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Effects of nitrogen addition and seasonal change on arbuscular mycorrhizal fungi community diversity in a poplar plantation

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Arbuscular mycorrhizal (AM) fungi play a crucial role in carbon (C), nitrogen (N), and phosphorous (P) biogeochemical cycling. Therefore, it is essential to determine the seasonal responses of the AM fungal community to N addition to understanding better the ecological processes against a background of intensified N deposition. Based on an ongoing field simulation experiment with five N addition levels (0, 5, 10, 15, and 30 $gN \cdot m^{-2} \cdot a^{-1}$) in a 5-year-old poplar plantation at Dongtai Forest Farm in Yancheng, Jiangsu province, eastern China, soil physicochemical properties, the root colonization rate, and the rhizosphere soil AM fungal community diversity and composition in four seasons (summer, autumn, winter, and spring) were investigated. Meanwhile, the relationships between the characteristics of the AM fungal community and soil environmental factors were analyzed. High-throughput sequencing showed that the dominant genera in the poplar plantation were *Glomus* (average relative abundance 87.52%), *Diversispora* (9.62%), and *Acaulospora* (1.85%). The addition of N significantly increased the root colonization rate in spring. The diversity of the AM fungal community (Chao and Shannon indexes) was primarily affected by seasonal change rather than N addition, and the diversity in summer was significantly lower than in the other three seasons. Redundancy analysis showed that soil temperature, available P, total P, and pH significantly affected the structure of the AM fungal community. It can be concluded N addition primarily influenced the root colonization rate, whereas seasonal change had a notable effect on the AM fungal community diversity. Although seasonal change and N addition greatly influenced the composition, seasonal change exerted a more substantial effect than N addition. These results will improve our understanding of the underground ecological processes in poplar plantation ecosystems.

KEYWORDS

nitrogen deposition, arbuscular mycorrhizal fungi, composition and diversity, season, plantation, eastern China

1. Introduction

Global nitrogen (N) deposition caused by anthropogenic activities is increasing dramatically (Penuelas et al., 2013). The atmospheric N deposition rates have exceeded 20 kg N·ha^{-1}·a^{-1} in the forest ecosystems of central and eastern China (Liu et al., 2011). Intensive deposition of reactive N can induce soil nutrient imbalances, which can alter plant productivity, abundance, and ecosystem function (Bobbink et al., 2010), as well as the soil microbial community diversity and composition in forest ecosystems (Tian et al., 2017; Wang et al., 2018).

Arbuscular mycorrhizal (AM) fungi can form a symbiosis with most terrestrial plants, thereby improving nutrient absorption and stress tolerance. In return, host plants provide photosynthetic products for the growth of AM fungi (Smith and Read, 2008). Consequently, AM fungal associations link the below- and above-ground communities in ecosystems and play crucial roles in carbon (C), N, and phosphorous (P) biogeochemical cycling. The high N availability and soil acidification caused by N deposition can influence AM fungal communities, thereby altering the related soil ecological processes (Lilleskov et al., 2019). Despite this recognition, the effect of N deposition on AM fungal communities remains ambiguous and is the subject of intense scientific debate because previous studies have reported negative (Chen et al., 2017; Williams et al., 2017; Treseder et al., 2018), positive results (Egerton-Warburton et al., 2007), or no effects (Mueller and Bohannan, 2015; Maitra et al., 2021). These discrepancies could have been caused by differences in host plant and ecosystem types, soil nutrient contents (N and P), and N addition amounts and durations (Han et al., 2020). Therefore, it is critical to conduct more studies on this topic better to understand AM fungi's roles in ecosystem function.

AM fungi belong to the new Glomeromycota phylum; there is one class, four orders, 11 families, 25 genera, and about 300 species in this phylum (Redecker et al., 2013). Different AM fungi have contrasting growth strategies, hyphae production, and nutrient absorption abilities (Chagnon et al., 2013). For example, species within the Gigaporaceae family tend to allocate more biomass to external hyphae than intraradical structures, whereas species of Glomeraceae display the opposite tendency (Maherali and Klironomos, 2007). More external hyphae mean more photosynthetic products are needed to support the growth of AM fungi and their hyphal networks. Thus, the AM fungal communities tend to shift from Gigasporaceae to Glomeraceae upon anthropogenic N enrichment and experimental fertilization (Treseder et al., 2018). Furthermore, the soil N/P ratio or P availability also affects the response of AM fungi to N addition. The high production of external hyphae always represents competitive soil exploration and high P solubilization. Soil P limitation stimulated by N addition would increase the abundance of Gigasporaceae members (Egerton-Warburton et al., 2007). These results indicated that different groups of AM fungi might have contrasting responses to N addition in various environments.

Seasonal change can influence AM fungi by modifying climatic factors and the C supply derived from host plants (Dumbrell et al., 2011; Maitra et al., 2021). Seasonal variations of the AM fungal community have been studied in arable, wetland, grassland, and forest habitats. Generally, the density of spores, external hyphal length, and root colonization rates were higher in summer, or the growing period, than in winter and early spring (Mandyam and Jumpponen, 2008). Meanwhile, other studies suggested that AM fungi were not affected by season (Santos-Gonzalez et al., 2007; Maitra et al., 2021). Traditionally, the temporal dynamics of AM fungal community have been described based on the morphological analysis of their characteristic structures of AM fungi. However, some AM fungal structures cannot be precisely identified through their morphologies. Therefore, the resulting compositions will likely differ from field AM fungal communities (Davison et al., 2012). By contrast, molecular techniques can overcome the limitations of the traditional method. Therefore, DNA-based and morphological techniques can be used in ecological studies of the AM fungal community (Vieira et al., 2018).

As a critical tree species for afforestation, poplar has been widely planted in China, especially in the eastern coastal area (Fang, 2008). This region suffers severe N deposition (Liu et al., 2011). Based on a long-term experiment with five N addition levels (0, 5, 10, 15, and 30 gN·m^{-2}·a^{-1}) in a poplar plantation in Dongtai Forest Farm, Jiangsu Province, researchers found changes in soil organic carbon, soil fauna, and the microbial community structure (Bian et al., 2019; Yu et al., 2021). However, the response of AM fungi to N addition in the poplar plantation remains unknown. This study sampled poplar roots and rhizosphere soils in four seasons (summer, autumn, winter, and spring) after 6 years of continuous N additions. The effects of N addition and seasonal change on AM fungi were examined by traditional root colonization identification and high-throughput sequencing. We tested two hypotheses: (1) N addition and seasonal change could alter the AM fungal colonization rate and AM fungal community diversity and composition, and (2) N addition and seasonal change-induced variations of soil physicochemical properties contribute to the variation in the AM fungal community.

2. Materials and methods

2.1. Site description and soil sampling

The study site is located at Dongtai Forest Farm in Yancheng, Jiangsu province, eastern China (E120° 49′, N32° 52′). The region experiences a subtropical northern monsoon climate. The mean annual precipitation is 1,050 mm, and the mean annual temperature is 14.6°C. The growing season extends from May to October, whereas the non-growth season is from November to April. The soil is classified as fluvisol, according to the Food and Agriculture Organization (FAO) of the United Nations (Ge et al., 2018).

The farm was reclaimed from the coastal wetlands by constructing coastal levees. Poplar is one of the main tree species planted on the farm, and the area of the poplar plantation covers approximately 2000 hm^2. The N addition experiment started in May 2012 and involved five N levels in 5-year-old poplar plantations (*Populus deltoides* L. '35'). We selected three sites (25 × 190 m), with the distance between any two sites being >1 km. Each site was divided into five plots (25 × 30 m) with a 10 m wide buffer zone. Five N addition treatments were randomly applied to the plots: N_0 (control), N_1 (5 $gN•m^{-2}•a^{-1}$), N_2 (10 $gN•m^{-2}•a^{-1}$), N_3 (15 $gN•m^{-2}•a^{-1}$), and N_4 (30 $gN•m^{-2}•a^{-1}$). Nitrogen in the form of NH_4NO_3 has been applied annually during the growing season. Fertilizer with different N concentrations was sprayed into the corresponding plots below the canopy, and the control plots received the same amount of deionized water.

Root and soil samples were collected in July 2018, October 2018, January 2019, and April 2019 (representing summer, autumn, winter, and spring, respectively). Fine roots (< 2 mm) and rhizosphere soils of five poplars with the same growth rate were randomly sampled from each plot. Five samples from the same plot were mixed homogeneously into one sample, resulting in 60 sub-samples. The rhizosphere soils were sieved with a 2 mm mesh and subdivided into two parts. The first part was air-dried for soil physicochemical properties analyses. The other part was stored at −80°C for DNA extraction. The fine roots were washed and cut into 1 cm fragments to determine the proportion of the root colonized by AM fungi.

2.2. Root colonization rate and soil physicochemical properties determination

The root colonization rate was measured using the grid-crossing method (McGonigle et al., 1990). The average soil moisture and temperature of the sampling month were measured using an automatic sensor. A glass electrode determined soil pH in a 1:5 soil/water (w/v) suspension. Soil total nitrogen (TN) and total carbon (TC) were measured using an elemental analyzer (2,400 II, Perkin Elmer, Waltham, MA, United States). Soil-available phosphorus (AP) was extracted with 0.5 M $NaHCO_3$, and total phosphorus (TP) was determined after digestion by alkali fusion. AP and TP were quantified using the Mo-Sb colorimetric method. Ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were quantified using indophenol blue colorimetry and ultraviolet spectrophotometry, respectively (Lu, 2000).

2.3. DNA extraction, amplification, and sequencing

Total DNA was extracted from 0.5 g of frozen soil using a FastDNA kit (MP, Santa Ana, CA, United States). AM fungal 18S rDNA for Miseq pyrosequencing was amplified using a two-step PCR. In the first PCR, the primer pair AML1 and AML2 were used (Lee et al., 2008). The obtained products were used as templates for the second PCR using primers AMV4-5NF and AMDGR (Van Geel et al., 2014). PCR reactions were performed in a 20 µl reaction mixture containing 4 µl of 5× FastPfu buffer, 0.8 µl of each primer, 2 µl of dNTPs, 0.4 µl of DNA polymerase, 0.2 µl of bovine serum albumin (BSA), and 10 ng of DNA template. The PCR condition consisted of an initial denaturation at 95°C for 3 min, 30 cycles of denaturation (95°C for 30 s, 55°C for 30 s, and 72°C for 45 s), and a final extension at 72°C for 10 min. The PCR products were purified using an Axy Prep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, United States) and then quantified using a Quanti Fluor™-ST (Promega, San Luis Obispo, CA, USA). The purified PCR products were sequenced on a Miseq PE250 pyrosequencer (Illumina, San Diego, CA, United States) at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China).

2.4. Bioinformatics and statistical analysis

Raw sequence data were quality-filtered and spliced using FASTP (version 0.19.6[1]) and FLASH (version 1.2.11[2]) software, respectively. Briefly, reads with a quality score < 20, ambiguous nucleotides, reads shorter than 200 bp (excluding barcode and primer sequences), or lacking a complete barcode were removed and excluded from further analysis. The remaining sequences were then clustered into operational taxonomic units (OTUs) at 97% similarity using UPARSE (version 7.1[3]). The taxonomic identification of OTUs was performed by consulting the MaarjAM database (version 81[4]). The Chao and Shannon indexes were calculated after normalizing the read number to 10,561 reads (the minimum sequence number of all samples) in Mothur (Schloss et al., 2009), and the AM fungal community composition was assessed at the genus and species levels. The Bray-Curtis distances of community compositions (based on the OTU compositions) under five N additions and four seasons were calculated using nonmetric multidimensional scaling (NMDS), and the significance of community differences was assessed by analysis of similarities (ANOSIM) in the vegan package in R 3.6 (Dixon, 2003). Additionally, the relationships between the AM fungal community composition and soil physicochemical properties were evaluated using redundancy analysis (RDA). Only the environmental factors with a variance inflation factor (VIF) of less than 10 were retained in the RDA analysis. The significance of the correlation between environmental factors and the community composition was determined using a Monte Carlo test in R (Dixon, 2003). To identify the main and interactive effects of seasonal changes and N addition treatments on the root

1 https://github.com/OpenGene/fastp
2 https://ccb.jhu.edu/software/FLASH/index.shtml
3 http://drive5.com/uparse/
4 https://www.maarjam.botany.ut.ee/

colonization rate and the AM fungal community diversity and composition, multivariate analysis of variance (MANOVA) was performed in SPSS 18.0 for Windows (IBM Corp., Armonk, NY, United States). Lastly, associations between AM fungal community characteristics (including the root colonization rate, diversity, and the relative abundance of AM fungal groups) and environmental factors were determined using Spearman.

3. Results

3.1. Root AM fungal colonization rate

N addition significantly affected the root colonization rate; however, the effect of seasonal change and their interaction was not significant (Figure 1). Generally, with the increase in the level of N addition, the root colonization rate showed an increasing trend. In spring, the root colonization rate under N_0 treatment was significantly lower than that under N addition ($p < 0.05$).

3.2. Arbuscular mycorrhizal fungal community diversity

A total of 1,264,310 high-quality sequences were identified from all 60 samples (ranging from 10,561 to 24,418 reads per sample; mean 21,068), clustered into 227 OTUs. Rarefaction curves suggested that the sequencing depth in this study was sufficient to reflect the community structure in the samples (Supplementary Figure 1). Seasonal changes significantly affected the Chao and Shannon indexes of the AM fungal community ($p < 0.01$, Table 1); however, the effect of N addition and their interaction was insignificant. Compared with summer, the Chao and Shannon indexes of AM fungal community were significantly higher in winter, spring, and autumn.

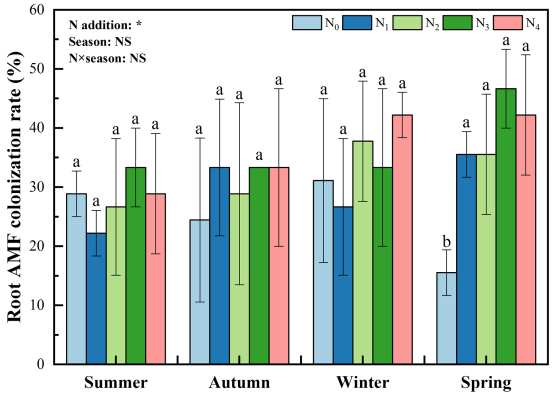

FIGURE 1
Seasonal change in poplar roots' arbuscular mycorrhizal (AM) fungal colonization rate with different nitrogen additions. N_0, N_1, N_2, N_3, and N_4 were 0, 5, 10, 15, and 30 $gN \cdot m^{-2} \cdot a^{-1}$, respectively. The values are the means of three replicates ± SD. Different lowercase letters within the same season indicate significant differences among nitrogen additions at the 0.05 level. Two-way ANOVA outputs are shown in the figure. NS, not significant; *, $p<0.05$.

3.3. Arbuscular mycorrhizal fungal community composition

Analysis of Similarity (ANOSIM) revealed that the structures of the AM fungal community were significantly influenced by seasonal changes ($R = 0.554$, $p = 0.001$; Figure 2) and N treatments ($R = 0.05$, $p = 0.031$). ANOSIM analysis was also performed on AM fungal community structures among different N additions in the same season. We found that there were significant differences in AM fungal community structures between N additions in spring ($R = 0.772$, $p = 0.001$), winter ($R = 0.704$, $p = 0.001$), and summer ($R = 0.387$, $p = 0.009$), but no in autumn ($R = 0.039$, $p = 0.358$).

The AM fungal community in the poplar plantations was assigned to 4 orders, 7 families, 8 genera, and 43 species. Figure 3 and Supplementary Figure 2 present the AM fungal community compositions on the genus and species level, respectively. The genera *Glomus* (average relative abundance, 87.52%), *Diversispora* (9.62%), *Acaulospora* (1.85%), unclassified Glomeromycetes (0.61%), *Scutellospora* (0.22%), *Archaeospora* (0.12%), unclassified Diversisporaceae (0.05%), and *Paraglomus* (0.005%) were detected in the samples. The genus *Glomus* showed the highest abundance, consisting of 28 species. The genus *Diversispora* could be further divided into three main species: *Diversispora* VTX00060, *Diversispora* VTX00040, and unclassified *Diversispora* (Supplementary Figure 2).

The relative abundances of *Glomus*, *Diversispora*, *Acaulospora*, and *Paraglomus* were significantly affected by N addition treatments and seasonal changes. The abundances of *Glomus* in winter (98.3%) and autumn (93.3%) were higher than those in spring (78.8%) and summer (79.7%). In comparison, the abundances of *Diversispora*, *Acaulospora*, and *Paraglomus* in winter and autumn were lower than those in spring and summer ($p < 0.05$, Figure 3). Compared with N_0 treatment, N addition significantly decreased the relative abundances of *Glomus* and had a trend to increase the relative abundances of *Acaulospora* and *Diversispora*. The relative abundances of *Diversispora* and *Paraglomus* under N_2 treatment were higher than those under the other treatments, while the highest relative abundances of *Glomus* (92.6%) and *Acaulospora* (5.39%) were under N_0 and N_1 treatments, respectively.

3.4. Drivers of the root colonization rate and the AM fungal community diversity and composition

Soil pH, total P, C/N ratio, NO_3^--N, and NH_4^+-N were significantly affected by seasonal change, N additions, and their interaction effects (Table 2). Soil total C, available P, moisture, and

TABLE 1 Seasonal changes in the Chao and Shannon indexes of the AM fungal community in the poplar plantations with different nitrogen additions.

Treatment	Chao				Shannon			
	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring
N_0	57.17 ± 6.92a	76.44 ± 6.71a	61.17 ± 11.97a	75.90 ± 13.13a	1.84 ± 0.51a	2.70 ± 0.31a	2.76 ± 0.10a	3.09 ± 0.14a
N_1	52.56 ± 13.80a	73.72 ± 6.71a	55.58 ± 8.84a	62.61 ± 10.07a	1.97 ± 0.80a	2.76 ± 0.53a	2.72 ± 0.08a	2.80 ± 0.19b
N_2	51.21 ± 1.49a	72.75 ± 6.47a	61.44 ± 6.87a	73.87 ± 3.38a	2.64 ± 0.12a	2.87 ± 0.34a	2.81 ± 0.11a	2.89 ± 0.03ab
N_3	47.17 ± 8.14ab	78.44 ± 3.86a	61.50 ± 8.85a	71.64 ± 10.43a	1.75 ± 0.63a	2.59 ± 0.89a	2.85 ± 0.07a	3.11 ± 0.08a
N_4	34.03 ± 4.65b	75.17 ± 22.25a	60.00 ± 13.81a	75.30 ± 15.75a	1.63 ± 0.60a	2.58 ± 0.88a	2.88 ± 0.19a	3.00 ± 0.17ab
Significance caused by:								
N addition	NS				NS			
Season	**				**			
N × season	NS				NS			

N_0, N_1, N_2, N_3, and N_4 were 0, 5, 10, 15, and 30 $gN \cdot m^{-2} \cdot a^{-1}$, respectively. The values are the means of three replicates ± SD. Different lowercase letters within the same column indicate significant differences among nitrogen addition treatments at the 0.05 level. Two-way ANOVA outputs are shown in the table: NS, not significant; **, $p < 0.01$.

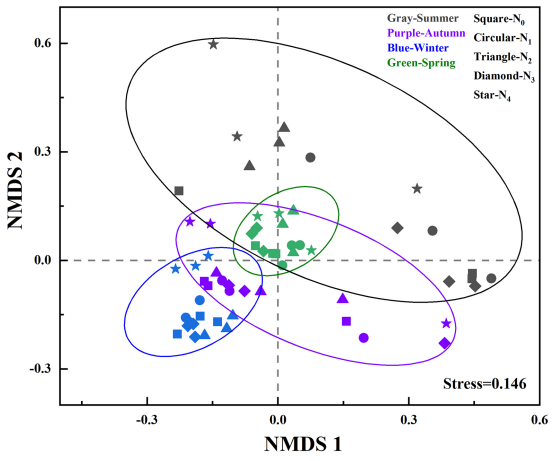

FIGURE 2
Nonmetric multidimensional scaling (NMDS) of seasonal changes in the soil AM fungal community compositions (based on Bray-Curtis distances and the presence of OTUs) in the poplar plantations under different nitrogen additions. N_0, N_1, N_2, N_3, and N_4 were 0, 5, 10, 15, and 30 $gN \cdot m^{-2} \cdot a^{-1}$, respectively.

temperature were significantly influenced by seasonal change. Specifically, NO_3^--N concentrations increased dramatically from N_0 (6.37 $mg \cdot kg^{-1}$) to N_4 (19.42 $mg \cdot kg^{-1}$) treatment ($p < 0.01$). Soil temperature decreased from summer (July 2018, 20.23°C) to winter (January 2019, 5.55°C) and then increased from winter to spring (April 2019, 14.64°C). For soil moisture, the order was winter > spring > summer >autumn.

The Chao index was largely associated with NH_4^+-N, available P, pH, NO_3^--N, total P, and total C. The Shannon index was significantly associated with total P, pH, available P, temperature, and total C ($p < 0.05$, Table 3). NH_4^+-N ($R = -0.477$, $p < 0.01$) and total P ($R = -0.494$, $p < 0.01$) showed the highest correlation with the Chao index and the Shannon index, respectively. The root AM fungal colonization rate was significantly related to the C/N ratio, total C, pH, available P, and NO_3^--N ($p < 0.05$, Table 3).

Nine environmental factors (pH, total C, total P, total N, available P, C/N ratio, NO_3^--N, NH_4^+-N, and soil temperature) were chosen for the RDA analysis, which explained 35.45% (32.89% for the first two axes) of the total variances of the AM fungal community ($p = 0.001$, Figure 4). The Monte Carlo test indicated that the structure of the AM fungal community correlated with soil temperature, available P, total P, and pH ($p < 0.05$, Table 4). The correlation analysis between the relative abundance of the AM fungal genera and environmental factors showed that the relative abundances of *Glomus*, *Diversispora*, and *Acaulospora* correlated significantly with total N, NO_3^--N, and temperature (Supplementary Table 1). The relative abundance of *Scutellospora* correlated positively with available P, total N, and temperature, while *Archaeospora* correlated significantly with total N, moisture, and NH_4^+-N ($p < 0.05$, Supplementary Table 1).

4. Discussion

N addition positively affected the root AM fungal colonization rate in the poplar plantations. Specifically, root colonization rates under N additions were significantly higher than under N_0 treatments in spring (Figure 1). The exchange of P and C is the fundamental function of the plant-AM fungal symbiosis; therefore, the soil P availability can strongly influence the response of AM fungi to N fertilization (Williams et al., 2017). In P-deficient soils, N fertilization-induced P limitation would stimulate plant C allocation to AM fungi to acquire sufficient P. However, in P-rich soil, plant dependency on AM fungi might be reduced, resulting in a decrease in C allocation to AM fungi (Johnson et al., 2013; Han et al., 2020). The soils under the poplar plantation in our study belong to the former category; thus, N addition increased the root AM fungal colonization rate. The correlation analysis also

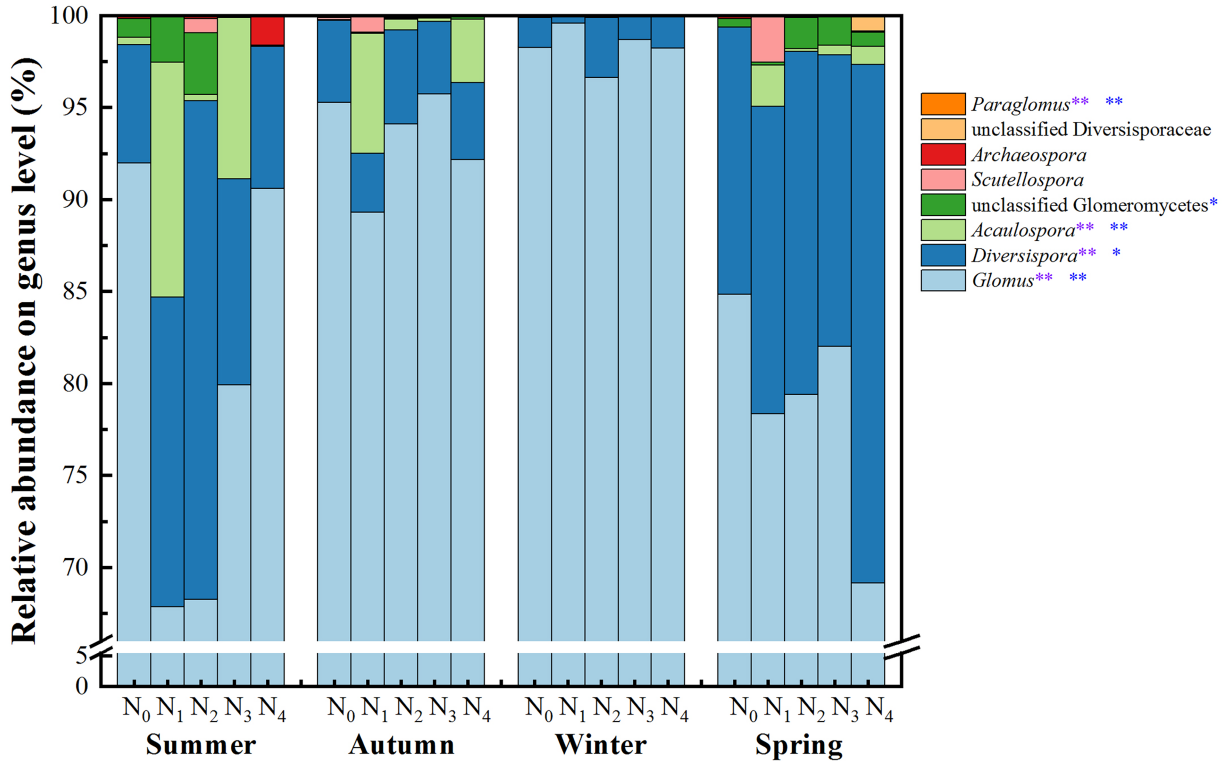

FIGURE 3
Average relative abundances of the AM fungal groups at the genus level in the poplar plantations under different nitrogen additions and seasons. N_0, N_1, N_2, N_3, and N_4 were 0, 5, 10, 15, and 30 $gN \cdot m^{-2} \cdot a^{-1}$, respectively. The values are the means of three replicates. Two-way ANOVA outputs are shown in the figure: the purple * and ** indicate significant differences among different seasons at the 0.05 and 0.01 levels, respectively, and the blue * and ** indicate significant differences among nitrogen additions at the 0.05 and 0.01 levels, respectively.

showed that the colonization rate correlated negatively with soil available P ($p < 0.05$, Table 3).

Seasonal variation played a notable role in the diversity of the AM fungal community. AM fungi must establish symbiosis with living plant roots to complete their life history; therefore, some studies found strong relationships between the phenology of plants and their mycorrhizal responsiveness. AM fungal characteristics were higher in the growing period than in the dormant season (Mandyam and Jumpponen, 2008). However, in the present study, the Chao and Shannon indexes were significantly higher in autumn, spring, and winter than in summer (Table 1). They tended to be negatively correlated with soil temperature (Table 3). Higher diversity in the colder seasons probably resulted from more even competition for limited C availability among AM fungal species (Dumbrell et al., 2011). In addition, poplar can form associations with AM fungi and ectomycorrhizal (EM) fungi simultaneously (Neville et al., 2002), and increased colonization by EM fungi in summer might influence AM fungal growth.

Although N addition and season influenced the AM fungal community compositions, seasonal change exerted a more substantial effect than N addition (Figure 2). Dumbrell et al. (2011) observed distinct AM fungal compositions in the cooler and warmer seasons. Similarly, the AM fungal assemblages in summer and winter were completely separate in the NMDS plots (Figure 2). The RDA results showed that the AM fungal community composition correlated significantly with temperature (Table 4). The mechanisms of the effect of temperature on the AM fungal communities can be classified as either indirect, by altering soil nutrient availability and host plants' C allocation, or direct, by affecting AM fungal growth (Cotton, 2018). The RDA results also showed that the AM fungal community composition was greatly influenced by available P, total P, and pH (Table 4). N addition and season directly affected soil pH, P, and N availability (Table 2). Consequently, the effects of N addition and season on the AM fungal community composition were partially mediated through the soil (Zheng et al., 2014; Ji et al., 2021). Moreover, previous studies reported an indirect plant-mediated effect of N addition on AM fungal community composition in grassland ecosystems (Liu et al., 2012). In the poplar plantation ecosystem, nine environmental factors chosen for the RDA analysis explained 35.45% of the total variances of AM fungal community. Therefore, other environmental factors that affected the AMF community structure were not considered, e.g., the composition and diversity of the plant community. In future research, their

TABLE 2 Seasonal changes in soil physicochemical properties in the poplar plantations with different nitrogen additions.

Season	Treatment	pH	Total N (%)	Total P ($mg \cdot kg^{-1}$)	Total C (%)	C/N ratio	Available P ($mg \cdot kg^{-1}$)	NH_4^+-N ($mg \cdot kg^{-1}$)	NO_3^--N ($mg \cdot kg^{-1}$)	Moisture (%)	Temperature (°C)
Summer	N_0	8.38b	0.20a	871.95ab	1.29b	6.46a	50.22ab	8.53b	8.24c	21.0a	20.23a
	N_1	8.38b	0.17a	911.55a	1.28b	7.55a	57.48a	10.50a	9.94c	20.8a	20.24a
	N_2	8.51a	0.17a	814.07b	1.30b	7.49a	48.54b	7.20c	10.77c	21.2a	20.22a
	N_3	8.50a	0.19a	837.19ab	1.38b	7.35a	53.98ab	9.23b	15.33b	20.9a	20.21a
	N_4	8.36b	0.19a	800.65b	1.68a	9.40a	50.02ab	9.13b	21.41a	21.0a	20.22a
Autumn	N_0	8.52b	0.26ab	815.20a	1.70a	7.54b	26.73ab	4.85ab	3.12c	18.1a	17.33a
	N_1	8.51b	0.66a	789.17a	1.74a	4.86b	21.38b	5.40a	2.87c	18.0a	17.35a
	N_2	8.50b	0.42ab	796.61a	1.36a	4.04b	38.54a	5.28a	11.48b	18.0a	17.31a
	N_3	8.57a	0.04b	785.29a	1.63a	52.15a	19.09b	3.86c	11.07b	18.1a	17.31a
	N_4	8.32c	0.13ab	749.56a	1.56a	11.86b	19.70b	4.18bc	27.53a	17.9a	17.31a
Winter	N_0	8.39c	0.15a	638.34bc	1.55a	10.13b	18.63a	10.51a	12.37c	33.7a	5.52a
	N_1	8.50b	0.14ab	615.03c	1.51a	10.54b	17.16a	8.28bc	12.55c	33.9a	5.53a
	N_2	8.60a	0.16a	682.88a	1.53a	9.63b	23.27a	7.46c	13.90b	33.4a	5.58a
	N_3	8.63a	0.16a	655.06ab	1.70a	10.78b	23.37a	9.87ab	16.34a	34.1a	5.54a
	N_4	8.56ab	0.13b	633.14bc	1.56a	12.31a	20.97a	9.30abc	16.79a	33.6a	5.56a
Spring	N_0	8.63bc	0.14b	599.37ab	1.54d	10.76bc	22.30a	9.49a	1.75b	27.9a	14.63a
	N_1	8.59c	0.21a	609.63a	2.92a	13.85a	21.08a	7.76b	5.28b	27.8a	14.63a
	N_2	8.65b	0.21a	588.53b	2.35b	11.19bc	21.64a	6.16c	10.49a	27.8a	14.63a
	N_3	8.78a	0.19a	599.16ab	2.13bc	11.47b	24.23a	9.26a	11.75a	27.9a	14.62a
	N_4	8.64bc	0.21a	597.26ab	1.97c	9.56c	17.67a	7.31b	11.96a	27.9a	14.66a
Significance caused by:											
N addition		**	NS	*	NS	**	NS	**	**	NS	NS
Season		**	NS	**	**	*	**	**	**	**	**
N × season		**	NS	*	**	**	NS	**	**	NS	NS

N_0, N_1, N_2, N_3, and N_4 were 0, 5, 10, 15, and 30 $gN \cdot m^{-2} \cdot a^{-1}$, respectively. The values are the means of three replicates. The means followed by different letters in the same column and season are significantly different ($p < 0.05$). Two-way ANOVA outputs are shown in the table. NS, not significant; **, $p < 0.01$; *, $p < 0.05$.

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TABLE 3 Spearman correlation coefficients between the diversity of the AM fungal community and environmental factors (n=60).

Factors	pH	Total N	Total P	Total C	C/N ratio	Available P	NH_4^+-N	NO_3^--N	Moisture	Temperature
Chao index	0.333**	0.123	−0.286*	0.286*	0.177	−0.376**	−0.477**	−0.313*	−0.231	−0.177
Shannon index	0.477**	0.000	−0.494**	0.305*	0.253	−0.466**	−0.175	−0.098	0.091	−0.382**
Colonization rate	0.301*	−0.096	−0.232	0.322*	0.323*	−0.285*	−0.050	0.259*	0.203	−0.237

** and * indicate significant correlations at $p < 0.01$ and $p < 0.05$, respectively.

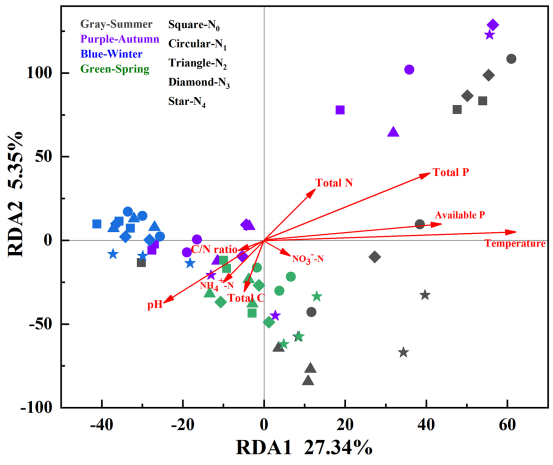

FIGURE 4
RDA for soil AM fungal community compositions (based on the species level) and environmental factors in the poplar plantations under different nitrogen additions in four seasons. N_0, N_1, N_2, N_3, and N_4 were 0, 5, 10, 15, and 30 gN·m^{-2}·a^{-1}, respectively.

impacts on the AM fungal community could be further investigated.

Generally, the AM fungal communities in poplar plantations were assigned to 4 orders, 7 families, 8 genera, and 43 species using high-throughput sequencing. The dominant genera were *Glomus* (average relative abundance 87.52%), *Diversispora* (9.62%), and *Acaulospora* (1.85%) (Figure 3). Using molecular techniques, former studies examined the structure of AM fungal communities in some forest ecosystems. In a subtropical forest, the dominant AM fungal groups across three seasons were Glomeraceae (78.82%), Archaesporaceae (11.06%), Claroideoglomeraceae (3.49%), Gigasporaceae (3.33%), Acaulosporaceae (1.61%), and Diversisporaceae (1.60%) (Maitra et al., 2021). In a rainforest, the AMF community was dominated by *Glomus* (96.72%), *Acaulospora* (2.42%), and *Scutellospora* (0.63%) (Pereira et al., 2022). Similarly, the genera *Glomus*, *Diversispora*, *Scutellospora*, and *Paraglomus* accounted for more than 95% of the sequences in a natural mixed broadleaf-conifer forest (Ji et al., 2021). Therefore, the dominant groups of the AM fungal community in different forest ecosystems vary. The study of the structure of the AM fungal community in the ecosystem of a poplar plantation can provide references for the exploration of their function.

Furthermore, in this study, N addition and season influenced the relative abundances of the dominant AM fungal groups (Figure 3). Compared with N_0 treatment, N addition significantly decreased the relative abundances of *Glomus*, and had a trend toward increasing the relative abundances of *Diversispora* and *Acaulospora*, as demonstrated in previous investigations (Liu et al., 2015; Chen et al., 2017). The AM fungal community structure shifts were related to the competitive dynamics among AM fungi, changes in environmental conditions, or both (Dumbrell et al., 2011). Fertilization has been shown to have significant effects on the AM fungal population. For example, suppose the host plant reduces the allocation of C to the mycorrhiza under N addition. In that case, the intensified competition among AM fungi might result in a dominance of the AMF taxa, which can adapt to environments rich in N and limited in C (Johnson et al., 2015).

Moreover, because different AM fungi have contrasting soil exploration and P solubilization abilities, soil P availability affected the dependence of host plants on mycorrhizae (Treseder et al., 2018). The abundances of *Glomus* in winter and autumn were higher than in spring and summer, while the abundances of *Diversispora* and *Acaulospora* showed an opposite trend. The genus *Glomus* possessed a high sporulation rate and colonization ability *via* spore dispersal or mycelium fragments (Daniell et al., 2001); therefore, they were more likely to survive in relatively severe winter and autumn conditions.

5. Conclusion

In this study, we analyzed the seasonal changes of the root AM fungal colonization rate after six-year N addition in poplar using traditional morphological identification. We also detected the composition and diversity of AM fungal community in the rhizosphere soil using high-throughput sequencing. Our results showed that N addition primarily influenced the root colonization rate. In contrast, seasonal change had a notable effect on the Chao and Shannon indexes

TABLE 4 Relationships between the composition of the arbuscular mycorrhizal (AM) fungal community and environmental factors revealed by Monte Carlo tests (n=60).

	pH	Total N	Total P	Total C	C/N ratio	Available P	NH_4^+-N	NO_3^--N	Temperature
R^2	**0.100**	0.045	**0.242**	0.042	0.004	**0.306**	0.028	0.012	**0.648**
P	**0.050**	0.268	**0.001**	0.318	0.928	**0.001**	0.460	0.700	**0.001**

Significant correlations are marked in bold.

of the AM fungal community. Meanwhile, both the seasonal change and N addition impacted the composition of the AM fungal community. Soil temperature, available P, total P, and pH were the main drivers for the seasonal dynamics of AM fungal community in this poplar plantation.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI; SAMN31857539- SAMN31857598.

Author contributions

SP, ZG, and LM: designed the project and provided the funding. SP and MB: collected the samples and conducted the laboratory analysis. SP, WX, and ZG: performed the bioinformatics and statistical analysis. SP: wrote the original draft of the paper. LM: revised the manuscript and contributed to the conception. All authors have read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.1101698/full#supplementary-material

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