Long-term N addition reduced the diversity of Arbuscular mycorrhizal fungi and understory herb of the Korean pine plantation in northern China

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Abstract

With the development of agriculture and industry, the increase in nitrogen (N) deposition has caused widespread concern among scientists. Although emission reduction policies have slowed N releases in Europe and North America, the threat to biodiversity cannot be ignored. Arbuscular mycorrhizal (AM) fungi play an important role in the establishment and maintenance of plant communities in forest ecosystems, both their distribution and diversity have vital ecological functions. Therefore, we analyzed the effects of long-term N addition on AM fungi and understory herbaceous plants in Korean pine plantation in northern China, measured the soil properties, AM fungal and herbaceous community structure and diversity with different concentrations of NH4NO3 (0,20,40,80 kg N ha-1 year-1) applied for 7 consecutive years. The results showed that long-term nitrogen fertilization affected soil properties, decreased soil pH, increased soil ammonium content, and caused significant fluctuations in P elements. Nitrogen application improved the stability of soil aggregates by increasing the content of GRSP (glomus-related soil protein); N addition changed the AM fungal community composition, and Glomus genus was more adaptable to the acidic environment treated with the highest nitrogen concentration; with the increase of N treatment, The species of AM fungi and herbaceous plants and the biomass of fine roots decreased. In summary, we concluded that long-term understory application of different concentrations of N altered soil pH, changed the distribution of N, P elements, and the soil aggregate fractions, reduced AM fungal and herb diversity. The importance of AM fungi in maintaining forest ecosystem diversity under the pressure of global change was verified.

1. Introduction

The dramatic increase in atmospheric emissions of reactive nitrogen (N) from human activities (industrial and agricultural activities and fossil fuel combustion) since the Industrial Revolution has also led to the rapid increase in N deposition to terrestrial and aquatic ecosystems ((Ackerman, Millet , & Chen, 2019). N deposition provides a new source of fertilizer for plants, but excessive N input can also affect biogeochemical cycles and alter ecosystem structure and function (Yu et al., 2019). Forest ecosystem species diversity is an important indicator reflecting the relationship between plants and the environment, determining the structure and ecological functions of forest communities, which are mutually constrained and synergistic with environmental factors. Moderate N input will alleviate forest N limitation to a certain extent and promote vegetation growth. On the other hand, excessive N deposition induces ecological problems such as soil acidification, reduction of biodiversity, and degradation of forest functions(Gao et al., 2019; Hong, Gan , & Chen, 2019; Vuorenmaa et al., 2018). The results of a worldwide network of long-term monitoring of N deposition since the twentieth century show that China faces more severe N deposition than the United

States, European countries, and other countries in East Asia(Zhang et al., 2021). Nitrogen deposition remains a threat to the biodiversity and stability of forest ecosystems (Weldon, Merder, Ferretti , & Grandin, 2022).

Herbaceous plants have the highest species diversity in forest ecosystems and contribute significantly to forest ecosystem structure and function, but are more sensitive to atmospheric changes and N deposition(GILLIAM, 2007; McDonnell, Clark, Reinds, Sullivan , & Knees, 2022; Thrippleton, Bugmann, Kramer-Priewasser , & Snell, 2016). It has been demonstrated that excessive N input can have complex effects on herbaceous community structure and plant biological characteristics, such as reducing herbaceous diversity and altering herbaceous root chemistry and biomass(Li et al., 2015). However, most studies have focused on tropical forests and temperate grasslands, and not enough studies have been conducted on herbaceous plants in temperate forests.

The functions of soil microorganisms in forest ecosystems should not be underestimated, among which AM fungi can establish symbiotic relationships with most herbaceous plants and play an important role in the establishment and maintenance of plant communities((Mariotte et al., 2013; Smith & Read, 2008; Wang et al., 2019). Arbuscular mycorrhizal (AM) fungi can promote plant growth((Hoeksema et al., 2010; Xie et al., 2022)and improve plant resilience(Chen, Arato, Borghi, Nouri , & Reinhardt, 2018; Ruiz-Lozano et al., 2016), and mycelium secretion-GRSP can also promote soil aggregation(Gao, Wang , & Wu, 2019), affecting host plants in a range of direct and indirect ways. Most mycorrhizal fungi depend on the host plant for existence and reproduction and AM fungi are thought to be susceptible to environmental conditions, such as climate((Compant, van der Heijden , & Sessitsch, 2010), plant species((Kivlin, Hawkes , & Treseder, 2011), and soil properties, etc. Excessive N input indirectly affects the structure and diversity of AM fungi through changes by soil factors (Boeraeve et al., 2022; Ma et al., 2021), and thus affects the ecological function of AM fungi. The results of the studies are not the same around the world, which is due to the influence of the experimental conditions. A global meta-analysis showed that the negative effect of N addition on AM fungi was mainly a reduction in total AM fungal abundance and did not significantly affect the diversity and structure of AM fungi(Han, Feng, Han , & Zhu, 2020). However, in a tropical simulated N deposition experiment, it was found that N addition mainly reduced the diversity and abundance of AM fungi(Camenzind et al., 2014). AM fungi have been shown to contribute to the maintenance of forest herbaceous diversity under N application conditions(Smith & Stephan, 2021), But studies in northern forest lands are scarce.

In this experiment, AM fungi, herbaceous plants, and soil properties were investigated in a northeastern temperate Korean pine plantation ecosystem that was subjected to a 7-year N addition experiment. We hypothesized that (1) Long-term nitrogen fertilization will change the soil pH value, increase soil GRSP content and thus improve the stability of soil aggregates. (2) Long-term N addition will change the structure and diversity of AM fungal communities, thus affecting the ecological functions of AM fungi. (3) Long-term N addition would directly change AMF diversity by reduce pH, and indirectly by change herbaceous plant composition.

2. Materials and methods

2.1 Study site

The experiment sample site is located in the Korean pine plantation in Liangshui National Nature Reserve, Heilongjiang Province, China (128°53'20"E, 47deg10'50"N), Natural N deposition is 12.93 kg N ha^{-1} $year^{-1}$. The area has a distinct temperate continental monsoon climate with an average annual temperature of -0.3degC, of which the average annual precipitation of 676 mm, a frost-free period of 100-120 d, and an annual snowpack period of 130-150 d. The Korean pine plantation in this test site was established in 1954 after a clear-cutting of the mixed broadleaved-Korean pine forest. The soil is dark-brown forest soil (using the Chinese classification), The dominated species is Korean pine, and undergrowth associated herbs were *Oxalis corniculate* , *Mitella nuda* , etc.

2.2 Experimental design

The simulated N deposition experiment commenced in 2014, the sample plots were treated with N application from June to mid-September. The required NH_4NO_3 for each treatment was dissolved in 20 L of water, and CK only adds 20 L of water. Each sample square 5 m x 30 m, bounded by a 10 m wide buffer strip, with a total of four gradients of CK (0 kg N $ha^{-1}year^{-1}$), LN (20 kg N ha^{-1} $year^{-1}$), MN (40 kg N ha^{-1} $year^{-1}$) and HN (80 kg N ha^{-1} $year^{-1}$) ((Song, Zhang, Muller , & Jin, 2019). There were three replicates in each treatment with completely randomized design.

2.3 Soil sample collection and pre-treatment

Soil samples were collected in mid-July 2020. Soil samples were collected using soil samplers (0-15 cm deep, 5 cm inside diameter), and five random samples were collected from each plot and mixed to a composite sample. Soil samples were sieved and divided into three parts: One part was stored at -80degC for soil microbial DNA extraction, another part was air-dried and used for soil chemical property determination, the rest part was used to determine the composition of soil aggregates and measures the content of GRSP.

2.4 Soil physicochemical properties

The air-dried soil samples were sieved through soil sieves with 2 mm, 1 mm and 0.25 mm aperture, and four groups of soil samples were obtained (>2 mm, 2-1 mm, 1-0.25 mm, <0.25 mm) (Muruganandam, Israel , & Robarge, 2009), use an electronic balance to weigh the weight of each particle size. The soil structural stability was evaluated by the mean weight diameter (MWD) of water-stable aggregate (Zhao, Chen, Hu , & Li, 2017). The content of easily extractable glomalin-related soil protein (EE-GRSP) and total glomalin-related soil protein (T-GRSP) was determined by improving the assay method according to Janos et al. (2008). The EE-GRSP was extracted from 0.5 g soil with 4 ml of 20 mmol L^{-1} of sodium citrate (pH=7.0) and autoclaved at 121 for 30 min. The T-GRSP was extracted from 0.5 g soil with 4 ml of 50 mmol L^{-1} of sodium citrate (pH=8.0) and autoclaved at 121 for 60 min.

The supernatant was collected by centrifugation at 10000 rpm for 10 min at 4. The T-GRSP extraction was performed 6 times until the solution was straw-colored ((Gao, Zhou, Ling, Hu , & Chen, 2017). An spectrophotometer (595 nm) was used to determine the GRSP content using bovine serum protein as the standard product.

The rest air-dried soil samples were used to determine soil pH by pH meter (FiveEasy FE20) on a mixture of water to soil ratio of 2.5:1. TC (total carbon), TOC (total organic carbon), and total N (TN) were determined by the analyzer (multi N/C 2100 TOC/TN Analyze, Germany, Analytikjene). Soil ammonium N (NH_4^+-N) and nitrate N (NO_3^--N) were determined by continuous flow analyzer (BRAN+LUEBBEAA3, Germany), and the extract solution was 2 mol*L^{-1} KCl, and soil total phosphorus (TP) and available phosphorus (AP) were determined by the molybdenum antimony anti-colorimetric method.

2.5 Herbaceous plant diversity, fine root biomass, and AM fungal colonization rate survey

We explored herbaceous plant diversity in mid-July 2020. Three small randomly sample squares (1 mx1 m) were set in each sample square, the species of herbaceous plants, the number of individuals of the species (counted as a single plant in the above-ground part), height, and cover were investigated. The soil was collected at a depth of 0-15 cm using a soil auger, and the fine roots (<2 mm in diameter) of herbaceous plants were selected and their fine root biomass was measured(Li et al., 2021). We determined the AM fungal colonization rate of fine roots from herbs by a modified aniline blue staining method ((Zubek, Błaszkowski , & Mleczko, 2011).

2.6 AM fungal community structure and diversity

2.6.1 Isolation and identification of AM fungal spores

The wet sieving method was used to obtain spores. The spores were placed under a stereomicroscope for count and observation. Then transferred spores to polyvinyl alcohol-lactic acid-glycerol (PVLG) with a pipette, and morphological characteristics were examined microscopically. It included spore color, shape, size, sporiferous saccule, spore wall thickness and type, subtending hyphae width and shape. The spores

were identified according to the photos and feature and feature descriptions from *VA mycorrhizal fungi identification manual* (Schenck & Perez, 1990), INVAM (https://invam.ku.edu/), International AM fungal classification system (http://www.amf-phylogeny.com/).

AM fungal spore density, species richness, relative abundance (RA), frequency, importance value (I), and species alpha diversity index were calculated per 10 g of air-dried soil sample. The degree of AM fungal dominance was assigned into four categories according to their important values (I), I > 50% means dominant species, 30% < I [?] 50% means subdominant species, 10% < I [?] 30% means accompanying species and I [?] 10% means rare species.

2.6.2 High throughput analysis of AM fungi

Total DNA was extracted from mixed soil (0.5 g) using Fast DNA® Spin Kit for Soil. And then AM fungus-specific primers AML1 and AML2 were selected as the first round of primers ((Lee, Lee , & Young, 2008), AMV4.5NF (AAGCTCGTAGTTGAATTTCG) and AMDGR (CCCAACTATCCCTAT-TAATCAT) were selected as the second round of primers for amplification with the nested PCR amplification method ((Sato, Suyama, Saito , & Sugawara, 2005). The PCR amplification products were detected and recovered by 2% agarose gel electrophoresis, and Illumina's MiseqPE300 platform was used for sequencing. Raw sequences were uploaded to the NCBI Sequence Read Archive (SRP426700).

2.7 Statistical analysis

One-way ANOVA and Duncan's multiple tests ($P < 0.05$) were performed using IBM SPSS Statistics 26 (IBM Corporation, New York) software to compare the means of plant, AM fungi, and soil physicochemical property data. \soutTwo-way ANOVA was used to analysis the interaction effect between N addition and soil agglomerate particle size on GRSP. The correlation between soil physicochemical properties and herbaceous plants diversity was calculated by Pearson's test (two-tailed) at two significant levels, $P < 0.05$ and$P < 0.01$. RDA analysis was used to reveal the effect of soil properties on the community composition of AM fungi. Structural equation modeling (SEM) was used to predict causal pathways of N addition, key soil physicochemical indicators, and AM fungi on herbaceous plant diversity. Build prior models and evaluate model fit using chi-square, RMSEA, and GFI.

3. Result

3.1 Soil physical and chemical properties

3.1.1 Soil chemical properties

The soil pH gradually decreased with the increase of N addition concentration, and the pH value in HN treatment significantly decreased to 5.24 ($P<0.05$) (Table 1). TP content was significantly increased by 16.7% in the MN treatment. The AP content was significantly reduced after N addition. There were significant changes in NH_4^+-N and NO_3^--N content. The NH_4^+-N content increased with the concentration of N addition increasingly, and there was a significant increase in MN and HN treatments. The NO_3^--N content in the LN treatment was significantly increased and was significantly decreased in the HN treatment.

3.1.2 Soil aggregate and glomalin-related soil protein

The weight percentage of soil aggregate varied with its size fractions (Fig.1). In all N addition treatments, MWD values ranged from 1.48-1.74 mm. The content of large aggregates (2-5 mm) and MWD value was significantly higher in the HN treatment compared to the CK.

N addition exerted significant effect on the EE-GRSP and T-GRSP content of soil aggregate. The contents of EE-GRSP ranged from 1.36-4.29, and T-GRSP ranged from 3.73-13.50 $mg{\cdot}g^{-1}$ (Fig.2). \soutThough two-way ANOVA analyze, there were interaction effect between N addition and aggregate fractions on GRSP ($P<0.01$). Among all treatments, the GRSP content of soil aggregate <0.25 mm was the highest; GRSP content varied according to the amount of N addition. N addition increased the content of EE-GRSP and

T-GRSP in soil aggregates, and reached the maximum in the MN treatment. N addition increased the EE-GRSP content of soil aggregates by 60.6% to 118.7%, 12.0% to 77.2%, 5.0% to 60.5%, and 16.3% to 56.2% for each particle size (<0.25 mm, 0.25-1 mm, 1-2 mm, and 2-5 mm), respectively. N addition increased the T-GRSP content of soil aggregates by 45.5% to 87.6%, 25.5% to 88.4%, 44.5% to 105.2% and 51.1% to 86.4% for each particle size, respectively.

3.1.3 Relationship between the stability of soil aggregates and the content of GRSP content under N addition conditions

In the present study, Korean pine plantation soils were dominated by medium aggregates (1-2 mm) with MWD values ranging from 1.48 to 1.74 mm, which have been identified as an indicator of soil stability, and long-term (7 years) HN treatment (80 kg N $ha^{-1}year^{-1}$) increased the soil macro-aggregates content and thus improved soil stability. Pearson correlation analysis of undisturbed soil GRSP content and MWD showed that both EE-GRSP and T-GRSP were significantly positively correlated with MWD values (P <0.01) (Fig. 3).

3.2 Understory herbs

3.2.1 Herb plants communities

There were 33 species of herb plants were investigated in this experiment (Table 2). N addition significantly altered the dominance of the first five plants in Table 2 (P <0.05), which were *Chrysosplenium sinicum, Aegopodium alpestre, Dryopteris crassirhizoma, Eranthis stellata* and*Phryma leptostachya* . Compared with CK, 9.1% of the species were not detected in the LN treatment, 27.3% in the MN treatment, and 33.3% in the HN treatment.

3.2.2 Herb diversity, fine root biomass, and AM fungal colonization rate

The Shannon index and Simpson index of herb plants decreased gradually with increasing gradient of N addition concentration, and the MN and HN treatments were significantly lower than CK (Table 3); The fine root biomass and AM fungal colonization rate decreased significantly with the increase of N addition concentration gradient, and the biomass decreased by 78.6% in the HN treatment, the AM fungal colonization rate decreased significantly in the MN and HN treatments, and decreased by 21.95% in the HN treatment. These indicated that herb diversity and AM fungal colonization rate of fine roots were significantly reduced when the applied N concentration exceeded 40 kg N $ha^{-1}year^{-1}$ in this experiment. The fine root biomass of herb plants was significantly reduced when the applied N concentration exceeded 20 kg N ha^{-1} $year^{-1}$.

3.3 AM fungal community structure and diversity

3.3.1 Morphological identification of AM fungi

There were 4461 AM fungal spores isolated from all soil samples, and 9 genera and 20 species were identified morphologically. Among them, 7 species belonging to *Acaulospora* are the most diverse, accounting for 35% of the total number of species, followed by the genus*Glomus* with four species, accounting for 20% of the total number of species.

The relative abundance (RA) and importance values (I) of AM fungal spores under the N application treatment were analyzed (Table 4). The soil samples from the CK had the most abundant spore species, with 17 AM fungal species identified, while*Acaulospora spinosa* and *Claroideoglomus lamellosum* only appeared in CK and not found in other treatment. Gradual reduction of spore species in soil samples with increasing N addition concentration, and 14 species of AM fungi were isolated and identified in the LN treatment. There were also 14 species in the MN treatment, in which*Sclerocystis sinuosa* was unique and never found in other treatments. It was found that HN treatment reached the least AM fungal species of 10. In summary, the increasing of N addition concentration gradually reduced the spore species.

3.3.2 Spore density, species abundance and diversity index of AM fungi

The highest AM fungal spore density was found in the MN treatment with 40.13 spores g^{-1} (Table 5), followed by the HN and LN treatments with 36.9 and 36.43 spores g^{-1}, and the lowest 35.27 spores g^{-1} in CK. With increasing N addition concentration, the species abundance of AM fungi gradually decreased, with a significant decrease in the LN and MN treatment ($P<0.05$), and there were only 8 species g^{-1} in the HN treatment. All AM fungal species diversity differed significantly in the N addition treatment compared to the CK, both Simpson's index and Shannon's index were highest in the CK, the LN treatment was significantly lower compared to the CK and MN treatments, while the HN treatment had the lowest diversity index.

3.3.3 Bioinformatical analyses

In the present study, we identified 398299 sequences from the total dataset, optimized to 391960 sequences. The coverage of OTUs in all soil types was more than 99%. The CK had the largest OTUs richness of AM fungi (Table 6). The highest AM fungal diversity was found in the CK by Shannon index and Simpson index analysis, and gradually decreased with the increase of N addition concentration, and the lowest AM fungal diversity was found in the high N treatment.

The results of the community structural composition showed that the majority of OTUs sequences belonged to *Glomus* (Fig. 4). The relative abundance of the*Glomus* increased with N addition and was significantly higher than CK in the HN treatment. In contrast, the relative abundance of *Paraglomus* reduced by 96.71% in HN treatment compared to CK.

The AM fungal community composition and relative abundance at the species level was shown in Fig.4. The dominant species in the CK was*Glomus* -Glo3-VTX00074, *Acaulospora* -VTX00272 in the LN treatment, *Diversispora* -unclassified in MN treatment, and*Glomus* -MO-G5-VTX00219 in HN treatment. It can be seen that the N addition had a significant effect on AM fungal community composition in Korean pine plantation. *Glomus* -MO-G18-VTX00064 is a special species for the CK and did not appear in other treatments.*Glomus* -group-B-*Glomus* -acnaGlo7-VTX00057 was a special and unique specie for the LN treatment. *Glomus* -MO-G22-VTX00125,*Glomus* -Wirsel-OTU13-VTX00140 were special and unique species for the MN treatment.

3.3.4 Comparative and analysis of morphological and molecular identification results of AM fungi

The result of morphological identification showed that there were 9 genus and 20 species of AM fungi after wet sieving, while high-throughput sequencing amplified and sequenced the DNA extracted from the mixed soil from each small sample, and 4 genera and 11 virtual species were identified. The genus *Diversispora* detected by high-throughput sequencing means was not found in the morphological identification, while morphological identification revealed six genera which were not identified by high-throughput. The change trend of AM fungal diversity calculated by the two methods was the same, and the AM fungal diversity index used in the subsequent analysis was the result of morphological identification.

3.4 Relationship between soil physicochemical properties and AM fungal community composition under N addition conditions

The RDA analysis of AM fungi and soil physicochemical properties showed that six soil physicochemical factors could explain 82.15% of the variation in AM fungal communities as shown in Fig. 5. Soil pH and NO_3^--N、AP、NH_4^+-N and TN content significantly influenced the distribution of AM fungal species.

3.5 Relationship between changes in environmental factors and herb diversity under N addition conditions

Long-term N addition reduced the diversity of understory herbaceous plants. The correlation between the α diversity index of herbs and biochemical factors was analyzed by Pearson correlation. The diversity of understory herbaceous plants was significantly and positively correlated with the diversity of AM fungi (P <0.05; Fig.6), and the colonization rate of AM fungi (R^2 =0.66). The diversity of herbs was significantly and positively correlated with total nitrogen content (P <0.05), significantly and negatively correlated with

NH_4^+-N content (P <0.01), and significantly and positively correlated with pH (R^2 =0.67). Additionally, herbaceous plant fine root biomass was significantly and positively correlated with diversity (P <0.01).

A structural equation model (SEM) was developed to illustrate the effect of each factor on herb diversity under N addition conditions (Fig. 7), The P-value of $\chi 2$ was higher than 0.05 indicated that the model structure was reasonable. The SEM throughput analysis largely (R^2 > 90%) explained the effects of biotic as well as abiotic factors on herb diversity. N addition indirectly affected AM fungal diversity and plant diversity though soil pH value, ammonium N, and AP content. Among them, AM fungal diversity was the strongest pathway affecting plant diversity.

4. Discussion

4.1 Effect of N addition on soil properties

Soil acidification is caused by nitrogen deposition, and soil pH decreases linearly with increasing N application (Tian & Niu, 2015). In this study, long-term HN treatment (80 kg N $ha^{-1}year^{-1}$) reduced soil pH, while long-term low and medium N treatments had no significant effect on soil pH. The reason may be that soils in terrestrial ecosystems are more sensitive to N inputs and forest ecosystems are more stable than other types of ecosystems.

MN treatment had a significant effect on Soil TP content, while AP content decreased to varying degrees with N addition. This may be because soil acidification activates aluminum ions and iron ions in the soil, causing more phosphorus to be adsorbed by the soil, resulting in a decrease in AP content. When plants and microorganisms absorb and utilize AP, they get a corresponding supplement from the phosphorus element of other components, which leads to the reduction of AP content in different degrees.

Soil TN content was stable, ammonium content increased linearly with N application, and nitrate content fluctuated with N application. The result of Qin et al. (2022) showed long-term nitrogen application can change the soil ammonium and nitrate content. Besides, total N increased significantly with the increase of N addition. The stability of whole nitrogen content in this experiment may be because the forest ecosystem is more stable and can resist a certain concentration of N addition.

As the gradient of N addition concentration increased, there was a tendency for the easily EE-GRSP content in the in-situ soil to increase, and the T-GRSP content was significantly higher in the HN treatment than CK, probably due to the significant acidification of the soil, and it has been shown that acidic soil had high GRSP content (Agnihotri et al., 2022). On the contrary, short-term N addition reduced the GRSP content in the soil (Huang et al., 2022; Jia et al., 2021), the reason for this contrasting result may be that short-term N additions cannot significantly change soil acidity and alkalinity, but could lead to a decrease in GRSP by changing soil dissolved organic carbon and N/P ratio. The proportion of large soil aggregates and GRSP content was significantly elevated in the HN treatment. Pearson correlation analysis of in-situ soil GRSP content and MWD showed that both EE-GRSP and T-GRSP were significantly and positively correlated with MWD values (Fig.3), which is further evidence of the importance of AM fungi in soil stability.

4.2 Effect of N addition on the structure and diversity of AM fungi community

Different N addition content and local soil conditions affect plants and soil microorganisms (Cao et al., 2020; Zeng et al., 2021). In this study, both high-throughput sequencing and traditional spore morphological identification were used to investigate the community structure and diversity of woodland AM fungi. Morphological identification has inconsistencies, limitations, and chances, but can be improved by means such as increasing the sample size. High-throughput sequencing technology has been widely used by researchers, but stills has some limitations. Due to the variety of microorganisms, the current database cannot cover all the microorganisms or lacks accurate species information, resulting in the omission of some low-abundance species, so we combine the two methods to accurately reflect the diversity and structural characteristics of AM fungi (Chaudhary, Nolimal, Sosa-Hernandez, Egan , & Kastens, 2020). Both high-throughput sequencing results and morphological identification results showed that N addition altered AM fungi community composition and reduced AM fungal alpha diversity.

It is clear from the RDA analysis (Fig. 5) that pH significantly affected the community composition of AM fungi in Korean pine plantation, and there was research reported that soil pH was one of the most important abiotic factors affecting the ecological distribution of AM fungi (Davison et al., 2021). The abundance of *Glomus*increased gradually (Fig. 4) with increasing N addition concentration and was significantly higher in the HN treatment, the main reason for this phenomenon being that *Glomus* showed a better ecological advantage in acidic soils. In the experiments of (Cao et al., 2020), short-term HN treatment (80 kg N $ha^{-1}year^{-1}$) also reduced fungal diversity and changed community composition. It is believed that this phenomenon is caused by the change of P elements in the soil due to N addition, which causes plants to select AM fungi with high phosphorus absorption capacity

4.3 Effect of N addition on understory herb plants

Cases of nitrogen deposition-induced soil acidification leading to reduced plant diversity have been reported (Kimmel et al., 2020).In this study, we found that the diversity of understory herb plants in the forest also decreased with decreasing soil pH value. In addition, excess N can also cause nutrient imbalance. Large-scale input of exogenous N can break the original stable stoichiometry, thus causing a nutrient imbalance in the soil and plants, which can also lead to the loss of some species of plants. N deposition indirectly affected plant species diversity by altering the composition of soil microbial communities (Bobbink et al., 2010). In the present study, it was also found that the varies of understory plant diversity was linearly and positively correlated with AM fungal diversity and AM fungal colonization rate (Fig.6), this also verified that the function of AM fungi in maintaining the stability of ecosystems and preserving biodiversity should not be underestimated. In addition, plant fine root biomass was also significantly reduced by long-term N addition, and a small amount of N input increased plant root biomass (Frew, 2022), In contrast, excessive N addition decreased the fine root biomass with increasing time of N application. The SEM clearly shows that AM fungal diversity is the strongest influence pathway affecting herb diversity under N addition conditions (Fig. 7), which is further evidence of the ability of AM fungi in maintaining understory herb diversity.

5. Conclusion

N addition in temperate Korean pine plantation ecosystems altered AM fungal community structure, reduced AM fungal and herb diversity, decreased soil pH, increased inorganic N concentration, and decreased soil fast-acting phosphorus content. We suggested that the N application first changed the soil characteristics and then changed the composition and diversity of the soil AM fungal, and the changes in the AM fungal community in turn affect the distribution of soil aggregates, and the above-mentioned factors (biotic and abiotic) in the subsurface part together affect the plant community and diversity. Considering the importance of AM fungi in the ecosystem, future research should explore the pathways by which AM fungi regulate the ecological functions under N additional conditions to improve our understanding of the ecological functions of AM communities under global environmental change conditions.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Author contributions

All authors made significant contributions to this study. FS and GJ provided the conceptualization. FS and WW framed the methodology. WW and YF wrote and prepared the original draft. XW, RW and XH did the review and editing. MZ and KL oversaw the project administration. FS was responsible for funding acquisition. All authors contributed to the article and approved the submitted version

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Declaration of Competing Interest

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.

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