

Article

The Addition of a High Concentration of Phosphorus Reduces the Diversity of Arbuscular Mycorrhizal Fungi in Temperate Agroecosystems

Feng Miao ^{1,†}, Senlin Wang ^{2,†}, Yuxiang Yuan ², Yun Chen ², Erhui Guo ^{3,*} and Yuan Li ^{4,5,*} 

- ¹ College of Agronomy, Henan Agricultural University, No. 63 Agricultural Road, Zhengzhou 450002, China
² College of Life Sciences, Henan Agricultural University, No. 63 Agricultural Road, Zhengzhou 450002, China
³ College of Forestry, Henan Agricultural University, No. 63 Agricultural Road, Zhengzhou 450002, China
⁴ School of Mechanical Engineering, Henan Institute of Technology, Xinxiang 453000, China
⁵ School of Materials Science and Engineering, Zhengzhou University, Zhengzhou 450001, China
* Correspondence: guoerhui@henau.edu.cn (E.G.); liyuanliuxin@126.com (Y.L.)
† These authors contributed equally to this work.

Abstract: Phosphorus (P) is an essential macronutrient crucial for both plant growth and crop production, playing a pivotal role in agriculture since the early 20th century. The symbiotic relationship between AMF and plants serves as a classic illustration. These fungi play a regulatory role in the growth and development of plants, especially in facilitating the absorption of P and carbon molecules by plants. While there has been a growing body of research on the community assembly of arbuscular mycorrhizal fungi (AMF) in recent decades, our knowledge of the processes governing the coexistence of these AMF communities influenced by P in agroecosystems remains limited. To investigate the impact of various P fertilizers on AMF communities in temperate agroecosystems, this study was conducted using soils sourced from wheat–maize rotation farmland at Henan Agricultural University Yuanyang Base. With the Illumina MiSeq high-throughput sequencing technique, we systematically examined the taxonomic composition of soil AMF at the Yuanyang Base of Henan Agricultural University in a wheat–maize rotation agricultural field. Our primary objective was to unravel the mechanisms behind AMF community assembly and stability under varying P gradient fertilization conditions. Nonmetric Multidimensional Scaling (NMDS) analysis revealed significant differences among AMF communities in field soil subjected to various treatments ($p < 0.05$). A torus translations test demonstrated positive associations with the three treatments in 36 out of the 51 examined AMF operational taxonomic units (OTUs), making up 70.59% ($p < 0.05$) of the results. Furthermore, 37.84% (14/37) of the OTUs displayed preferences for the low P concentration treatment, while 34.29% (12/35) and 32.26% (10/31) favored medium and high P concentrations, respectively. An analysis of the Normalized Stochasticity Ratio (NST) and Checkerboard Score (C-score) indicated that in temperate agroecosystems, deterministic processes predominantly governed AMF in all treatment groups, with high P conditions exerting a stronger influence than low or medium P conditions. This study underscores the profound impact of long-term P fertilizer application on AMF community structures within temperate agricultural systems employing wheat–maize rotation. Additionally, it highlights the dominant role played by deterministic processes in shaping the assembly of AMF communities in these temperate agricultural systems that use P fertilizers. These findings emphasize the need for balanced nutrient management, particularly concerning P, to ensure the stability of AMF communities.

Keywords: arbuscular mycorrhizal fungi; torus translations test; wheat and maize rotation; temperate agroecosystems; microbial community



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1. Introduction

Intensive cropping systems, as the primary grain production method, heavily rely on the use of phosphorus (P) fertilizers. These systems are known for their high resource input and remarkable productivity [1–3]. It is noteworthy that China, with less than a tenth of the world's arable land, sustains one-fifth of the world's population. Notably, between 1961 and 2009, agricultural food production expanded by a significant 3.4-fold. Consequently, the usage of nitrogen (N) and P fertilizers in agriculture has increased substantially, by 37-fold and 91-fold, respectively, in Northern China [4]. In food production, it is crucial to prioritize sustainable agricultural development with environmental considerations [5]. The issue of excessive fertilization has raised widespread concerns, particularly regarding eutrophication [6], greenhouse gas emissions [7], and soil acidification [8]. The North China Plain (NCP), one of the largest wheat production regions in China, plays a vital role by contributing 50% of the national wheat output [9]. This region is known for its irrigation practices, intensive wheat–maize double cropping system, and significant potential for high yields [10,11].

P is an essential macronutrient crucial for both plant growth and crop production, playing a pivotal role in agriculture since the early 20th century. Reasonable P addition can address low P stock and availability in agricultural soils due to factors such as biological activity, atmospheric deposition, erosion, and crop removal [12]. However, over the past few decades, some countries with intensive agricultural production have witnessed the overuse of phosphate fertilizers and animal manure. In contrast, many regions worldwide, notably in Africa, face agricultural yield constraints due to low soil P levels and limited or no P fertilizer application [13]. Given its nonrenewable nature, there are projections that P reserves could be depleted within the next 70 to 300 years. Simultaneously, the demand for P is expected to peak in the coming decades [14,15]. Therefore, the utmost priority lies in its effective and judicious utilization.

Arbuscular mycorrhizal fungi (AMF), classified within the Glomeromycota phylum, are beneficial to plants in several ways and play a crucial role in facilitating the uptake of essential nutrients like P and trace elements, including Cu, Zn, Al, Mn, Mg, and Fe, by host plants [16–20]. Mycorrhizal fungi form symbiotic relationships with more than 80% of terrestrial plants [21], and plants colonized by AMF tend to show increased biomass and productivity due to improved mineral nutrition [22,23]. In natural environments, the composition and richness of AMF communities are often influenced by soil chemistry, particularly the availability of macronutrients [24,25]. Lin et al. found that factors like N deposition and P addition significantly affect colonization rates and spore density [26].

As we delve deeper into understanding the complex interactions between P and AMF in cropland ecosystems, it becomes essential to investigate how changes in P availability affect the dynamics of these beneficial symbiotic relationships. The symbiotic relationship between AMF and plants, which serves as a classic illustration [27], involves these fungi playing a regulatory role in plant growth and development. Within the root cortical cells of host plants, AMF establish intricate fungal structures called arbuscules, which facilitate the exchange of inorganic minerals, particularly P and carbon molecules. Nevertheless, the effects of P addition on the diversity and community composition of AMF in agroecosystems are still unclear.

Meanwhile, exploring the mechanisms of AMF community aggregation and coexistence is of paramount importance in agroecosystems. The microbial community assembly is concurrently influenced by deterministic and stochastic factors [28–30]. Deterministic processes involve biotic and abiotic factors, that is, deterministic processes involving interspecies interactions and environmental filtering [31]. Stochastic processes include random birth, death, dispersal, extinction, and speciation, which affect community assembly and consider that all species are ecologically equivalent [32,33]. Understanding microbial community assembly patterns can contribute to biodiversity preservation, ecosystem restoration, agricultural management, and disease control [34–36]. Although, studies on AMF community assembly have increased in recent decades. For instance, a review on

the elements of community assembly and coexistence of AMF highlighted recent studies using molecular methods in 2016 [37]; two articles reported that coexisting fungi were more phylogenetically clustered than the random communities defined by null models [38], and root-colonizing and soilborne communities of AMF differ among soybean fields with contrasting historical land use [39], respectively. Nevertheless, information about the processes that eventually drive the assembly of coexisting AMF species by P in agroecosystems remains limited.

We investigated the effects of different concentrations of P fertilizer on AMF communities in temperate agroecosystems. Three hypotheses were tested: (1) P amendments can influence AMF diversity, possibly because the addition of nutrients reduces the dependence of host plants on AMF [40]. (2) Adding excessive amounts of P might affect AMF community assembly patterns and reduce the diversity of AMF in temperate agroecosystems. (3) Deterministic processes play a dominant role in AMF assembly in temperate agricultural systems where P fertilizers are applied.

2. Material and Methods Introduction

2.1. Study Site and Physical and Chemical Properties of Soil

This study was conducted using soils from the wheat–maize rotation farmland in Henan Agricultural University Yuanyang Base (located in Henan Province, China, 113°42' E, 35°01' N). The predominant soil texture in this region is sandy. The collection site experiences year-round rainfall and has an average yearly precipitation of 615.1 mm, with a mean temperature of 14.5 °C. The physical and chemical properties of a 0–20 cm surface soil are shown in Table 1 [41].

Table 1. The physical and chemical properties of a 0–20 cm surface soil.

Physical and Chemical Properties	Unit	Amount
pH	/	7.8
organic matter	g·kg ⁻¹	17.61
total nitrogen (TN)	g·kg ⁻¹	0.82
total phosphorus (TP)	g·kg ⁻¹	0.43
available nitrogen (AN)	mg·kg ⁻¹	84.20
available nitrogen (AP)	mg·kg ⁻¹	18.2
available kalium (AK)	mg·kg ⁻¹	108.00

2.2. Experimental Design, Sample Collection, and Measured Soil Properties

A field test was initiated in April 2018. A winter wheat (*Triticum aestivum*) and summer maize (*Zea mays*) alternate planting system was used in all treatments yearly prior to the experiment. The plowing method was applied to winter wheat and summer maize in a double-cropping system. Harvesting was conducted once a year, and fertilizers were applied in the fall. Winter wheat was sowed in early October and harvested in early June of the following year, while summer maize was sowed in early June and harvested in mid-September of the following year. Winter wheat was sowed with a seeding plant density of 190 seeds·m⁻², and summer maize with 7 seeds·m⁻². The field test had a randomized plot design, and the P concentrations were as follows: three P levels (20, 60, and 120 kg/hm²) were set as low, medium, or high (Table 2). Field management was performed according to local agronomic practices, which include tillage, irrigation, fertilizer application, pesticide, and weed control.

Table 2. Annual fertilizer rate in the treatments of long-term experiment.

Treatment	Low P (kg/hm ²)	Medium P (kg/hm ²)	High P (kg/hm ²)
N	150	150	150
P(P ₂ O ₅)	20	60	120
K(K ₂ O)	90	90	90

After the removal of surface litter, fresh soil was sampled from the root soil of mature wheat. Soil samples were collected in April 2021. Nine subplots were distributed in three 10 m × 10 m plots, and three soil cores within each subplot were composited together as an individual sample (15 cm depth below the surface of the soil; 5 cm diameter). Nine soil samples were obtained. After passing through a 2 mm sieve, a subsample of the soil from each plot was stored at −80 °C for subsequent molecular analysis.

To study the effect of soil physical and chemical properties on AMF, we measured available P (AP) using the sodium bicarbonate extraction molybdenum antimony anticolorimetric method (JingHua, China) and available potassium (AK) using flame photometry. Nitrate nitrogen (NO₃[−]) was measured with a flow autoanalyzer (FIA Compact, Hamburg, Germany) [42].

2.3. DNA Extraction and Illumina MiSeq Sequencing

Soil microbial DNA was extracted according to the instructions of the Fast DNA SPIN kit for soil (MP Biomedicals, Santa Ana, CA, USA). AMF 18S rRNA genes were amplified using AMV4.5NF 5'-AAGCTCGTAGTTGAATTTTCG-3' and AMDGR 5'-CCCAACTATCCCTA TTAATCAT-3' for the construction of AMF community profiles.

Polymerase chain reaction (PCR) amplification was performed in a 20 µL reaction system. TransGen AP221-02: TransStart FastPfu DNA polymerase (TransGen Biotech, Beijing, China) with an ABI GeneAmp 9700 sequence detection system (ABI, Foster City, CA, USA) was used. The following thermal cycling conditions of PCR were used: denaturation step at 95 °C for 3 min, amplification through 30 cycles at a melting temperature of 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s. Finally, a 10 min extension step at 72 °C was conducted.

Primer AMV4.5NF/AMDGR was selected with a high proportion of AMF in soil samples to precisely portray AMF communities [43,44]. A unique barcode (5 bp) in the reverse primer was used for each sample. Amplicon sequencing was carried out using the Illumina MiSeq platform of Major Biotechnology Co., Ltd. (Shanghai, China).

The sequencing of AMF raw data yielded 286,054 reads. The pairs of reads were spliced into sequences according to the direct overlap relationship of paired-end reads. The quality of reads and splicing effect were controlled and filtered, and the correction of the sequence direction was made according to the end of the box sequence.

Operational taxonomic units (OTUs) with 97% similarity cutoff were clustered using UPARSE (version 7.1, <http://drive5.com/uparse/> (accessed on 3 September 2021)) from AMF. Chimeric sequences were identified and removed. The taxonomy of each OTU representative sequence was analyzed by RDP Classifier (<http://rdp.cme.msu.edu/> (accessed on 7 September 2021)) against the 18S rRNA database at a confidence threshold of 0.7.

2.4. Statistical Analysis

The OTUs of AMF defined at 97% sequence similarity were used for statistical analyses [45]. To detect the otherness of microbial composition in the three phosphate fertilizer treatment groups, we conducted Nonmetric Multidimensional Scaling (NMDS) analysis. The composition of the AMF was analyzed in three treatments by ordination using NMDS with Bray–Curtis dissimilarity. Significant differences based on 999 permutations were explored using permutational multivariate ANOVA (PERMANOVA), which was performed with the R package “vegan” [46]. To show the proportion of AMF in the three treatments at species and OTU levels, we used the R package “circlize” in R version 4.1.0 (available at <https://cran.r-project.org/web/packages/circlize/index.html> (accessed on 7 September 2021)) to make a chord diagram [47]. Redundancy analysis (RDA) was used to evaluate the difference in soil properties among different AMF communities. RDA was completed with the “ggord” package of R [48].

We used a torus translations test to determine whether species distribution preference species composition differed among treatments. The torus translation test is currently the most commonly used method for determining the association between a species and a habi-

tat [49–51]. It calculates the probability of the true distribution of a species in each habitat under the condition of random distribution and uses probability analysis to determine whether a species is significantly correlated with a certain type of habitat [52]. In addition, the test considers the spatial autocorrelation of species distribution. The associations of all OTUs with three treatments (positive correlations, $p \leq 0.05$) were analyzed. Details of the method are provided by Harms et al. [49].

The Normalized Stochasticity Ratio (NST) was used in studying the mechanisms underlying soil microbial community assembly based on Jaccard dissimilarity and determining whether community assembly is deterministic or stochastic through quantitative evaluation (<50% or >50%, respectively) [53]. We determined whether the AMF communities were clustered or overdispersed by analyzing the deviation of each observed metric from the average of the null model (Checkerboard Score (C-score)) [54]. The standardized effect size (SES) higher or lower than the expected values was interpreted as overdispersion or underdispersion, respectively, and the magnitude of SES was interpreted as the level of influence of deterministic process on assembly [55]. The C-score was evaluated based on 30,000 simulations. The sequential swap randomization algorithm with the package “EcoSimR” in R version 4.1.0 was used [56].

3. Results

3.1. Species Diversity of AMF under Different P Gradients

NMDS showed significant differences among field soil AMF communities under different treatments ($p < 0.05$; Figure 1A). The stacked bar plot and the chord plot showed OTUs belonging to Glomerales had the highest relative abundance (OTU21, OTU14, and OTU53; Figure 1B,C and Table S1). In the ranked richness of detected OTUs (at the genus level; Table S2), Glomerales claimed the top spot (with 33, 32, and 28 OTUs under low, medium, and high P conditions, respectively). Following closely were Diversisporales (with three, two, and two OTUs under low, medium, and high P conditions, respectively), and Scutellospora (with one, one, and one OTU under low, medium, and high P conditions, respectively).

In RDA, all canonical axes cumulatively explained 41.7% of the variance, and the first two canonical axes accounted for 20.82% and 13.29% of the variance separately (Figure 2A). The rarefaction curve gradually flattened as the number of measured sequences increased. The rarefaction curve showed high species richness and evenness under the low-P condition (Figure 2B).

3.2. Correlation of AMF Community under Three P Concentrations

Through torus-translations test, positive associations with the three treatments were observed in 36 out of the 51 (70.59%) examined AMF OTUs ($p < 0.05$). The torus translations test showed that 37.84% (14/37) of the OTUs tended to be distributed in the treatment with low P concentration, whereas 34.29% (12/35) and 32.26% (10/31) of the OTUs were distributed in the treatment with medium and high P concentrations, respectively. No AMF was negatively correlated with treatment (Figure 3A). The proportions of positive correlation between each treatment and microorganisms were 25.93% in the treatment with low P concentration, 22.22% in the treatment with medium P concentration, and 18.52% in the treatment with high P concentration (Figure 3B).

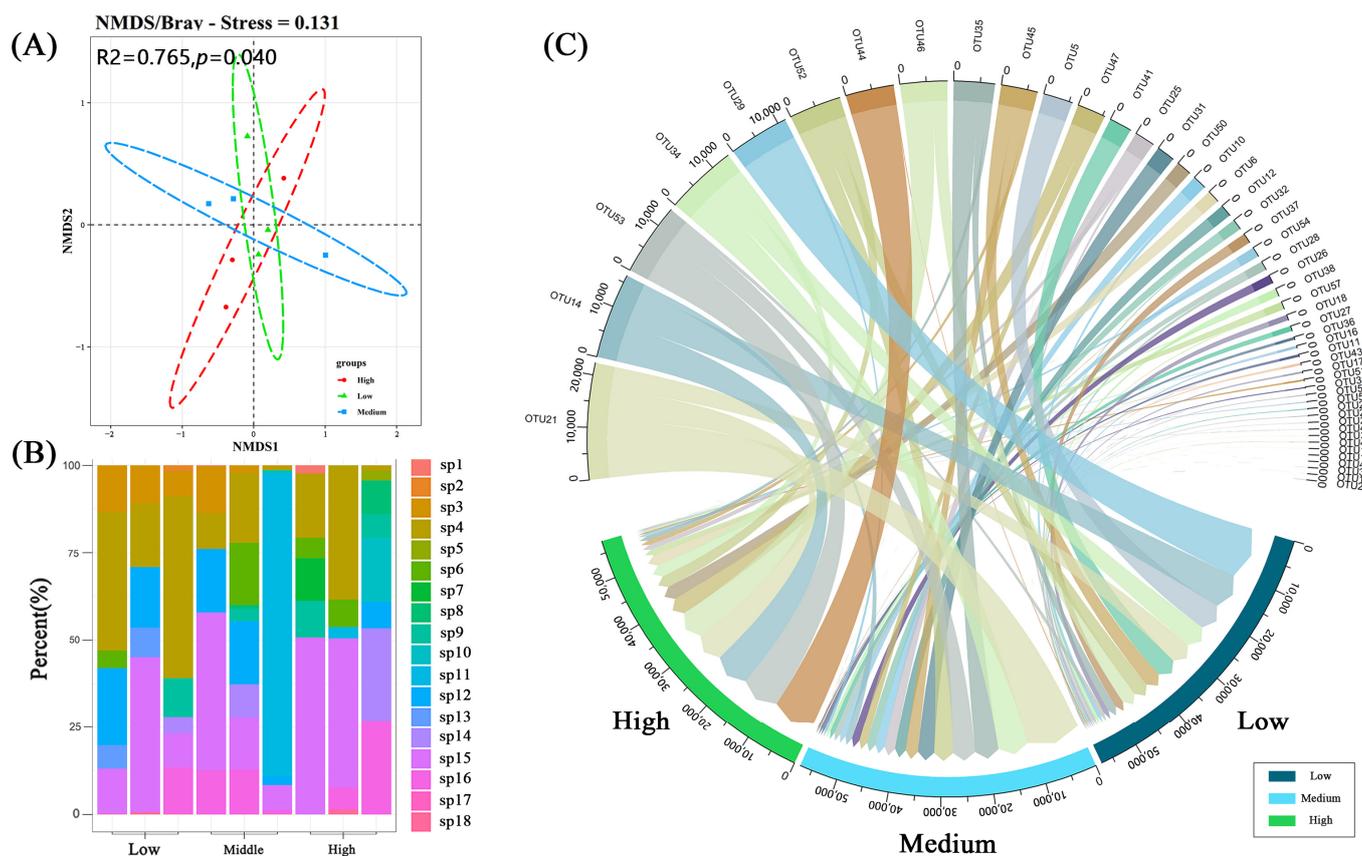


Figure 1. AMF OTU distribution under different P concentrations. **(A)** Natural dynamics of P-gradient-dependent AMF: NMDS plot of the Bray–Curtis-based dissimilarity matrix of AMF communities. **(B)** Stacked bar plot. At the species level, the percentage distribution of 18 AMF species in habitats under different P concentrations is shown. The abbreviations of species are shown in Supplementary Table S3. **(C)** Chord plot. At the OTU level, the percentage distribution of 18 AMF species in habitats under different P concentrations is shown. In the upper half of the circle, different colors are used to distinguish between different OTUs. In the lower half of the circle, each fan area represents habitats treated with high, medium, and low P concentrations. The width indicates the total amount of co-occurrence that connects an AMF OTU to a habitat.

3.3. Relative Importance of Deterministic and Stochastic Processes along the P Gradient

The NST values (Figure 4A) based on Jaccard confirmed that the AMF in the three treatments were predominately governed by deterministic processes in temperate agroecosystems. Furthermore, the C-score results showed that the SES of high P was the highest in the three treatments (Figure 4B), demonstrating that high P is more influenced by deterministic processes than low or medium P.

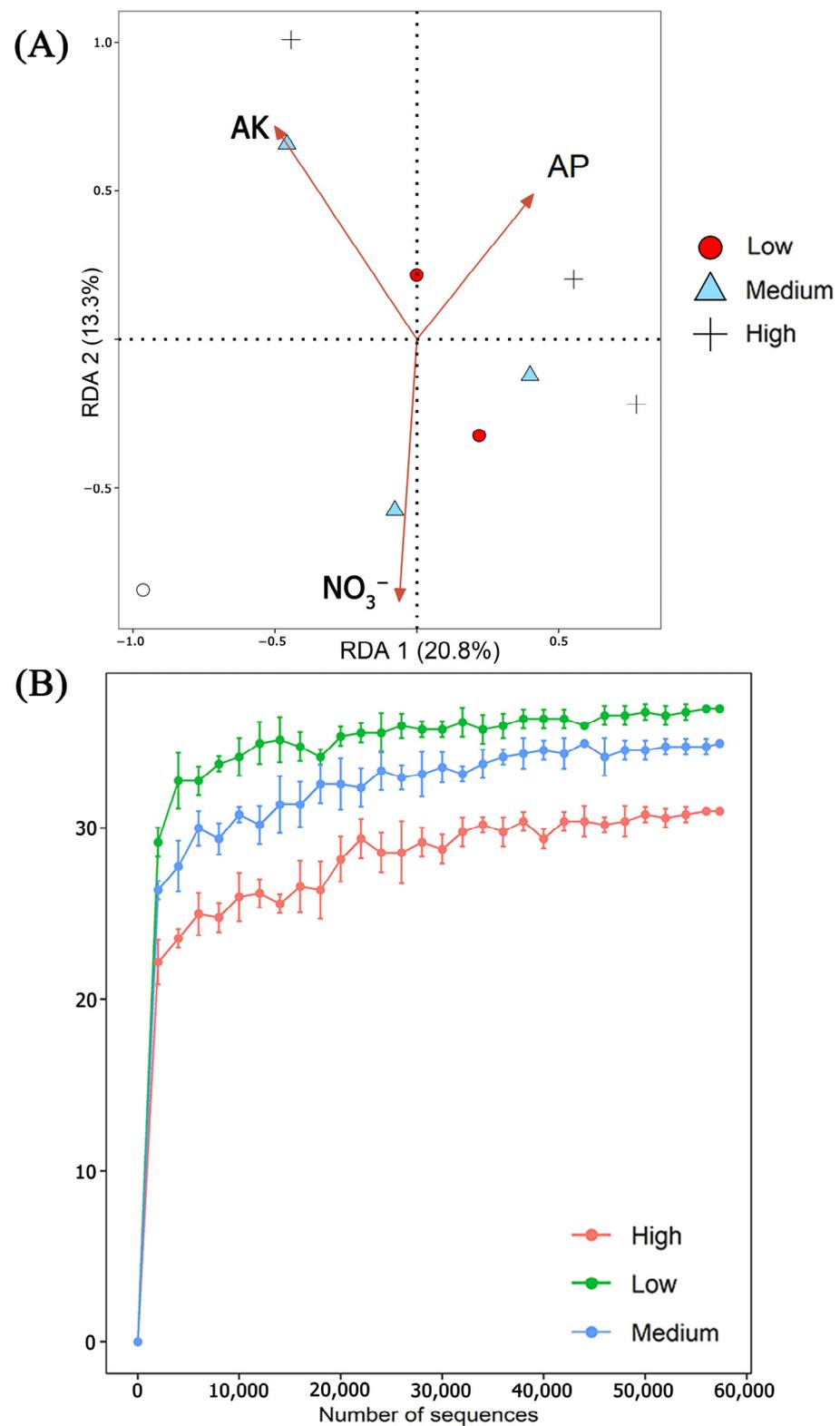


Figure 2. (A) RDA of AMF and soil properties under different P concentrations. The horizontal dashed line represents RDA Axis 1, while the vertical dashed line represents RDA Axis 2. (B) Rarefaction species richness curves. Rarefaction curve analysis was performed to deduce difference in AMF distribution among different P concentrations.

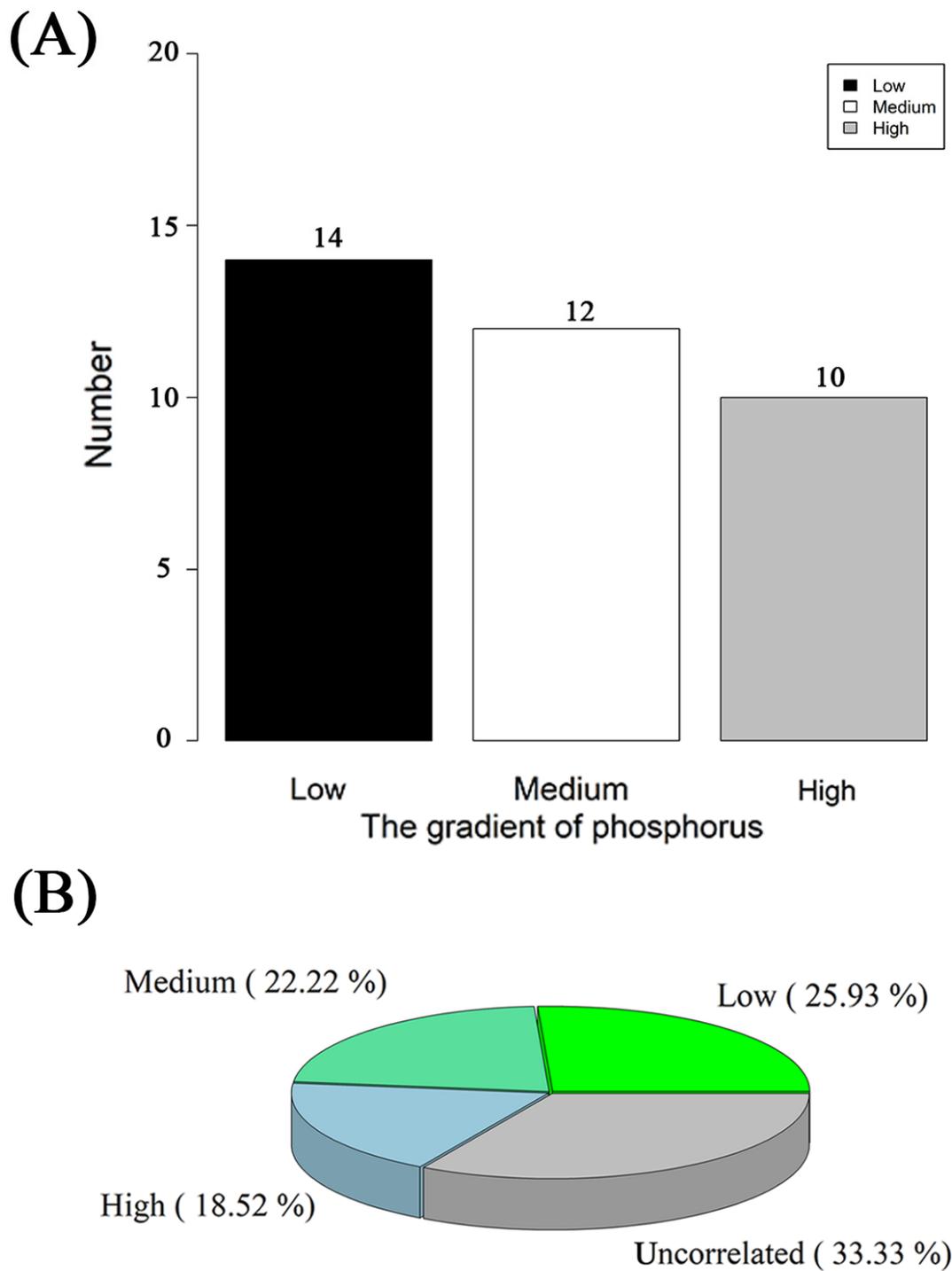


Figure 3. Differences in species distribution between communities and individuals. (A) Histogram of the number of OTUs positively correlated with the three habitats after the torus translation test. (B) Pie charts of the percentage of OTUs positively correlated with the three habitats after torus translation test.

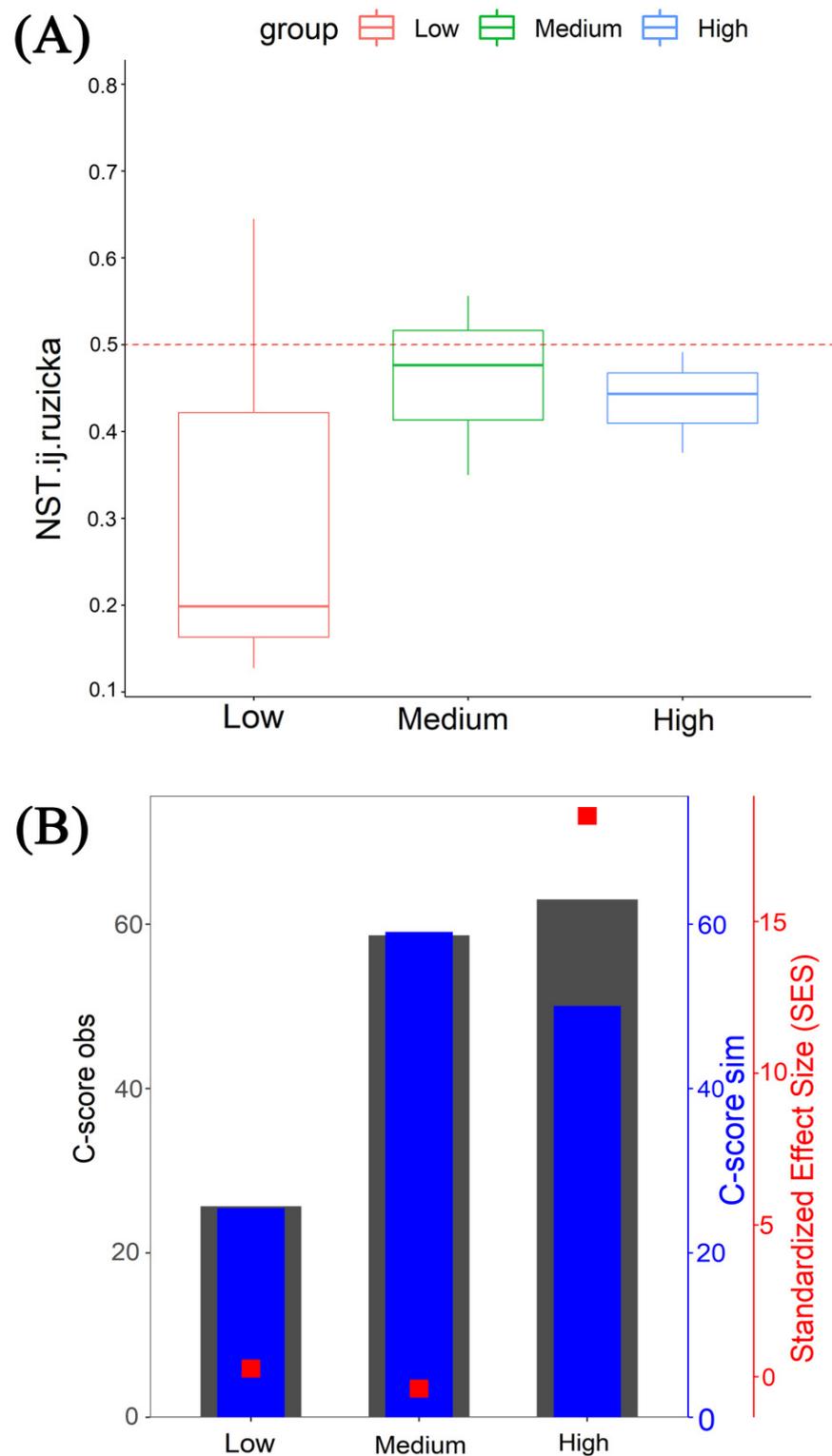


Figure 4. Ecological processes shaping AMF community assembly. (A) NST for AMF communities in temperate agroecosystem soils (NST < 50%: community assembly is more deterministic; NST > 50%: more stochastic). (B) C-score metric using null models. The values of observed C-score (C-score_{obs}, shown in gray) > simulated C-score (C-score_{sim}, shown in blue) indicate nonrandom co-occurrence patterns. Standardized effect sizes (shown in red) of -2 and >math>2</math> represent aggregation and segregation, respectively.

4. Discussion

The NCP is one of the foremost vital grain production regions in China and produces 67% of the nation's wheat and 28% of the nation's maize [9], and wheat–maize rotation is the main cropping system within the region [57,58]. Some studies have shown that long-term P fertilizer treatment significantly increases the available P content in soil [59]. AMF community structures were found to be correlated with the levels of available P content [60,61]. In our study, the structure of the AMF community in wheat–maize rotation root soil was obtained by sampling soil with different P concentrations. NMDS showed that soil AMF community structure was affected by different P treatments ($p < 0.05$). The soil properties were found to explain 41.7% of the variation in the OTU distribution among the three AMF communities. Kahiluoto et al. found that while a modest addition of P fertilizer can enhance AMF diversity, excessive application rates may significantly reduce AMF diversity and alter species composition in oilseed flax (*Linum usitatissimum* L.) field in temperate agricultural systems [62]. In our research, the relative abundance of Glomerales and Diversisporales was lower under the high-P condition than under the low- or medium-P conditions, and there were significant variations in AMF community structures among the three P concentration conditions. The long-term application of P fertilizer in soil significantly affected AMF community structure in temperate agricultural systems in wheat–maize rotation root soil.

Rarefaction curves clearly show a decline in AMF diversity with high P fertilizer concentration. This outcome is notably consistent with the findings of a previous study. Remarkably, a substantial body of literature encompassing fertilization experiments that encompass N and P additions within temperate systems corroborates this deleterious impact on AMF richness [63–67]. Our results align with previous studies, highlighting the robustness of this pattern in nutrient-driven alterations of AMF community structure and its ecological relevance.

The torus translation test results (Figure 3A,B) indicate that AMF tend to accumulate under low P concentrations. This may occur because plants reduce carbohydrate supply to root mycorrhiza in high-available-P soil environments [68,69]. In response to elevated plant-available P levels within the soil, specific AMF taxa that excel in capturing plant carbohydrates are favored [70]. As root-associated mutualistic symbionts, AMF communities mediate nutrient flow from the soil to the host plant in exchange for assimilated carbon [18]. High available P concentrations led to reduced matter exchange between plants and AMF communities, causing plants to acquire P directly from the soil. Consequently, AMF community diversity decreased [70–74]. Another possibility is that the addition of P affected the resource utilization of the AMF communities, reduced the diversity of microbial communities, and led to the narrowing of niche width. When the level of available P in soil was high, the AMF may have inhibited decomposition, and the hosts may have acquired low amounts of nutrients through the AMF pathway [75]. These processes may have affected AMF communities in symbiotic relationships with plants.

Generally, excess P fertilization under controlled conditions tends to reduce the relative abundance or diversity of AMF [76]. Nevertheless, recent findings suggest that high soil P supply may not consistently negatively affect AMF diversity in agricultural production. For instance, a study spanning three years in maize–soybean rotation systems found that the application of various organic and inorganic P fertilizers did not significantly impact soil or root-inhabiting AMF [77,78]. Most studies have shown that the excess application of P fertilizers to agricultural production reduces the diversity or relative abundance of AMF, which may be affected by spatial heterogeneity.

We found that shifts in P in field soil have a critical influence on the assembly of AMF communities, primarily by affecting the balance between deterministic and stochastic processes. Our results clearly show that deterministic processes play a dominant role in AMF assembly in temperate agricultural systems with P fertilizer application. This suggests that certain AMF species have a competitive advantage in such conditions, indicating varying sensitivities to P among AMF species. We speculate that certain AMF species exhibit high

sensitivity to low-P environments, where they can establish symbiotic relationships more effectively and aid in the efficient P uptake by plants. These AMF species typically play a crucial role in P-deficient soils, assisting plants in overcoming P limitations. Simultaneously, there are AMF species that display greater tolerance to high-P environments. They can persist under high-P conditions and continue to form symbiotic associations with plants. These particular AMF species might be more advantageous for plants growing in high-P soils as they can provide support for the uptake of other nutrients. This finding helps us better understand how AMF communities respond to changes in the external environment, and thus predict the likely evolution of AMF communities under different environmental conditions. Hence, future research should delve into investigating the diverse sensitivities of various AMF species to P.

Overall, deterministic processes play a dominant role in AMF assembly in temperate agricultural systems where P fertilizers are applied. Our findings hold valuable implications for refining and advancing our understanding of community assembly and coexistence models for AMF. By elucidating the factors and mechanisms that govern the assembly of AMF communities under varying P conditions, our study contributes to a more nuanced comprehension of ecological interactions in temperate agroecosystems.

While our study yielded some significant findings, there are also limitations and avenues for future improvement. Firstly, our experiments were conducted in a specific agricultural setting, and variations in geographical locations and soil types may yield different outcomes. Secondly, our primary focus was on assessing the impact of P on AMF communities, and we did not perform a comprehensive analysis of crop yield components or the colonization of AM fungi in plant roots. Therefore, future research should consider the integrated effects of these complex factors. Finally, despite implementing a series of measures to minimize errors in our study design and data analysis, the limitations imposed by sample size necessitate further replication experiments and statistical analyses to validate our results.

5. Conclusions

Our findings reveal that the prolonged application of P fertilizers significantly modifies the compositions of AMF communities within the root soil of temperate agricultural systems practicing wheat–maize rotation. Alterations in the P levels present in field soil exert a pivotal influence on the assembly mechanisms of AMF communities, predominantly by perturbing the equilibrium between deterministic and stochastic processes. Notably, under conditions of high P availability, deterministic processes exert a more pronounced impact compared to situations with low and medium P levels. In the context of temperate agricultural systems utilizing P fertilizers, deterministic processes emerge as the principal drivers governing AMF assembly.

These findings underscore the importance of balanced nutrient management, particularly regarding P, in maintaining AMF communities' stability. Future research could explore the complexity of AMF interactions under different climatic and soil conditions to explore adapting community assembly and coexistence models to AMF.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d15101045/s1>, Table S1: The AMF community abundance table of three P treatments; Table S2: Percentage distribution of AMF community under three P treatments; Table S3: Abbreviations for species of AMF.

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Conflicts of Interest: The authors declare no conflict of interest.

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