enhance the absorption capacity and stress resistance of plant nutrient elements, which is of great significance to maintain the balance and sustainable development of agricultural ecosystem (Hodge and Fitter, 2010; Rillig and Mummey, 2006).

Fertilization can improve plant biomass yield, change soil nutrient status and regulate soil microbial activity (Geisseler and Scow, 2014). Both the amount of fertilizer application and fertilizer type can affect the structure and function of soil microbial community. Studies have shown that long-term fertilization has an important impact on the structure and composition of AMF community (Wu et al., 2010). Wang et al., (2020b) found that long-term application of N fertilizer and N and P mixture could change the composition of soil AMF community in black soil farmland in Northeast China, while nitrogen application alone had no significant effect on AMF diversity. Nitrogen application would increase soil nitrogen concentration, thus reducing AMF biomass, species richness and diversity (Egerton-Warburton et al., 2007; Verbruggen et al., 2011). In areas where phosphorus content is scarce, a small amount of inorganic phosphorus fertilizer can change AMF community composition, contribute to mycorrhizal formation and protect AMF diversity (Alguacil et al., 2010). Chen et al. (2013) found that short-term addition of P fertilizer could significantly improve the diversity of AMF. Chen et al. (2014) studied the AMF biodiversity in the temperate grassland ecosystem, and the results showed that nitrogen application changed the species composition of AMF in this area, and P application had a significant impact on the richness of AMF. However, some studies found that adding different forms of phosphorus had no effect on AMF community composition (Beauregard et al., 2013). Furthermore, Adeyemi et al. (2019) found that AMF inoculation increased soybean growth and nodules by 3 to 5 times. Wang et al. (2020a) showed that reasonable N and P addition was beneficial to the infection of AMF and wheat root system, and played a positive role in promoting plant growth and food health. Overall, applying N and P fertilizer in a reasonable proportion can not only improve AMF community structure, but also play an important role in maintaining the stability of farmland ecosystem (Johnson et al., 2010).

The Loess Plateau is an important grain-producing area in northwest China. Serious soil and water loss, poor fertilizer conservation ability and fragile ecological environment are the problems that need to be solved in this region (Zhang et al., 2011). Long-term fertilization is one of the important agricultural production methods in the Loess Plateau (Wu et al., 2010). As the most commonly used inorganic fertilizers, N and P fertilizer can not only improve the soil nutrient structure (Hao et al., 2015), promote plant growth and increase crop yield (Zhang et al., 2018), but also have a certain impact on the diversity of soil microbial community (Gryndler et al., 2006; Paungfoo-Lonhienne et al., 2015; Wallenstein et al., 2006). In order to deal with the problem of food security, the diversity of soil microbial community and the yield of food crops in the Loess Plateau under fertilization are particularly important. However, there are few studies on the changes of soil AMF community under long-term fertilization.

In this study, long-term N and P addition with different concentration gradients was applied to farmland soil in the Loess Plateau, high-throughput sequencing based on Illumina Miseq platform was designed to reveal the effects of long-term N and P addition on AMF community composition and diversity, and to explore the potential relationship between soil environmental factors, AMF community and crop yield. In this regard, we hypothesized: (1) Long-term N and P additions will alter AMF community composition, diversity, and species richness. (2) AMF community and crop yield are affected by soil environmental factors, as well as the amount and method of N and P fertilizer additions.

Materials And Methods

Site description

The study was conducted at the Changwu Agricultural Ecology Experimental Station of the Chinese Academy of Sciences, located in Changwu county, Shaanxi province, China (35°14' N, 107°41' E, Fig. 1), At an altitude of 1200 m, an average annual precipitation of 580 mm, and an average annual temperature of 9.1°C, which is a typical dry farming area. The soil type is Heilu soil (Cumulic Haplustoll, USDA classification). This long-term positioning study started in 1984. The planting mode was continuous wheat cropping. The experimental field was managed by the high-yield model. The initial soil nutrient status before the study was: organic matter 10.50 g kg^{-1}, total nitrogen 0.80 g kg^{-1}, total phosphorus 1.26 g kg^{-1}, pH = 8.10.

Study design

In this study, three N and P concentration gradients of low, medium and high were set, and a total of 9 treatments of 3 × 3 complete interaction experiments. N fertilizer is urea (CON_2H_4), P fertilizer is superphosphate ($Ca(H_2PO_4)_2$, calculating with the content of P_2O_5). We set the nitrogen and phosphorus application amount as 0 kg hm^{-2} at low concentration, 90 kg hm^{-2} at medium concentration, and 180 kg hm^{-2} at high concentration. The specific 9 treatments are: CK (control, N and P fertilizer were 0 kg hm^{-2}), N_{12} (N fertilizer alone was 90 kg hm^{-2}), N_{24} (N fertilizer alone was 180 kg hm^{-2}), P_{12} (P fertilizer alone was 90 kg hm^{-2}), $N_{12}P_{12}$ (N and P fertilizer were each 90 kg hm^{-2}), $N_{24}P_{12}$ (N fertilizer was 180 kg hm^{-2}, P fertilizer was 90 kg hm^{-2}), P_{24} (P fertilizer alone was 180 kg hm^{-2}), $N_{12}P_{24}$ (N fertilizer was 90 kg hm^{-2}, P fertilizer was 180 kg hm^{-2}), $N_{24}P_{24}$ (N fertilizer was 180 kg hm^{-2}, P fertilizer was 180 kg hm^{-2}). Each treatment had three replicates, and each plot area was 36 m^2 (9 m × 4 m). The experimental crop was winter wheat, and the sowing rate of wheat was 180 kg hm^{-2}. The fertilizer was spread on the surface one week before sowing ,and the ploughed into the soil.

Sample collection

The samples were collected in May 2021. Five-point sampling method was adopted to take root soil at a depth of 0 ~ 20cm. The collected soil samples were sealed and labeled, and brought back to the laboratory in time for pretreatment. After sifting by 2 mm, about 1/3 of the soil was taken out for air drying to determine soil physical and chemical indexes. Plant root samples were carefully rinsed with distilled water and stored in a refrigerator at -20℃ for DNA extraction. Crop yield (including wheat yield, aboveground biomass, kernels per spike, plant height and thousand seed weight) was measured after harvesting.

Soil physicochemical analysis

Soil pH was determined at a water-soil ratio of 2.5:1; soil total carbon (TC) was determined by carbon analyzer (Vario TOC, Elementar, Hanau, Germany); soil total nitrogen (TN) was determined by automatic Kjeldahl nitrogen analyzer; soil total phosphorus (TP) was determined by H_2SO_4-$HClO_4$ digestion molybdenum antimony anti-colorimetric method. Soil ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N): with 0.5 M K_2SO_4 solution after leaching, then they were determined by a continuous flow analyzer (Autoanalyzer 3, Bran-Luebbe, Germany). Soil available phosphorus (AP) was determined by Olsen method (Bao, 2005). Soil total organic phosphorus (OP) adopts high temperature burning method (Saunders and Williams, 1995). The soil organic carbon (SOC) was determined by potassium dichromate oxidation and external heating method.

Rhizosphere AMF biomass, AMF root colonization rate and soil spore density

The phospholippid fatty acids (PLFA) in the soil can be used to represent the biomass and structure of the corresponding microbial community (Frostegard et al., 1999). We using 19:0 methyl lipids as internal standard, the fatty acids were analyzed by MIDI Sherlock microbial identification system (Microbial ID, Inc., Newark, DE, USA). There are certain PLFAs that were used as biomarkers for indicator organisms to calculate the presence and abundance of particular microbial groups, the 16:1 ω5 cis as arbuscular mycorrhizal fungi (Zelles, 1997).

The AMF root colonization rate was determined using the method of Phillips and Hayman (1970). Briefly, fresh fine roots were washed with water and then cut into root segments of about 1 cm, digested in 10% KOH solution at 90°C for 30 min, acidified with hydrochloric acid for 5 min, stained with 5% ink vinegar, and observed under a stereomicroscope after fading. The grid crossing method was used to calculate the AMF root colonization rate. The spores were collected by the sucrose-wet sieve decantation method (An et al., 1990), and the soil spore density (SD) was calculated as the number of spores per 50 g of air-dried soil.

DNA extraction and high-throughput sequencing

Root DNA extraction and high-throughput sequencing: weigh 0.1 g of fresh root samples in a mortar, freeze them quickly with liquid nitrogen and grind them, and then use a high-efficiency plant genomic DNA extraction kit (Hi-DNAsecure Plant Kit, Tiangen Biotechnology, China) to extract root AM fungal DNA. The steps follow the instructions. DNA concentration and purity were determined with a NanoDrop-1000 ultramicro spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). The V4 region fragment of SSU rDNA gene was amplified (Lumini et al., 2010), and the primer sequences were AMV4.5NF (5'-AAGCTCGTAGTTGAATTTCG-3') and AMDGR (5'-CCCAACTATCCCTATTAATCAT-3'). The PCR products were purified by agarose gel electrophoresis (2%, W/V) and agarose gel DNA extraction kit (Axygen, USA), respectively. The purified products were sequenced on Illumina Miseq platform (Shanghai Personal Biotechnology Co. Ltd., Shanghai, China). The raw reads from sequencing were deposited to the NCBI Sequence Read Archive (SRA) database under the accession number PRJNA824302.

Bioinformatics analysis of sequence data

High-throughput raw data processing was performed using QIIME2 (Bolyen et al., 2019), and high-quality sequences screened for quality control were merged and divided into different operational taxonomic units (OTUs) based on 97% sequence similarity based on the UCLUST sequence alignment tool, and select the most abundant sequence in each OTU as the representative sequence of the OTU. The AMF alignment database is MaarjAM database (http://maarjam.botany.ut.ee). Based on the QIIME2 software, Alpha diversity analysis and Beta diversity analysis were performed on the samples based on OTU clustering, and the sample flora distribution map was drawn according to the taxonomic analysis results to compare differences.

Statistical analysis

One-way analysis of variance (ANOVA) was performed on the data using SPSS (19.1) statistical software (SPSS, Chicago, IL, United States), and Duncan's multiple comparisons were used to determine the significance between treatments. Pearson correlation analysis was used to evaluate the relationship between AMF community and crop yield. Redundancy analysis (RDA) on the relationship between soil environmental variables and AMF communities was performed using CANOCO 5.0. Structural equation model (SEM) was used to obtain the interaction mechanism among AMF community diversity, soil environmental factors and crop yield under long-term N and P addition. The SEM analysis was performed under the criteria of a hypothetical relational conceptual model, and we hypothesized that long-term N and P additions would alter soil physicochemical properties and crop yield, thereby affecting AMF community diversity and composition.

Results

Basic physical and chemical properties of soil

The Basic physicochemical properties of soil under long-term N and P addition (Table 1). Long-term N and P treatments reduced soil pH, but the decrease amplitude was different. Soil pH under N_{24}, $N_{24}P_{12}$ and $N_{24}P_{24}$ treatment was significantly lower than CK. Compared with CK, NH_4^+-N was significantly decreased under N_{24} and P_{12}, while NH_4^+-N and NO_3^--N were significantly increased under other treatments, and TC was significantly decreased.

Table 1
Basic physicochemical properties of soil.

Treatment	pH	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	TN (g kg^{-1})	TC (g kg^{-1})	SOC (mg kg^{-1})	AP (mg kg^{-1})	TP (g kg^{-1})	OP (mg kg^{-1})
CK	8.22 ± 0.02a	0.29 ± 0.02bc	11.13 ± 0.04e	0.85 ± 0.01c	17.49 ± 0.16a	6.95 ± 0.08d	7.37 ± 0.27e	0.73 ± 0.00e	0.08 ± 0.01def
N_{12}	8.20 ± 0.04a	0.32 ± 0.02abc	13.50 ± 0.17c	0.72 ± 0.03d	16.85 ± 0.25bc	6.33 ± 0.24e	2.53 ± 0.09f	0.67 ± 0.00f	0.10 ± 0.01cde
N_{24}	7.92 ± 0.01b	0.23 ± 0.03bc	25.11 ± 0.11a	0.90 ± 0.03bc	16.00 ± 0.28e	7.70 ± 0.33c	4.30 ± 0.40f	0.69 ± 0.01ef	0.44 ± 0.02a
P_{12}	8.15 ± 0.05a	0.22 ± 0.01c	11.13 ± 0.06e	0.75 ± 0.01d	17.04 ± 0.16abc	7.10 ± 0.01d	42.80 ± 0.40c	1.06 ± 0.00c	0.06 ± 0.01f
$N_{12}P_{12}$	8.22 ± 0.03a	0.33 ± 0.06abc	11.46 ± 0.07d	0.75 ± 0.03d	16.96 ± 0.16abc	7.92 ± 0.06bc	23.27 ± 1.09d	0.96 ± 0.02d	0.13 ± 0.01c
$N_{24}P_{12}$	7.88 ± 0.03b	0.45 ± 0.01a	14.54 ± 0.12b	0.99 ± 0.03a	17.25 ± 0.02ab	8.52 ± 0.05a	25.00 ± 0.61d	0.94 ± 0.01d	0.17 ± 0.00b
P_{24}	8.23 ± 0.03a	0.38 ± 0.10ab	10.88 ± 0.02e	0.76 ± 0.01d	16.17 ± 0.02de	6.83 ± 0.06de	66.77 ± 1.47a	1.32 ± 0.01a	0.07 ± 0.01ef
$N_{12}P_{24}$	8.15 ± 0.06a	0.32 ± 0.03abc	11.59 ± 0.04d	0.85 ± 0.03c	16.57 ± 0.06cd	8.40 ± 0.13ab	48.20 ± 0.72b	1.32 ± 0.01a	0.11 ± 0.01cd
$N_{24}P_{24}$	7.95 ± 0.06b	0.32 ± 0.05abc	13.72 ± 0.14c	0.96 ± 0.01ab	17.01 ± 0.10abc	8.16 ± 0.30abc	47.23 ± 0.67b	1.25 ± 0.02b	0.11 ± 0.01cd
The values of different letters in the table are different at $P < 0.05$.									

Rhizosphere AMF biomass

Rhizosphere AMF biomass content under different N and P addition treatments (Fig. 2). The results showed that rhizosphere soil AMF content under different treatments ranged from 0.3 ~ 1.35nmol g^{-1}, and N_{12} had the lowest AMF content. $N_{24}P_{24}$ treatment was significantly higher than all treatments, and its content was 4.5 times that of N_{12}. Rhizosphere AMF biomass under N_{12} was significantly lower than CK, and there was no significant difference between N_{24} and CK. The rhizosphere AMF biomass was significantly higher under the N and P interaction treatments than in the single N or P fertilizer, and no fertilizer treatments.

AMF colonization rate, soil spore density

The application of N and P with different concentration gradients and their interaction had different effects on the AMF colonization rate (Fig. 3A). $N_{24}P_{24}$ treatment had the highest AMF colonization rate, which was significantly higher than other treatments. Compared with CK, N_{12}, P_{12}, $N_{12}P_{12}$ and $N_{24}P_{12}$ treatments significantly reduced the AMF colonization rate, and $N_{24}P_{12}$ treatment had the lowest AMF colonization rate. For N and P alone, AMF colonization rate showed the following trend: $P_{24} > N_{24} > P_{12} > N_{12}$. Long-term N and P addition significantly increased soil spore density (Fig. 3B), and the soil spore density under $N_{24}P_{24}$ treatment was the highest, followed by N_{12} and P_{12}. There was no significant difference in soil spore density content under N_{24} and P_{24} treatments, but significant difference under N and P interaction.

Community structure composition and diversity of AMF

Sample sequencing depth and rationality

A certain amount of sequencing data is randomly selected from the samples to be tested to verify whether the data amount of each processed sample is reasonable. Taking the amount of sequence data extracted from the community samples as the horizontal axis and the number of corresponding species as the vertical axis, the microbial rarefaction curves under different treatments were drawn (Fig. 4). When the sequencing volume was about 40,000, the OTU number of the sample basically showed a saturated state, indicating that the sequencing volume adopted in this experiment was relatively reasonable and could relatively accurately reflect the AMF community of each treatment sample. A total of 1,425,112 high-quality sequences were generated by high-throughput sequencing, with an average of 52,781 in each sample. The Good's coverage value of all samples was greater than 99% at 97% similarity (Fig. 7), indicating that the sequencing depth has been used to evaluate the AMF community composition and diversity of this sample.

AMF community composition

At the genus level, AMF mainly included 6 genera under different N and P addition treatments (Fig. 5): *Paraglomus* (11.4% ~ 67.9%); *Glomus* (8.8% ~ 54.3%); *Claroideoglomus* (0.2% ~ 2.5%); *Diversispora* (0.02% ~ 1.35%); *Ambispora* (0.03% ~ 0.5%); *Archaeospora* (0.04%~0.2%), these species accounted for 66.3%~85.3% of the total AMF taxa. Under $N_{24}P_{24}$, *Paraglomus* had the lowest relative abundance (11.4%), and *Glomus* had the highest relative abundance (54.3%). According to ANOVA analysis (Supplementary Table S1), it was found that long-term N and P supplementation significantly changed AMF community composition. The relative abundance of *Paraglomus* in $N_{12}P_{24}$ and $N_{12}P_{12}$ treatments was significantly lower than that in N_{12} treatment. On the contrary, N and P interaction treatment significantly increases the relative abundance of *Glomus*, and the relative abundance of *Glomus* under $N_{12}P_{24}$ and $N_{24}P_{24}$ treatments is higher than that under other treatments. The relative abundance of *Diversispora* was the highest under N_{12}, and the addition of N and P at different concentrations had little effect on the relative abundance.

At the family level, it mainly includes 4 dominant species, namely: *Paraglomeraceae*, *Glomeraceae, Claroideoglomeraceae*, *Diversisporaceae*. The relative abundance of *Paraglomeraceae* was relatively high under CK, N_{24} and P_{12} treatments, and the relative abundance of species among these three treatments was relatively close (Fig. 6). The relative abundance of *Glomeraceae* was the highest under $N_{24}P_{24}$, and that of CK was the lowest. There was a significant difference in species abundance between N_{12} and other treatments, and *Diversisporaceae* was the unique dominant species. The relative abundance of *Claroideoglomeraceae* in N_{12} and P_{24} was significantly higher than that in other treatments.

AMF community diversity

Effects of long-term N and P supplementation on Alpha diversity in AMF communities (Fig. 7). The results show that compared with CK, Chao1 index increases under $N_{12}P_{12}$ and $N_{24}P_{24}$ treatment, and decreases significantly under N_{12}. The Shannon index and Simpson index under N_{12}, N_{24} and P_{12} treatments decreased significantly. The species diversity index was the lowest under P_{12}.

Beta diversity analysis of AMF community composition under different treatments based on Bray-Curtis distance (Fig. 8). By principal coordinates analysis (PCoA), the results showed that the distances between CK and the rest of the treatments were all far apart, indicating that different levels of N and P additions caused a large divergence in AMF community composition. P_{12} and P_{24} treated the negative half axis of PCoA2 axis, indicating similar AMF community composition. The N_{12}, N_{24}, $N_{12}P_{12}$, $N_{12}P_{24}$, $N_{24}P_{12}$, and $N_{24}P_{24}$ treatments were all located on the right side of the PCoA1 axis and were considered to have similar AMF community composition and changed with the added N and P concentrations.

Crop yield

Long-term addition of N and P fertilizer can increase wheat yield and aboveground biomass of soil to a certain extent (Table 2). Among them, N and P interaction treatment has the best effect. In general, the aboveground biomass yield (BY), wheat yield (WY), kernels per spike, plant height and thousand seed weight under N and P interaction treatment were significantly higher than those under N or P treatments alone. Under $N_{24}P_{24}$, the aboveground biomass, wheat yield and thousand seed weight were the highest, reaching 1855.13 g m^{-2} and 701.70 g m^{-2} and 56.40 g, respectively.

Table 2
Crop yield under different nitrogen and phosphorus addition treatments.

Treatment	Aboveground biomass ($g\ m^{-2}$)	Wheat yield ($g\ m^{-2}$)	Kernels per spike ($grain\ spike^{-1}$)	Plant height (cm)	Thousand seed weight (g)
CK	517.10 ± 2.9de	116.50 ± 2.8g	15.23 ± 0.2f	61.27 ± 0.2d	42.57 ± 0.1g
N_{12}	542.63 ± 6.2d	147.80 ± 2.8f	12.47 ± 0.2h	65.73 ± 0.2c	50.40 ± 0.1b
N_{24}	904.83 ± 27.2c	190.37 ± 2.9e	20.87 ± 0.1e	66.50 ± 0.2c	47.17 ± 0.1e
P_{12}	531.30 ± 11.7d	119.30 ± 0.0g	13.60 ± 0.1g	65.40 ± 0.5c	45.37 ± 0.1f
$N_{12}P_{12}$	1606.47 ± 15.4b	463.10 ± 2.9d	27.70 ± 0.1c	89.20 ± 0.4b	49.53 ± 0.1c
$N_{24}P_{12}$	1595.20 ± 12.6b	559.70 ± 2.8b	36.47 ± 0.2a	88.27 ± 0.2b	48.53 ± 0.1d
P_{24}	480.23 ± 2.9e	113.70 ± 2.8g	11.83 ± 0.2i	59.60 ± 0.8e	45.27 ± 0.1f
$N_{12}P_{24}$	1626.43 ± 7.9b	471.70 ± 2.8c	24.53 ± 0.2d	92.27 ± 0.2a	49.33 ± 0.1c
$N_{24}P_{24}$	1855.13 ± 29.3a	701.70 ± 2.9a	28.30 ± 0.2b	89.13 ± 0.3b	56.40 ± 0.1a

The values of different letters in the table are different at $P < 0.05$.

Relationship between AMF community structure, soil physicochemical properties and crop yield

Taking the top six species of relative abundance at the genus level in the AMF community as the response variable, and taking the soil physicochemical factors as the explanatory variables, the redundancy analysis (RDA) was carried out (Fig. 9). The first ordination axis explained 78.72% of the community variation, and the second ordination axis explained 1.42% of the community variation. Among them, the effect of TP content on the AMF community composition was the most obvious, which explained 39.7% of the AMF community composition ($P < 0.05$). According to the distribution of various environmental factors and the length of the connection, the main physical and chemical factors affecting the composition of the AMF community in the farming area under the long-term N and P addition were TP, AP, and pH ($P < 0.05$).

Taking crop yield as the response variable and soil environmental physicochemical factors as explanatory variables, the RDA was performed (Fig. 10). The first ordination axis explained 88.71% of crop yield variation, and the second ordination axis explained 0.64%. The SOC content contributed the most to crop yield, accounting for 70.8% of crop yield ($P < 0.05$); followed by AP, which accounted for 9.7% of crop yield ($P < 0.05$). Combined with the distribution of various environmental factors and the length of the connection, it can be seen that AMF infection rate, spore density and AP are positively correlated; the SOC and AP contents were the main influencing factors of crop yield changes under long-term N and P addition.

The Pearson correlation analysis between the top six species of AMF genus level relative abundance and crop yield is shown in the table (Table 3). The results showed that: at the genus level, *Glomus* had a significant positive correlation with aboveground biomass, wheat yield, kernels per spike, plant height, thousand seed weight and spore density. In contrast, *Paraglomus* showed a significant negative correlation with these metrics. *Claroideoglomus* was significantly negatively correlated with aboveground biomass, wheat yield, kernels per spike and plant height. There was a significant positive correlation between *Diversispora* and spore density. There was no significant correlation between AMF colonization rates and AMF taxa composition.

Table 3
Pearson correlation coefficient between the intergeneric abundance of arbuscular mycorrhizal fungi and crop yield.

genus		Aboveground biomass	Wheat yield	Kernels per spike	Plant height	Thousand seed weight	AMF colonization rates	SD
Paraglomus	*R*	**-0.791****	**-0.863****	**-0.594****	**-0.738****	**-0.865****	-0.169	**-0.418***
	P	0	0	0.001	0	0	0.399	0.03
Glomus	*R*	**0.782****	**0.841****	**0.588****	**0.752****	**0.814****	0.132	**0.384***
	P	0	0	0.001	0	0	0.51	0.048
Claroideoglomus	*R*	**-0.666****	**-0.617****	**-0.665****	**-0.602****	-0.241	-0.039	0.086
	P	0	0.001	0	0.001	0.225	0.846	0.67
Diversispora	*R*	-0.3	-0.232	-0.341	-0.201	0.232	-0.287	**0.529****
	P	0.129	0.245	0.081	0.315	0.244	0.146	0.005
Ambispora	*R*	**0.562****	**0.430***	0.303	**0.657****	0.241	0.061	0.035
	P	0.002	0.025	0.125	0	0.226	0.762	0.861
Archaeospora	*R*	0.102	0.008	-0.117	0.195	0.034	0.136	-0.033
	P	0.614	0.967	0.561	0.329	0.865	0.499	0.872
others	*R*	**0.620****	**0.694****	**0.499****	**.505****	**0.687****	0.225	0.302
	P	0.001	0	0.008	0.007	0	0.258	0.126

Notes: Bold values indicate significant levels. **indicates $P < 0.01$, * indicates $P < 0.05$.

Structural equation model (SEM) analysis of the interaction mechanism between soil physicochemical properties, AMF community structural diversity and wheat yield under long-term N and P addition (Fig. 11). The explanation rates for wheat yield and AMF diversity were found to be 97% and 24%, respectively. Wheat yield was directly affected by long-term N and P additions and the diversity of AMF community structure, and was indirectly affected by soil physicochemical properties. Long-term N and P addition mainly affects soil nutrient content indirectly by directly affecting soil pH. There was a certain negative correlation between soil AP and AMF community diversity. There were significant positive correlations between TP, TOC and wheat yield.

Discussion

Effects of long-term N and P addition on soil physicochemical properties, mycorrhizal infection and spore density

Long-term addition of nitrogen and phosphorus will change the physical and chemical properties of soil (Table 1). The research results show that long-term N and P addition will lead to a decrease in soil pH, which is due to the continuous accumulation of organic matter while N and P fertilizer promotes crop growth, resulting in the production of a large amount of organic acids (Cammeraat and Risch, 2008). Furthermore, the denitrification process of soil microorganisms produces a certain amount of H^+, which leads to soil acidification (Luo et al., 2015), and optimized fertilization strategies can be adopted to alleviate acidification. In this study, the long-term addition of N and P significantly increased the soil TP, which may be related to the reduction of the soil's adsorption capacity for phosphorus by fertilization. This is similar to the findings of (Li et al., 2019; Wang et al., 2010). The adsorption and desorption of PO_4^{3-} in soil largely affects the bioavailability of soil phosphorus, which may be one of the reasons for the significant increase in soil AP after long-term N and P addition (Tang et al., 2021). Under the interaction of N and P, the amount of stubble returned to the crops increased, which may be the reason for the significant increase in soil organic carbon content (E et al., 2016).

The degree of AMF colonization rates on plants is closely related to the N and P concentrations in the rhizosphere soil environment (Nouri et al., 2014). Lin et al. (2012) believed that phosphorus absorption was the main factor affecting the symbiosis of AMF, and the level of phosphorus nutrient supply would affect the infection rate of AMF (Guttay and Dandurand, 1989; Ultra et al., 2007). In this study, under the P_{12}, $N_{12}P_{12}$, and $N_{24}P_{12}$, the colonization rate of AMF was significantly lower than that of the CK, which may be due to the fact that under the condition of sufficient soil nutrients, the host plants are more inclined to rely on their own roots to absorb nutrients and reduce AMF colonization rates (Li et al., 2021; Luo et al., 2015; Zhang et al., 2020). The interaction of N and P resulted in a large amount of NO_3^- and PO_4^{3-} in the soil, which affected the change of soil pH and stimulated the sporulation ability of AMF (Khanam et al., 2006; Ren et al., 2014). This may be one of the reasons why the soil spore density was significantly lower than that under the condition of N and P interaction. The results of this study are consistent with those of Wu et al. (2010). AMF is characterized by obligate symbiosis, and its spore density and infection rate are affected by the combined effects of host plants, soil environment changes, and their own living strategies (Bainard et al., 2012; Liu and Zhang, 2019).

Effects of long-term N and P addition on community structure diversity and composition of AM fungi

Different levels of N and P additions in this study altered AMF richness and diversity, which is similar to previous findings (Camenzind et al., 2014; Qin et al., 2015). Lin et al. (2012) showed that long-term application of P fertilizers reduced the abundance of AMF, similar to the results of this study. Li et al. (2018) found that long-term targeted fertilization can change the physical and chemical properties of soil, thereby changing the environment in which AMF grows, and ultimately affecting the diversity of AMF community structure. Zhen et al. (2015) studies have shown that low soil phosphorus content is conducive to the development of AMF. However, in this study, the diversity of AMF community structure was significantly reduced under P_{12}, which may be caused by different climate, soil background environment and crop types. The application of N fertilizer will drive the change of AMF community structure, and studies have shown that AMF community diversity will decrease with the increase of N concentration (Zhu et al., 2018), which is similar to the results of this study. N_{12} treatment significantly reduced rhizosphere AMF biomass, while N and P interaction significantly increased AMF biomass and community structure diversity. This is due to the change in soil phosphorus availability caused by nitrogen application. N fertilizer can increase AMF richness when phosphorus is deficient and diversity (Chen et al., 2013; Egerton-Warburton et al., 2007). The soil environment is complex, and the mechanism of fertilization on the development of AMF is still controversial. Based on the trade balance model, it is believed that under normal circumstances, plants and AMF are mutually beneficial and symbiotic, but with the difference in the way and content of N and P addition, the relationship between AMF and plants will

change from mutualism to parasitism, resulting in different AMF community structures trends (Johnson et al., 2010). Wu et al. (2010) studied the AMF community structure in the dryland wheat rhizosphere on the Loess Plateau and found that *Paraglomeraceae*, *Glomus* and *Claroideoglomus* were the main dominant species in this area, which is consistent with the results of this study. Moreover, *Glomus* has strong adaptability and can tolerate relatively large habitat changes, and is widely distributed in my country (Zhang et al., 1998; Zhang et al., 2006; Zhao et al., 2017).

Potential relationships among soil nutrients, AMF community structure and yield

AMF plays an important role in the regulation of material exchange between host plant and soil systems (Wei et al., 2016). In farmland ecosystems, soil environmental factors are an important factor affecting microbial community composition and crop yield (Figs. 9 and 10). pH, as one of the important factors affecting soil microbial activity and community structure (Liu and Zhang, 2019), can not only directly affect the physiological state of AMF and change its ecological niche (Xu et al., 2017), but also can regulate soil chemical indirect effects such as properties and nutrient availability in turn affect AMF community composition (Hou et al., 2018; Maltz et al., 2019). The results of the RDA in this study showed that soil TP and AP contents were important factors affecting the AMF community composition (Fig. 9), which was consistent with the results of Luo et al. (2020). The mycorrhizal pathway has been confirmed by many scholars as the main way for plants to obtain phosphorus from soil (Püschel et al., 2021; Zhang et al., 2021). Studies have shown that AMF can induce the expression of phosphate transporter genes to promote the uptake of phosphorus nutrients by plants; when the phosphorus concentration is high, it will turn off the expression of specific phosphate transporter genes in arbuscules (Breuillin et al., 2010). Luo et al. (2019) and other studies have shown that AMF can help plants effectively utilize insoluble inorganic phosphorus in soil while mineralizing organic phosphorus, thereby improving the uptake efficiency of phosphorus by host plants. There was a certain correlation between AMF community diversity and composition and crop yield (Fig. 11, Table 3). In this study, *Glomus* had the most significant positive correlation with crop yield and thousand seed weight, because *Glomus* developed some specific survival strategies under long-term N and P addition (Liu et al., 2012). When the P concentration in the soil is relatively high, *Glomus* has a strong promotion effect on the absorption of phosphorus by crops (Chen et al., 2007). The AMF community and crops interact and influence each other. The large mycelial network of AMF continuously colonizes and extends in the soil, absorbing more nutrients from the soil and transferring them to the host plants, thereby increasing crop yields (Ruth et al., 2011). At the same time, plants allocate up to 20% of photosynthate to AMF to ensure their growth and nutrient transport (Peng et al., 1993). In recent years, a lot of research has focused on the application of different types of AM fungal inoculants to enhance the mycorrhizal effect of AMF and improve crop yield (Gianinazzi and Vosátka, 2004).

Conclusion

The interaction between soil-AMF-plant plays an important role in the stable balance of the ecosystem. The results of this study showed that long-term N and P addition had significant effects on AMF community structure and composition. Among them, long-term single application of N fertilizer significantly reduced the diversity of AMF community structure, while the interaction of N and P significantly increased the richness and diversity of AMF community. Soil AP, TP and pH were the main environmental factors affecting the changes of AMF community, and *Glomus* in AMF community was significantly positively correlated with wheat yield. In conclusion, for the loess dry plateau farmland ecosystem, long-term N and P additions lead to changes in soil physicochemical properties, which are considered to be the main factors affecting the AMF community structure and crop yield. Reasonable application of N and P is helpful to maximize the ecological and physiological functions of the AMF community, which is of great significance to realize the stable development of the ecosystem.

Abbreviations

AMF: arbuscular mycorrhizal fungi; N: nitrogen; P: phosphorus; TC: soil total carbon; TN: soil total nitrogen; TP: soil total phosphorus; NH_4^+-N: soil ammonium nitrogen; NO_3^--N: nitrate nitrogen; AP: soil available phosphorus; OP: soil total organic phosphorus; SOC: soil organic carbon; OTUs: operational taxonomic units; SD: soil spore density; BY: aboveground biomass yield; WY: wheat yield; RDA: redundancy analysis; SEM: structural equation model.

Declarations

The authors declare no conflict of interest.

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Data availability Not applicable.

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Figures

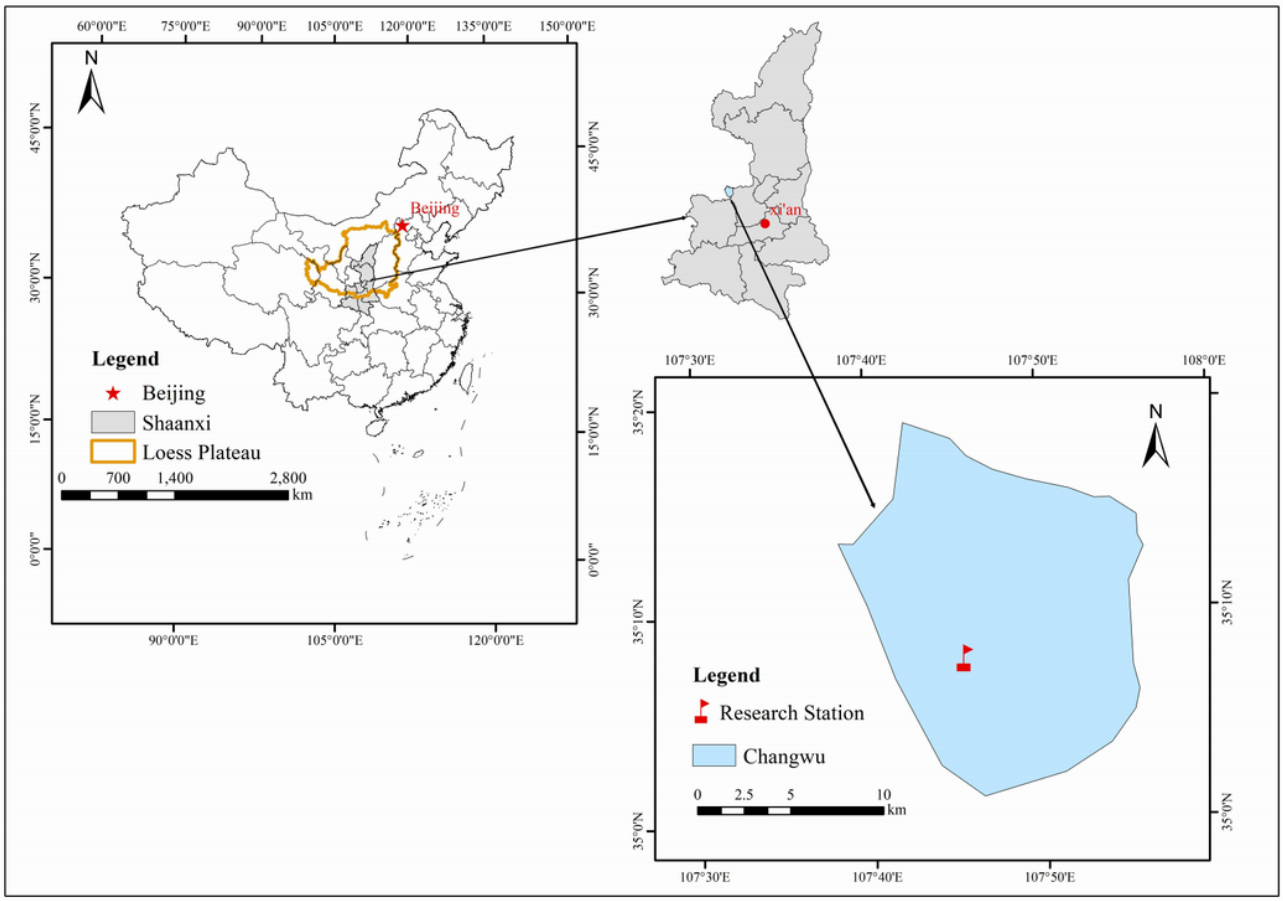

Figure 1

The research station is located at the Changwu Agricultural Ecology Experimental Station of the Chinese Academy of Sciences.

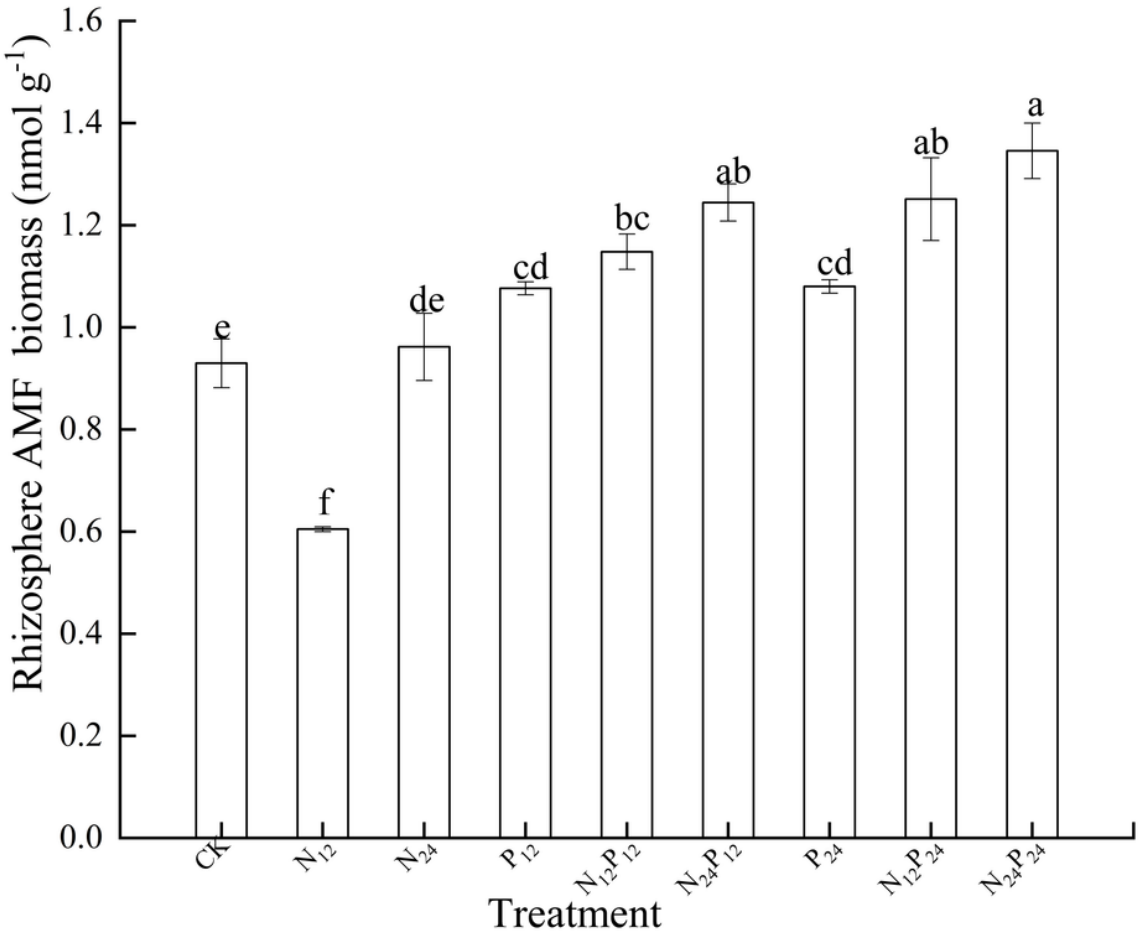

Figure 2

Rhizosphere AMF biomass of each treatment under different nitrogen and phosphorus treatments. The values of different letters in the figure are different in $P < 0.05$.

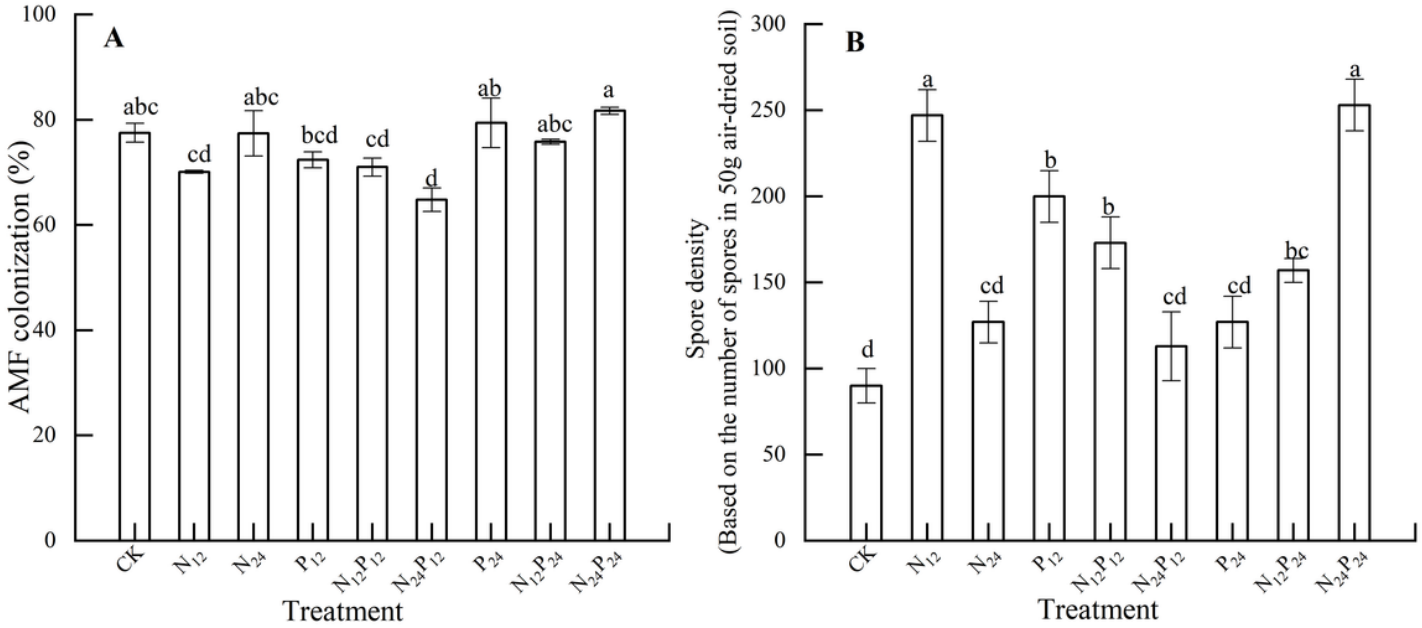

Figure 3

AMF colonization rate (A) and spore density (B) of each treatment under different nitrogen and phosphorus treatments. The values of different letters in the figure are different in $P < 0.05$.

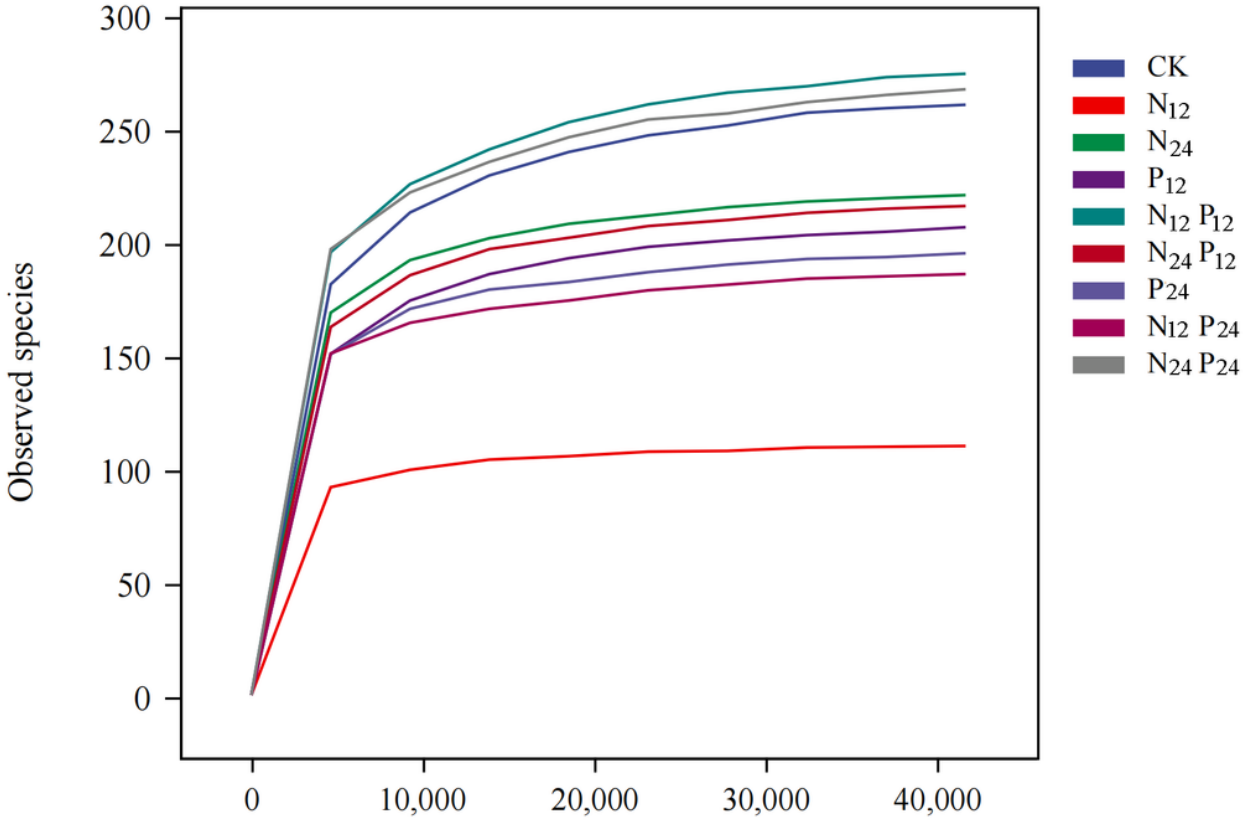

Figure 4

Rarefaction curve of samples under different nitrogen and phosphorus treatments.

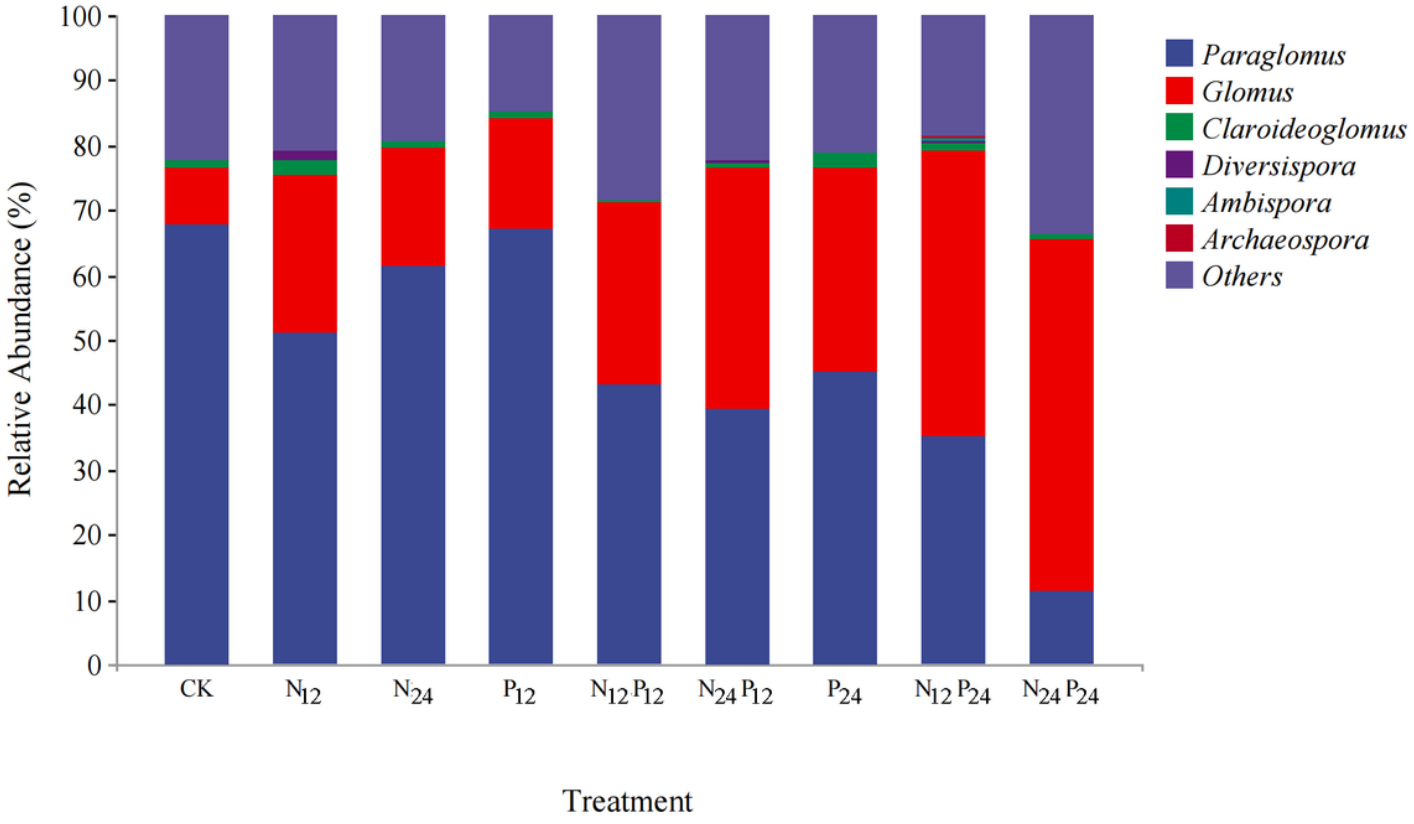

Figure 5

Relative abundance of AMF genus level species under different nitrogen and phosphorus treatments.

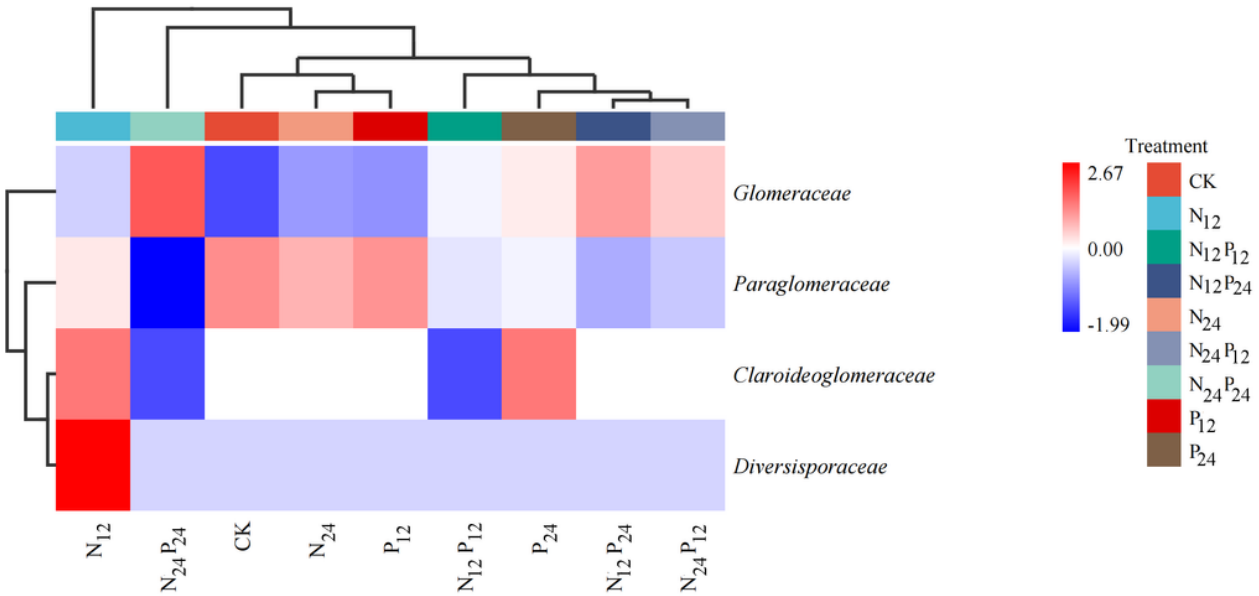

Figure 6

Heatmap of species interaction at AMF family level under different nitrogen and phosphorus treatments.

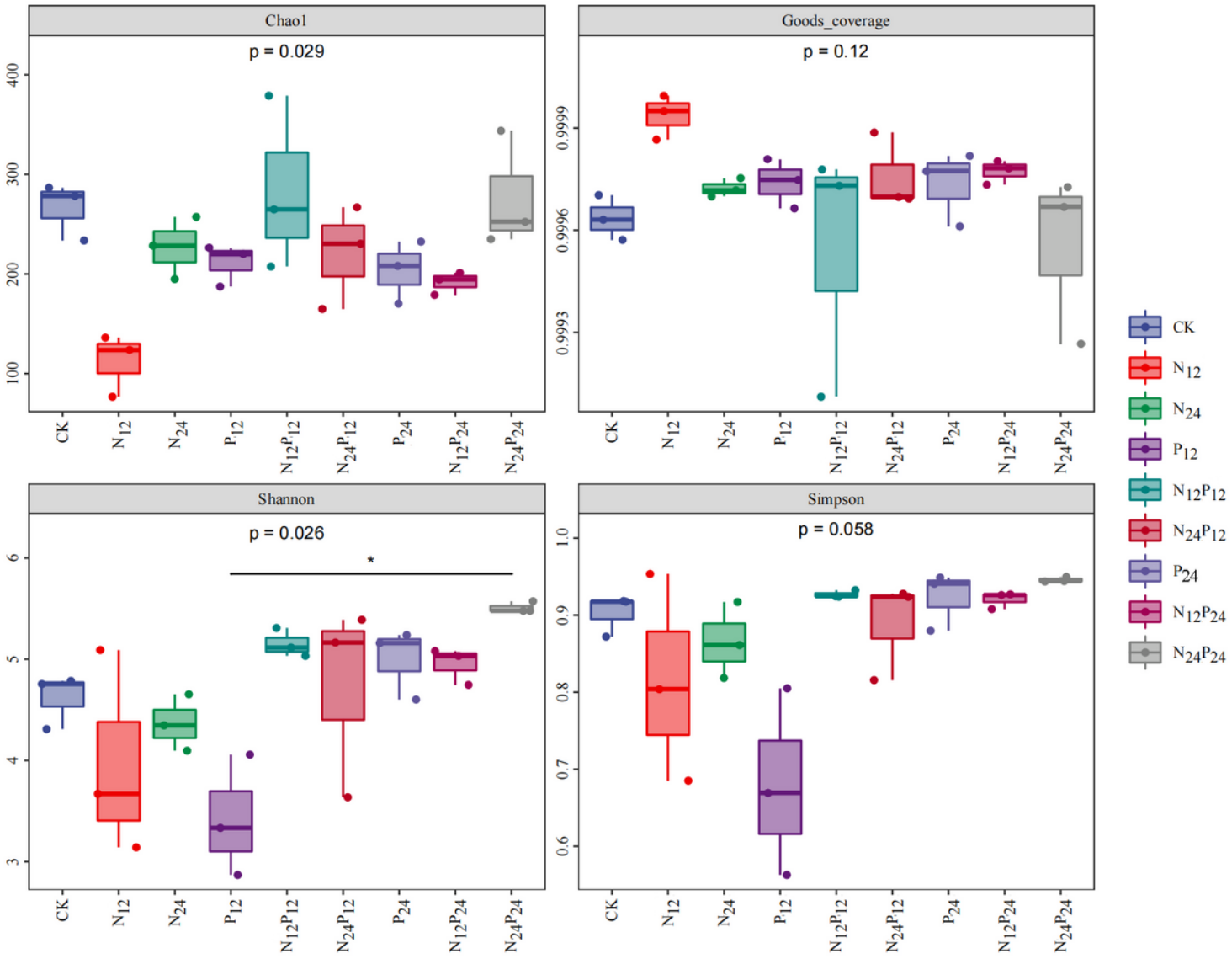

Figure 7

Diversity index of AMF community structure under different nitrogen and phosphorus addition treatments.

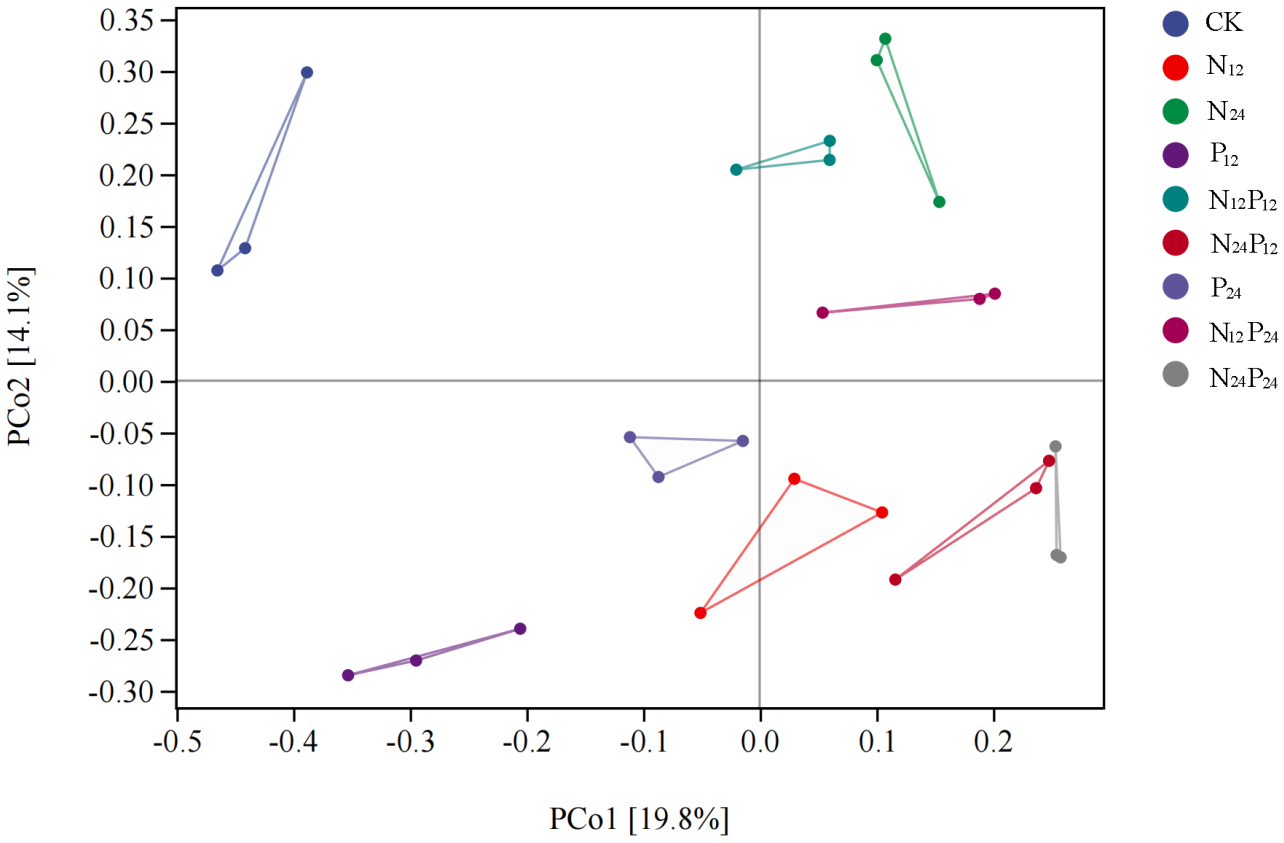

Figure 8

Principal coordinates analysis (PCoA) of AMF community composition under different nitrogen and phosphorus addition treatments.

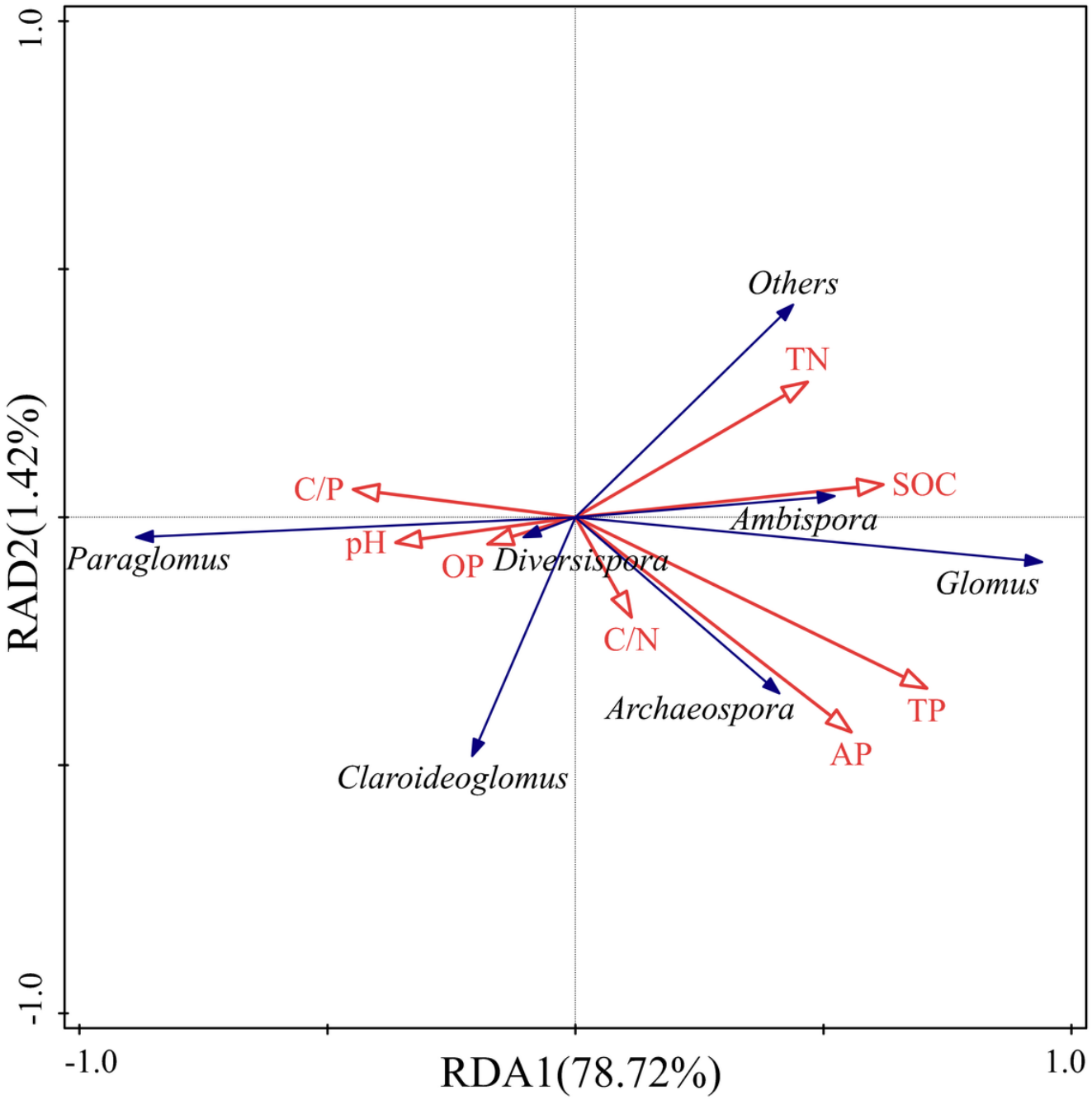

Figure 9

Redundancy analysis (RDA) of AMF community structure and soil environmental factors under different nitrogen and phosphorus supplemental treatments. C/N, the ratios of organic carbon to soil total nitrogen; C/P, the ratios of organic carbon to soil available phosphorus.

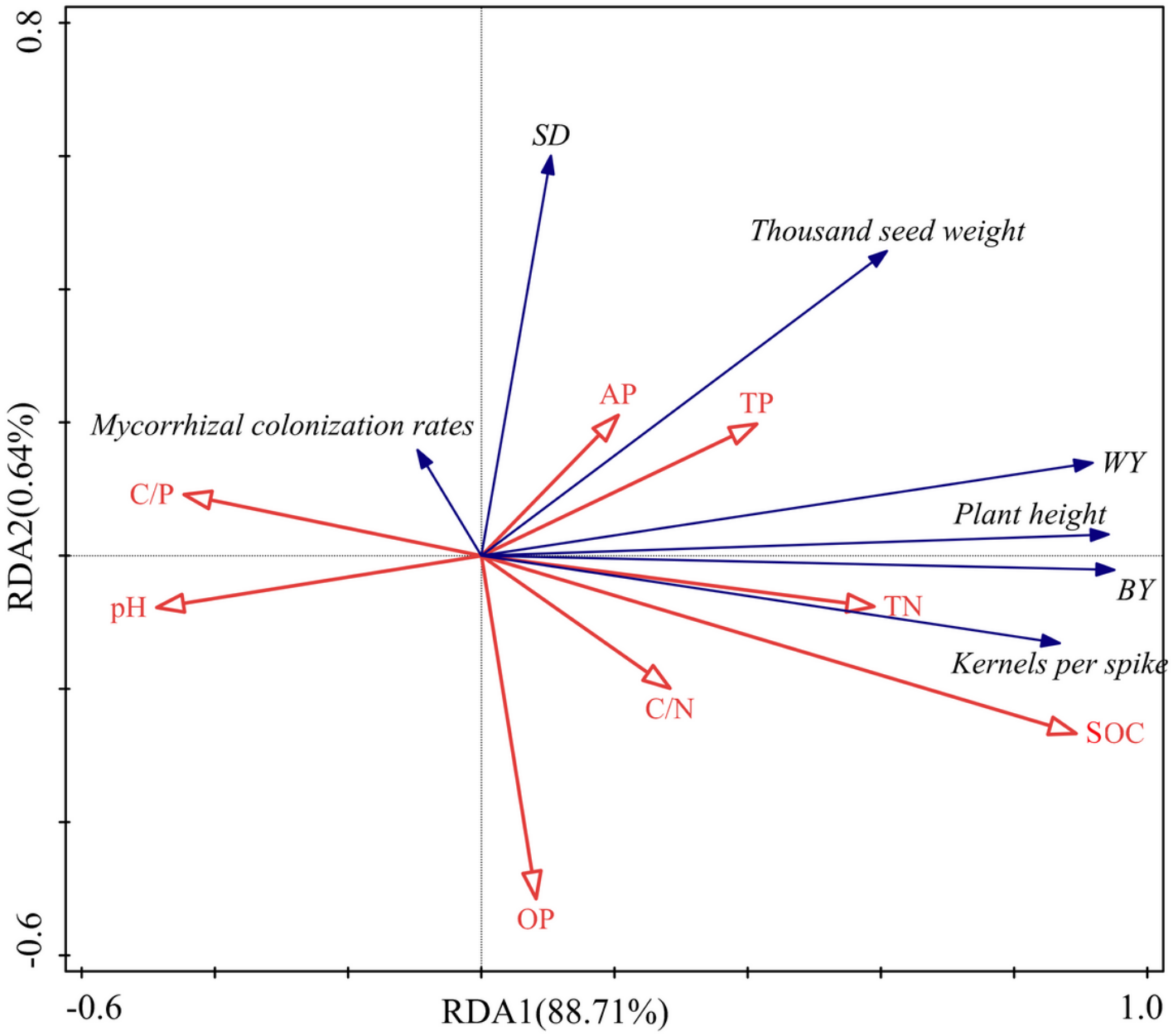

Figure 10

Redundancy analysis (RDA) of crop yield and soil environmental factors under different nitrogen and phosphorus supplemental treatments. BY, aboveground biomass yield; WY, wheat yield; SD, soil spore density; C/N, the ratios of organic carbon to soil total nitrogen; C/P, the ratios of organic carbon to soil available phosphorus.

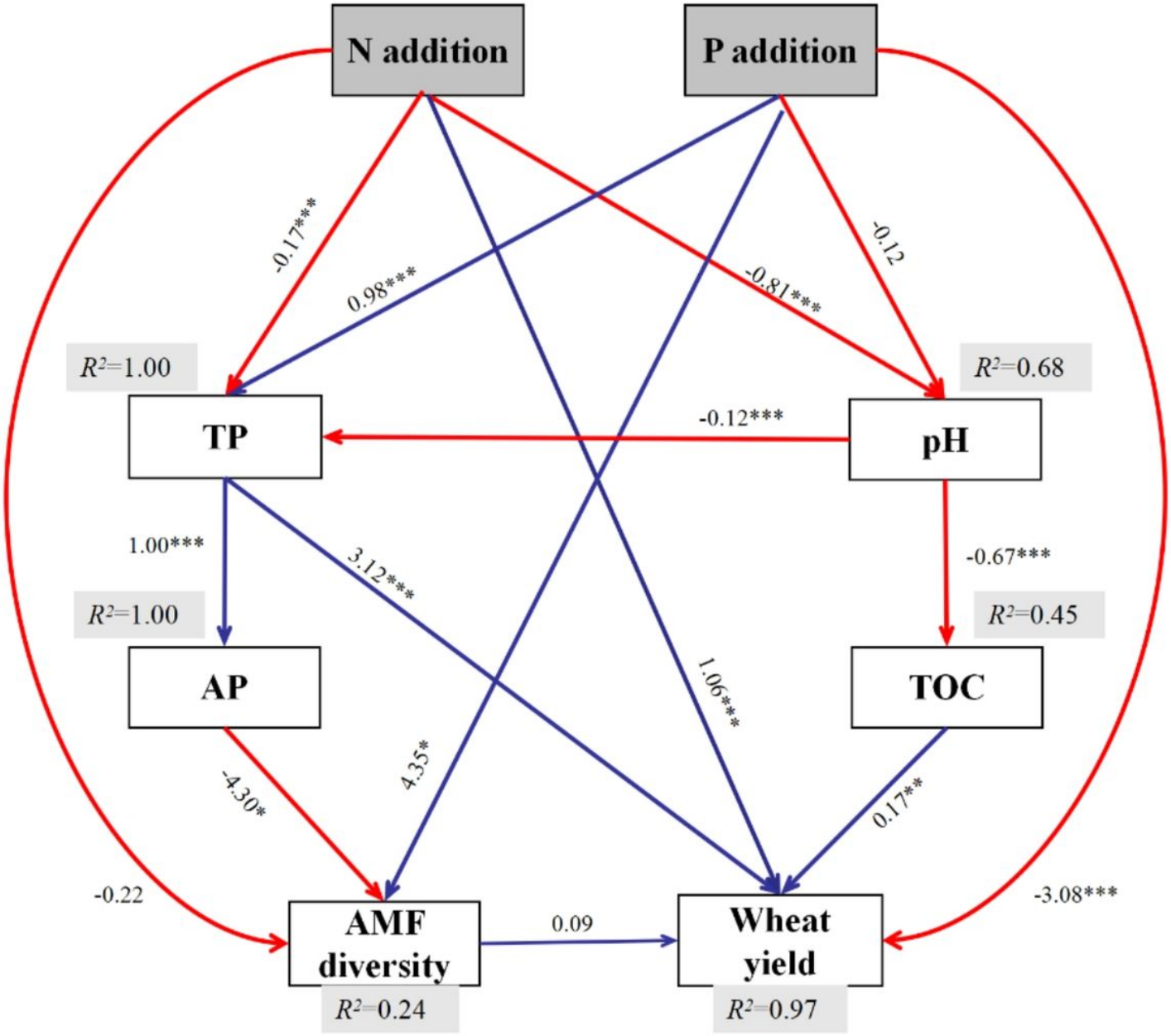

Figure 11

Structural equation modeling (SEM) of the effects of nitrogen and phosphorus additions and soil physicochemical properties on AMF community structure diversity and wheat yield. The model fits the data well, with χ^2=13.095， *df*=13，*P*=0.441，RMSEA=0.02，CFI=1.000，AIC=59.095，BIC=75.380. The number next to the path is the normalized path coefficient, where red is the negative effect, blue is the positive effect, and R^2 represents the explained rate of the variable in the model. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Supplementary Files

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- Supplementarymaterials.docx
- floatimage1.png