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Nitrogen Addition Does Not Change AMF Colonization but Alters AMF Composition in a Chinese Fir (*Cunninghamia lanceolata*) Plantation

Fan Wu †, Xiang Zheng †, Minmin Cao, Xin Guan and Jiang Jiang *

Collaborative Innovation Center of Sustainable Forestry in Southern China of Jiangsu Province, Key Labortory of Soil and Water Conservation and Ecological Restoration in Jiangsu Province, Nanjing Forestry University, Nanjing 210037, China; fanwu98@njfu.edu.cn (F.W.); xiang-zheng@outlook.com (X.Z.); healer_cao@outlook.com (M.C.); klein_blue@foxmail.com (X.G.)

* Correspondence: jiangjiang@njfu.edu.cn; Tel.: +86-159-5197-2417

† These authors contributed equally to this work.

Abstract: Aims: Our aim was to investigate how N addition affects arbuscular mycorrhizal fungal (AMF) growth in Chinese fir plantations. Methods: A Chinese fir plantation was treated with four different N addition treatments for one and half years starting in April 2019. AMF colonization, hyphal length density, community composition, and soil properties were under measurement. Results: N addition caused inapparent effects on AMF colonization, hyphal length density, and functional guilds (rhizophilic, edaphophilic, and ancestral). The predominant AMF species in the soil was *Septoglomus viscosum*. N addition altered AMF community and some rare species (e.g., *Entrophospora infrequens*) disappeared with N addition. Conclusion: AMF community structure was more sensitive to short-time N deposition than the symbiotic relationship between AMF and host plants.

Keywords: N addition; arbuscular mycorrhizal fungi; community composition; colonization; Chinese fir

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1. Introduction

Atmospheric N deposition has increased strongly over the last decades due to N use in agriculture, fossil fuels, and land conservation [1,2], and ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), and dissolved organic nitrogen deposition fluxes were up to 8.73, 8.73, and 9.98 kg N ha^{-1} yr^{-1}, respectively [3]. Increased N deposition raises carbon sinks, increases N availability and NPP in some ecosystems [4], and typically acidifies soil [5]. Meanwhile, N deposition, due to its increasing rate and duration, possibly has a negative impact with on the growth, composition, and function of soil microbes. [6].

Arbuscular mycorrhizal fungi (AMF) are ubiquitous components belonging to the phylum *Glomeromycota*. They can form mycorrhizal symbionts with approximately 80% of land plants [7]. AMF gain a significant presence in increasing the uptake of N and P to their host plants [8,9] and improving disease resistance, which determines the stability and versatility of ecosystem [10]. It should be noted that AMF have intra- and extra-radical structures [11]. Based on their patterns at the family level, AMF can be classified as edaphophilic, rhizophilic, or ancestral. Rhizophilic AMF help plants reduce root pathogen infection with fine root, and edaphophilic AMF promote plant nutrients with extraradical mycelium [12].

AMF structure and community composition can be influenced by climate change, but the factors that affact AMF parameters are not well understood. Decreasing pH and P availability caused by N addition can make a big difference in changing soil properties [13,14], so plants need AMF to overcome resource limitations. However, the impact of N application on AMF remains controversial. AMF colonization might be decreased [15,16],

promoted [17], or have no significant effect [18] by N addition. N addition can also enhance [18,19], suppress [20], or have no significant effect [21] on AMF spore density and hyphae. Some investigations showed that N addition treatments caused significant suppressive impacts on AMF abundance, richness, and diversity [22,23], whereas others found no significant impact [19,24]. Meanwhile, how these environmental factors shape the AMF community under N addition is not fully understood. A study observed that the relative abundance of AMF decreased the reduction of plant demand for N [25]. Importantly, the morphological modification of AMF caused by soil nutrient changes may be relevant to their composition owing to the differences of morphological internal responses to nutrient manipulations [26,27].

Chinese fir (*Cunninghamia lanceolata*) is a quick-growing timber tree species in south China [28]. On account of its high wood quality and termite resistance, it is frequently planted [29]. However, the productivity of the Chinese fir is declining due to soil acidification, soil aluminum poisoning, and unreasonable planting methods. Lu [30] showed that the number of AMF operational taxonomic units (OTUs) had an inverted V-shaped change with the age of the Chinese fir. Liu [14] found that the Chinese fir under N addition for 2 years would increase AMF colonization and diversity. Cao [31] observed that high N addition exerted a significant negative effect on AMF community. However, few studies showed the interactions of Chinese fir and AMF in terms of N deposition and nutrient availability. Therefore, we purposed to address these questions: (1) how does N addition affect AMF colonization and the diversity and composition of AMF in Chinese fir; (2) would the change of soil nutrients availability affect the responses of AMF groups?

2. Materials and Methods

2.1. Study Sites and Sampling

The soil experiment was carried out in the Fengyang Mountain Nature Reserve (28°53′56″ N, 190°10′56″ E), Zhejiang Province, China. The mean annual precipitation and annual temperature are 2400 mm and 12.3 °C. Soil properties (0–10 cm) are: total nitrogen (TN), 4.11 g kg^{-1}; total carbon (TC), 58.73 g kg^{-1}; NH_4^+-N, 15.71 mg g^{-1}; NO_3^--N, 14.66 mg g^{-1}; total phosphorus (TP), 0.3 g kg^{-1}; and bulk density, 0.76 g cm^{-3}. The Chinese fir was 39 years old with an average diameter at breast height of 21.75 cm and 13.2 m height.

The local N deposition was estimated to be 34 kg N ha^{-1} yr^{-1}. The N addition treatments included four different levels (four plots per treatment were considered as four independent repetitions, sixteen 10 m × 10 m plots in total) to simulate future climate change scenarios: no fertilization, 50 kg N ha^{-1} yr^{-1}, 100 kg N ha^{-1} yr^{-1}, and 200 kg N ha^{-1} yr^{-1}, which were regarded as control (CK), low-nitrogen (LN), medium-nitrogen (MN), and high-nitrogen (HN), respectively. Separated adjacent plots were separated by a 10-m-wide buffer strip. From April 2019, N was added monthly by spraying urea ($CO(NH_2)_2$) solution on the forest floor as N addition treatments. Meanwhile, CK plots were sprayed with the same volume of water.

Samples were collected in November 2020. Along the "S" route, six standard trees were selected in each plot. Weeds and litter around the sample trees were removed before collecting. Roots and rhizosphere soil around the average standard tree were collected in the 0–20 cm soil layer from four directions. The fine roots and about 1000 g of rhizosphere soil (soils adhering to roots by shaking off root surface) from six standard trees were collected and mixed as one root and one soil sample in each plot, and sixteen root and soil samples were collected. The soil samples were used for soil physicochemical analysis and the measurement of AMF colonization, spore isolation studies, and measured hyphal length density (HLD). The soil samples were air-dried, and the properties were measured, while the other soil samples were stored with root samples (washed using tap water) in the refrigerator at 4 °C until used.

2.2. Soil Properties Analysis

All the soil samples were sieved through a 2 mm sieve. Soil pH was measured in a soil-to-water ratio of 1:2.5 (*v/v*). Soil NH_4^+-N and NO_3^--N concentration were estimated by colorimetric analysis [32]. Soil available nitrogen (AN) is soil NH_4^+-N plus NO_3^--N. Soil available phosphorus (AP) and total phosphorus (TP) were extracted by sodium bicarbonate first, and their contents were followed by molybdenum blue colorimetry [33]. Soil total organic carbon was measured by TOC-L CPH/CPN [34]. Soil total carbon (C) and total nitrogen (N) were measured by an elemental analyzer (Vario MACRO cube, Elementar Trading Shanghai, Shanghai, China).

2.3. AMF Colonization and Extraradical Hphae Length Density

To highlight AMF structures, roots samples were strained following the methods described by Philips and Hayman [35], and the percentage of mycorrhizal colonization was estimated in the light of McGonigle et al. [36]. Extraradical HLD was determined on the basis of the description by Jakoben et al. [37].

2.4. AMF Spore Separation and Identification

The AMF spores used for identification were obtained from the 20 g air-dried rhizosphere soil samples following the wet sieving and decanting techniques as described by Brundrett [38]. In order to determine the spore density, the isolated intact healthy spores were extracted and placed on filter paper. We separated them by color, spore size, ornamentations, hyphal attachments, and wall layers, and then used a dissecting microscope at 45× magnification to observe them. Spores were mounted on slides in polyvinyl–lactic acid–glycerine (PVLG) and a mixture of PVLG with Melzer's (1:1; *v/v*). The spores were identified according to the International Culture Collection of Vesicular Arbuscular Mycorrhizal fungi (INVAM) (http://invam.caf.wvu.edu, last accessed in 21 May 2022) and recent advances in *Glomeromycota* taxonomy [39,40].

2.5. AMF Community Parameters and Statistical Analysis

The spore density (SD), frequency of occurrence (FO), relative abundance (RA), importance value (IA), Shannon–Wiener index (H), Simpson index (D), species richness (SR), and percentage were used to estimate the AMF community structure [41–43].

Correlation analysis was performed using SPSS 26.0 to test for relationship between the AMF community composition, parameters and soil properties. Venn was pictured for Venny 2.0. Graphical presentation was carried out using Origin 2022. Redundancy analysis (RDA) was conducted with Canoco 5.0 to reveal the influence of soil properties on the AMF communities.

3. Results

3.1. Effects of N Addition on Soil AMF Colonization, Spore Density, and External Hyphal Length Density

AMF colonization ($p = 0.824$), spore density ($p = 0.229$), and external hyphal length density ($p = 0.435$) were not significantly influenced by the nitrogen treatment (Table 1). The AMF colonization ranged from 74% to 88%. Spore density was highest in MN treatment, which averaged up to 365 spores per 1g air-dried soil, and spore density was lowest value in the control treatment. External HLD was highest in the control treatment, which averaged up to 5.10 m g^{-1}, and was the lowest in the LN treatment.

Table 1. Responses of arbuscular mycorrhizal fungi under different nitrogen (N) treatments.

	CK	LN	MN	HN	*F*-Value	*p*-Value
Colonization (%)	81.75 ± 2.25	84.75 ± 1.60	83.00 ± 3.10	82.00 ± 2.70	0.30	0.82
SD (spore g^{-1} air-dried soil)	255.69 ± 39.84	297.69 ± 32.33	364.63 ± 37.85	318.94 ± 41.76	1.65	0.23
HLD (m/g)	5.10 ± 0.95	3.53 ± 0.77	3.73 ± 0.46	4.53 ± 0.68	0.98	0.44
SR	21	21	20	19	-	-
H	1.89 ± 0.11	1.96 ± 0.11	1.67 ± 0.21	1.94 ± 0.01	1.02	0.42
D	0.79 ± 0.02	0.80 ± 0.03	0.69 ± 0.07	0.81 ± 0.01	1.92	0.18

SD, spore density; HLD, external hyphal length density; SR, species richness; H, Shannon–Wiener index; D, Simpson index. CK: no fertilization; LN: 50 kg N·ha^{-1}·yr^{-1}; MN: 100 kg N·ha^{-1}·yr^{-1}; HN: 200 kg N·ha^{-1}·yr^{-1}. Error bars represent ± SE of mean (n = 4).

3.2. Effects of N Addition on Soil AMF Community Composition

Although there was no significant effect of nitrogen treatment on the Shannon index and Simpson index, nitrogen addition decreased total spore richness (Table 1). The number of unique AMF species decreased gradually with N addition (Figure 1).

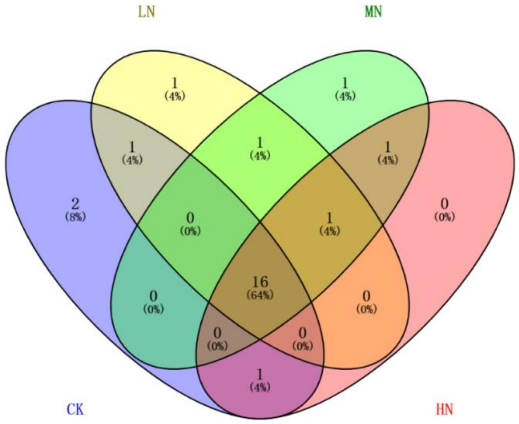


Figure 1. Venn diagram showing the unique and shared arbuscular mycorrhizal fungi species under different nitrogen treatments. CK: no fertilization; LN: 50 kg N ha^{-1} yr^{-1}; MN: 100 kg N ha^{-1} yr^{-1}; HN: 200 kg N ha^{-1} yr^{-1}.

In total, 26 AMF species of spore were detected in four nitrogen treatments (Figure 2; Table 2; Figure A1). These AMF belonged to 12 genera (Acaulospora, Archaeospora, Claroideoglomus, Dentiscutata, Diversispora, Entrophospora, Funneliformis, Gigaspora, Glomus, Rhizophagus, Sclerocystis, and Septoglomus) within six families (Acaulosporaceae, Ambisporaceae, Claroideoglomus, Diversisporaceae, Gigasporaceae, and Glomeraceae).

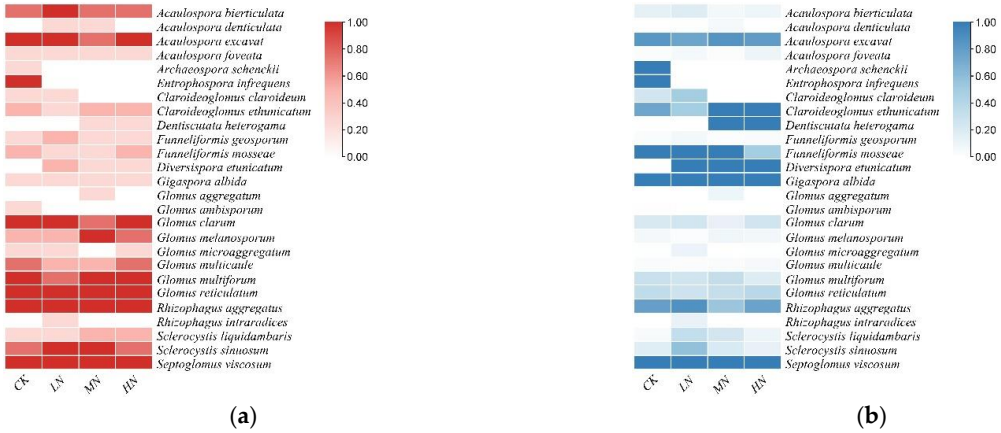


Figure 2. The frequency of occurrences (**a**) and relative abundance (**b**) among arbuscular mycorrhizal fungi under different nitrogen treatments. CK: no fertilization; LN: 50 kg N ha^{-1} yr^{-1}; MN: 100 kg N ha^{-1} yr^{-1}; HN: 200 kg N ha^{-1} yr^{-1}.

Table 2. Occurrence frequency, relative abundance, and important values of arbuscular mycorrhizal fungi species in Chinese fir under nitrogen treatment.

Species Number	AMF	Nitrogen Treatment				FO (%)	RA (%)	IV (%)
		CK	LN	MN	HN			
1	*Acaulospora bierticulata*	+	+	+	+	81.25%	11.79%	46.52%
2	*Acaulospora denticulata*	−	+	+	−	12.50%	1.59%	7.05%
3	*Acaulospora excavat*	+	+	+	+	93.75%	81.63%	87.69%
4	*Acaulospora foveata*	+	+	+	+	25.00%	4.99%	15.00%
5	*Archaeospora schenckii*	+	−	−	−	6.25%	0.91%	3.58%
6	*Claroideoglomus claroideum*	+	+	−	−	12.50%	15.00%	13.75%
7	*Claroideoglomus ethunicatum*	+	+	+	+	43.75%	85.00%	64.38%
8	*Dentiscutata heterogama*	−	−	+	+	12.50%	100.00%	56.25%
9	*Diversispora etunicatum*	−	+	+	+	25.00%	100.00%	62.50%
10	*Entrophospora infrequens*	+	−	−	−	6.25%	100.00%	53.13%
11	*Funneliformis mosseae*	+	+	+	+	37.50%	100.00%	68.75%
12	*Funneliformis geosporum*	+	+	+	+	31.25%	54.41%	42.83%
13	*Gigaspora albida*	−	+	+	+	25.00%	45.59%	35.30%
14	*Glomus aggregatum*	+	−	+	−	6.25%	100.00%	53.13%
15	*Glomus ambisporum*	+	−	−	−	6.25%	2.21%	4.23%
16	*Glomus clarum*	+	+	+	+	93.75%	0.12%	46.94%
17	*Glomus melanosporum*	+	+	+	+	68.75%	6.39%	37.57%
18	*Glomus microaggregatum*	+	+	−	+	18.75%	3.14%	10.95%
19	*Glomus multicaule*	+	+	+	+	62.50%	3.72%	33.11%
20	*Glomus multiforum*	+	+	+	+	93.75%	27.53%	60.64%
21	*Glomus reticulatum*	+	+	+	+	100.00%	34.61%	67.31%
22	*Rhizophagus aggregatus*	+	+	+	+	100.00%	97.39%	98.70%
23	*Rhizophagus intraradices*	−	+	−	−	6.25%	2.61%	4.43%
24	*Sclerocystis liquidambaris*	+	+	+	+	37.50%	40.98%	39.24%
25	*Sclerocystis sinuosum*	+	+	+	+	87.50%	59.02%	73.26%
26	*Septoglomus viscosum*	+	+	+	+	100.00%	100.00%	100.00%

FO, RA, and IV are the frequency of occurrences, relative abundance, and importance value, respectively. CK: no fertilization; LN: 50 kg N ha^{-1} yr^{-1}; MN: 100 kg N ha^{-1} yr^{-1}; HN: 200 kg N ha^{-1} yr^{-1}.

Acaulospora bireticulata, *Acaulospora excacat*, *Glomus clarum*, *Glomus melanosporum*, *Glomus multicaule*, *Glomus reticulatum*, *Rhizophagus aggregatus*, *Sclerocystis liquidambaris*, and *Septoglomus viscosum* were the dominant species.

Among all treatments, we observed that IV top five were: Septoglomus viscosum (100%) > *Rhizophagus aggregatus* (98.70%) > *Acaulospora excavat* (87.69%) > *Sclerocystis sinuosum* (73.26%) > *Glomus reticulatum* (67.31%) (Table 2).

For the CK treatment, Septoglomus viscosum and Entrophospora infrequens (100%) had the highest IV, followed by Acaulospora excavat (92.13%), Rhizophagus aggregatus (89.29%), and Funneliformis mosseae (75.00%). For the LN treatment, Septoglomus viscosum (100%) presented the highest IV, followed by Rhizophagus aggregatus (93.75%), Acaulospora excavat (87.62%), Sclerocystis sinuosum (79.17%), and Diversispora etunicatum (75.00%). For the MN treatment, Septoglomus viscosum was the highest IV, followed by Acaulospora excavat (80.56%), Rhizophagus aggregatus (77.38%), Claroideoglomus etunicatum (75.00%), and Glomus multiform (66.35%). For the HN treatment, Septoglomus viscosum had the highest IV, followed by Acaulospora excavat (90.42%), Rhizophagus aggregatus (88.33%), Claroideoglomus etunicatum (75.00%), and Glomus reticultaum (69.56%) (Figure 3).

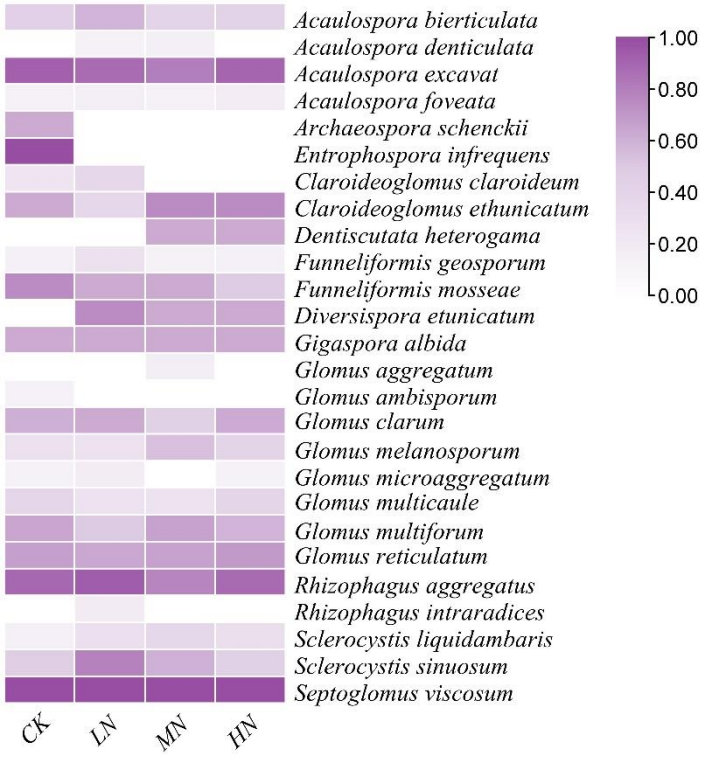


Figure 3. The importance value among arbuscular mycorrhizal fungi under different nitrogen treatments. CK: no fertilization; LN: 50 kg N ha^{-1} yr^{-1}; MN: 100 kg N ha^{-1} yr^{-1}; HN: 200 kg N ha^{-1} yr^{-1}.

3.3. Effects of N addition on Soil Nutrients

For the environmental factors measured, added N had insignificant influence on soil pH. However, N addition increased soil N under LN treatment (+15.05%). Under LN treatment, soil AP and TP were the highest. Soil N/P increased slightly with N addition (Table 3).

Table 3. Soil properties under different nitrogen (N) treatments.

	CK	LN	MN	HN	*F*-Value	*p*-Value
pH	4.00 ± 0.08	4.02 ± 0.18	3.83 ± 0.08	3.93 ± 0.08	0.57	0.64
TOC (g/kg)	1.39 ± 0.09	1.24 ± 0.26	1.36 ± 0.14	1.50 ± 0.11	1.35	0.30
NH_4^+-N (mg/kg)	28.07 ± 1.04	29.48 ± 1.92	32.48 ± 2.91	33.57 ± 3.05	1.16	0.37
AN (mg/kg)	61.43 ± 0.54	60.90 ± 2.60	64.52 ± 3.84	64.65 ± 2.76	0.54	0.67
AP (mg/kg)	7.82 ± 0.96	8.98 ± 0.52	8.03 ± 0.73	8.05 ± 0.59	0.51	0.69
C (g/kg)	89.78 ± 4.06	98.75 ±0.35	93.10 ± 5.25	91.68 ± 3.20	0.70	0.58
N (g/kg)	5.78 ± 0.25	6.65 ± 0.28	6.00 ± 0.28	5.65 ± 0.13	3.37	0.06
P (mg/kg)	234.64 ± 22.57	239.81 ± 21.02	228.52 ± 15.05	223.15 ± 25.56	0.12	0.95
C/N	15.54 ± 0.05	15.67 ± 0.59	16.55 ± 0.68	16.22 ± 0.33	1.03	0.42
N/P	25.31 ± 2.60	25.62 ± 2.78	26.73 ± 2.50	26.81 ± 1.70	0.10	0.96

TOC, total organic carbon; NH_4^+-N, ammonium nitrogen; AN, available nitrogen; AP, available phosphorus; C, total carbon; N, total nitrogen; P, total phosphorus. CK: no fertilization; LN: 50 kg N ha^{-1} yr^{-1}; MN: 100 kg N ha^{-1} yr^{-1}; HN: 200 kg N ha^{-1} yr^{-1}. One-way ANOVA and LSD were used to analyze the differences. Error bars indicate ± SE of mean (n = 4).

3.4. Relationship between Soil Properties and AMF

AMF colonization was negatively correlated with soil TOC ($p < 0.05$) (Table 4), external HLD was negatively connected with soil P ($p < 0.05$), spore density was positively related to soil C ($p < 0.05$), and species richness was in a positive correlation with soil TOC and C ($p < 0.05$). The Shannon indices presented a positive correlation with soil pH ($p < 0.05$).

Table 4. Spearman correlation coefficients between soil properties and parameters of arbuscular mycorrhizal fungi.

	pH	TOC	NH_4^+-N	AN	AP	C	N	P	C/N	N/P
Colonization	0.012	−0.643 *	−0.437	−0.571	−0.245	−0.14	−0.126	0.142	0.025	−0.112
HLD	−0.062	0.267	0.245	0.288	−0.531	−0.26	−0.197	−0.701 *	−0.154	0.564
SD	−0.338	0.214	0.008	0.251	0.161	0.679 *	0.477	0.283	0.489	0.002
SR	0.101	0.604 *	0.488	0.551	0.114	0.63 *	0.478	0.039	0.33	0.117
H	0.623 *	0.478	0.341	0.142	0.081	0.306	0.328	−0.023	−0.098	0.117
D	0.560	0.408	0.264	0.105	0.12	0.193	0.209	0.002	−0.066	0.07
Acaulospora	−0.378	−0.575	−0.506	−0.52	0.004	−0.332	−0.418	0.575	0.332	−0.708 *
Archaeospora	0.087	0.428	0.016	0.007	−0.237	−0.532	−0.537	−0.027	0.072	−0.25
Claroideoglomus	0.464	−0.303	0.302	−0.029	−0.047	0.077	0.175	0.071	−0.268	−0.045
Dentiscutata	0.014	0.244	0.434	0.451	0.17	0.453	0.349	0.165	0.203	−0.047
Diversispora	0.057	0.482	−0.205	−0.057	−0.051	−0.173	−0.304	0.384	0.399	−0.447
Entrophospora	0.087	0.428	0.016	0.007	−0.237	−0.532	−0.537	−0.027	0.072	−0.25
Funneliformis	0.05	0.764 **	0.175	0.266	−0.045	−0.163	−0.201	−0.093	0.113	−0.033
Gigaspora	−0.644 *	−0.385	−0.672 *	−0.466	0.042	−0.24	−0.419	0.636 *	0.581 *	−0.711 **
Glomus	0.341	−0.133	0.242	0.033	−0.086	0.199	0.279	−0.363	−0.258	0.393
Rhizophagus	0.275	−0.152	−0.376	−0.438	−0.378	0.023	0.081	−0.056	−0.124	0.208
Septogolomus	−0.138	0.252	0.23	0.404	0.21	0.179	0.193	−0.194	−0.104	0.288
Ancestral	−0.367	−0.508	−0.508	−0.524	−0.036	−0.425	−0.513	0.575	0.347	−0.757 **
Edaphophilic	−0.083	0.381	−0.341	−0.154	−0.04	−0.218	−0.381	0.505	0.509	−0.582 *
Rhizophilic	0.361	0.431	0.53	0.52	0.04	0.435	0.541	−0.617 *	−0.4	0.801 **

TOC, total organic carbon; NH_4^+-N, ammonium nitrogen; AN, available nitrogen; AP, available phosphorus; C, total carbon; N, total nitrogen; P, total phosphorus. HLD, external hyphal length density; SD, spore density; SR, species richness; H, Shannon–Wiener index; D, Simpson index. * $p < 0.05$. ** $p < 0.01$.

Archaeospora was in a negative correlation with soil N/P ($p < 0.05$), *Funneliformis* was positively correlated with soil TOC ($p < 0.01$), and *Gigaspora* was negatively connected with soil pH, NH_4^+-N ($p < 0.05$), and soil N/P ($p < 0.01$), but positively related to soil P and C/N ($p < 0.05$). Ancestral AMF was in a negative correlation with soil N/P ($p < 0.01$), edaphophilic AMF was negatively correlated with soil N/P ($p < 0.05$), and rhizophilic AMF was positively connected with soil N/P ($p < 0.01$) but negatively related to soil P ($p < 0.05$).

RDA was conducted to evaluate the relationship between the soil chemical properties and the AMF community (Figure 4). RDA1 accounted for 66.99% of the variations, while RDA2 accounted for 21.89%. Of the evaluated attributes, the N/P had significant conditional effects ($p < 0.01$), while the soil TOC ($p = 0.076$) and AN ($p = 0.092$) caused referential effects to the AMF communities.

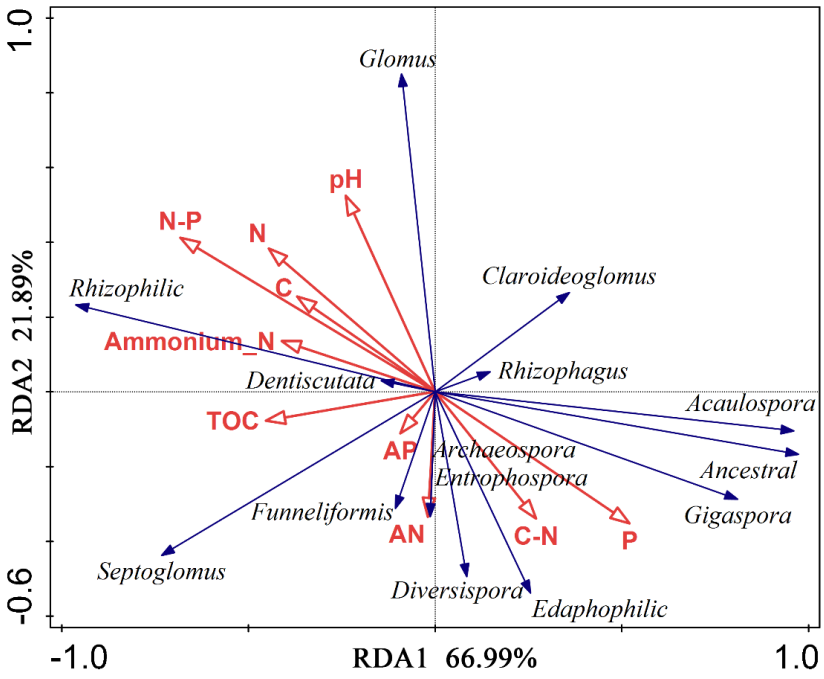


Figure 4. Redundancy analysis (RDA) at the genus level between the soil properties and arbuscular mycorrhizal fungi communities under different nitrogen treatments. TOC, total organic carbon; NH_4^+-N, ammonium nitrogen; AN, available nitrogen; AP, available phosphorus; C, total carbon; N, total nitrogen; P, total phosphorus. The red arrows represent soil properties, the blue arrows represent arbuscular mycorrhizal fungi communities.

4. Discussion

N addition can make a difference on AMF diversity and community composition, because with the increase of soil nutrients plants would have a strategy to acquire nutrients by fine roots or AMF [26,44]. Our results showed that N/P is a factor that has a significant effect on AMF communities (Figure 4). AMF community structure might have a connection with the dynamics of soil conditions and C-to-P trade between AMF and plants. It has been agreed that AMF exchanges P derived from soil for plant carbon [17,44–47]. In our study, N addition had insignificant influence on AMF colonization, external HLD, spore density, or diversity. This finding may be explained as the symbiotic relationship between AMF and root system being stable [48].

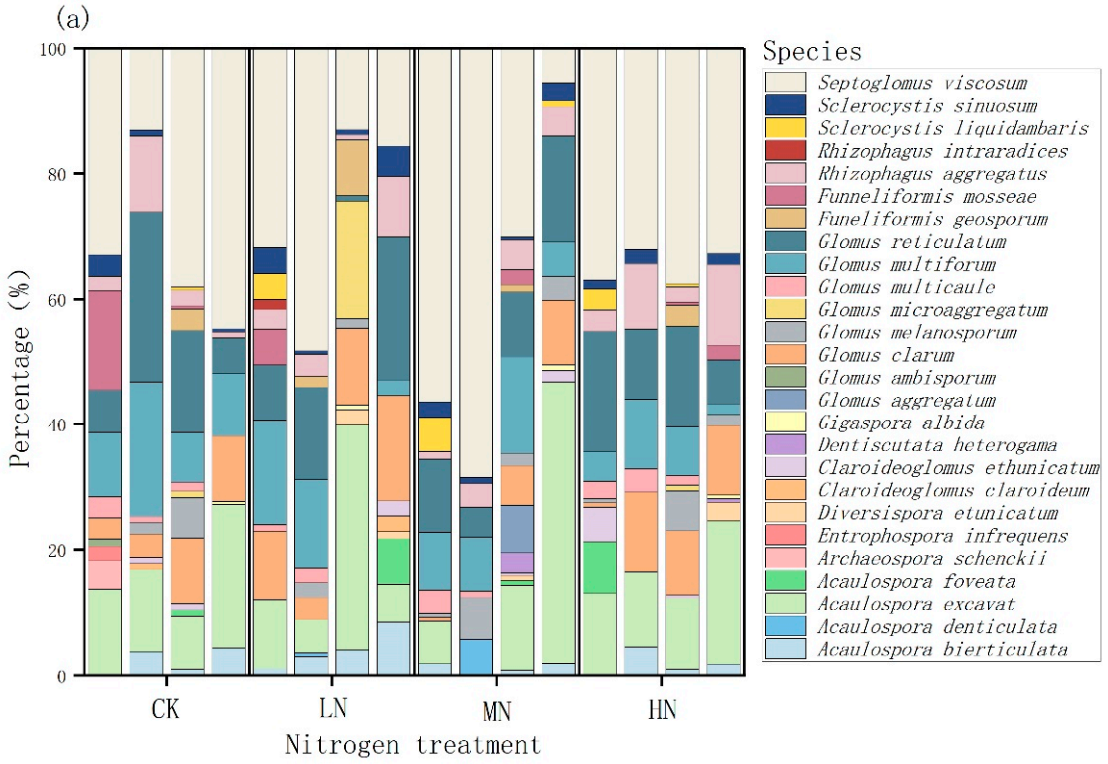
Generally, there is little specificity between AMF and the host plant, and several AMF species could colonize one AM plant at the same time [49,50]. However, different patterns of colonization with N addition suggest a preference for specific AMF species [51]. In our study, we observed that *Glomeraceae* was the most predominant family among all treatments; *Glomus*, *Septoglomus*, and *Acaulospora* were the predominant genera; and *Septoglomus viscosum* and *Acaulospora foveata* were the predominant species. Our results slightly disagree with previous studies, which presented that *Glomus* and *Acaulospora* as the predominant species due to their smaller size of spore, which helped them produce more spores in less time [48,52,53]. This discrepancy can be answered by the diverse species and age of the host plants. The high isolation frequencies of *Septoglomus viscosum* indicated that it has a strong ability to adapt to the environment (N deposition). *Septoglomus viscosum* can improve plant growth and production [54], which is mainly related to root and aboveground biomass [55].

Archaeospora schenckii, *Entrophospora infrequens*, and *Glomus ambisporum* disappeared with N treatments (Figure 3), which was in keeping with Eom [19], who also found that *Entrophospora infrequens* decreased with N addition. In P-rich (low N/P) soils, nitrogen application reduces underground carbon allocation, and rare species decrease as N increases. A previous study showed that the influence of N addition on AMF community appeared different, with added N promoting *Glomeraceae* in *A. Sieversiana*, while lessening them in *L. chinensis* [16]. Some *Glomus* species were sensitive to N application, which indicated that certain species of AMF play an indispensable role in host plant response to environmental change.

As identified in most surveys conducted in other fields [56–58], we also discovered *Funneliformis mosseae* presenting in our study, which was reported to promote plant nutrients [59]. *GmosAAP1*, an amino acid permease, has been characterized from the extraradical structure of *Funneliformis mosseae*, which indicates a potential for amino acid uptake [60]. Our study showed that with N addition, its importance decreased. This decrease may be explained by a decrease in the dependency on *Funneliformis mosseae* for N nutrient acquisition by the host plant.

Above all, our results showed that AMF, participating in and manage the soil nutrient cycle, could be sensitive to small changes in edatopes. Therefore, studying mycorrhizal symbionts increases the knowledge of climatic variation [61–63].

AMF were grouped into three guilds, namely, edaphophilic, rhizophilc, and ancestral. In our study, the percentage followed the order: rhizophilic > edaphophilic > ancestral (Figure 5c). N addition altered AMF species but did not change functional groups. Despite the addition of nitrogen, trees may reduce dependence on AMF. Meanwhile, a large group of rhizophilic guilds function to reduce root pathogen infection and primarily benefit plants with fine roots prone to pathogen infections.



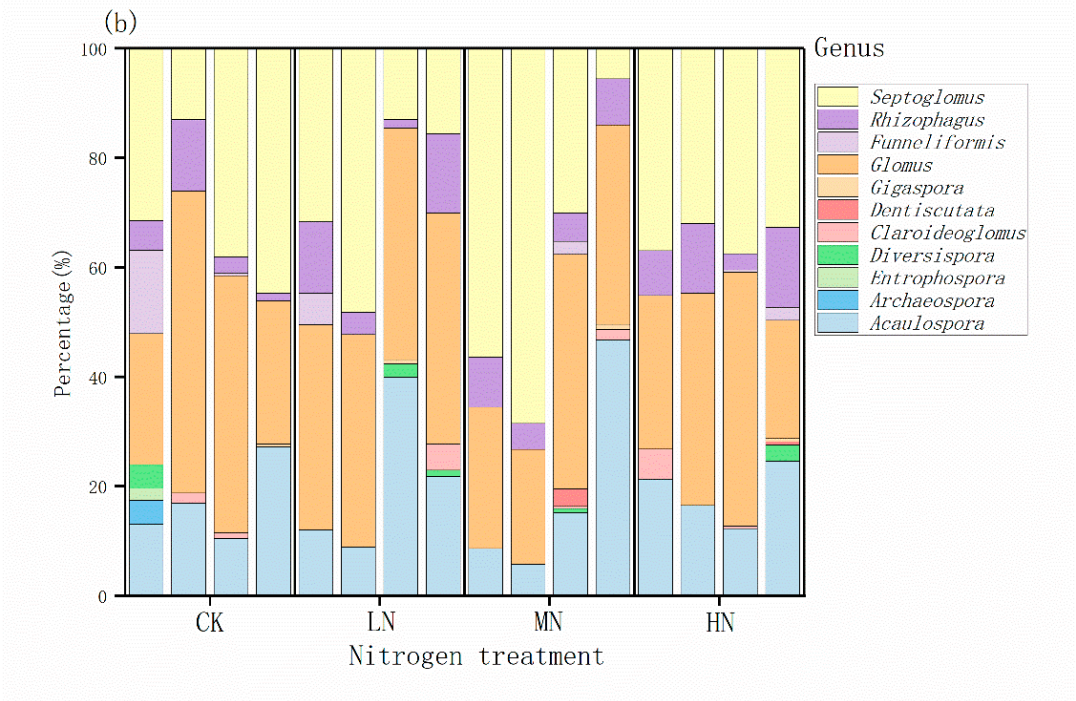


Figure 5. *Cont.*

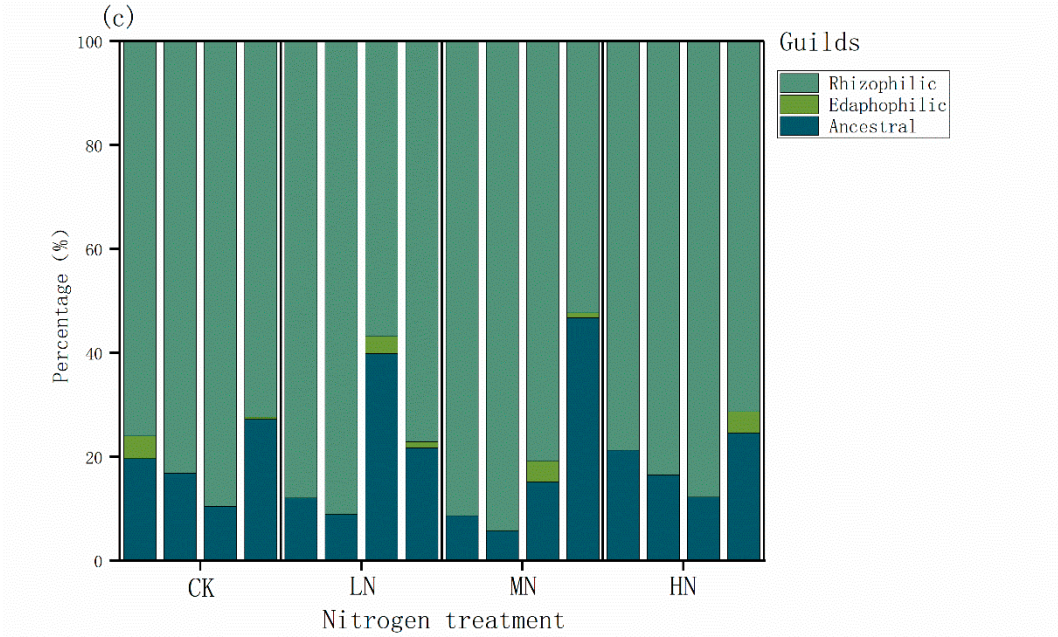


Figure 5. Arbuscular mycorrhizal fungi community composition at the genus (**a**), species (**b**), and classified guilds (**c**) under different nitrogen (N) treatments. CK: no fertilization; LN: 50 kg N ha^{-1} yr^{-1}; MN: 100 kg N ha^{-1} yr^{-1}; HN: 200 kg N ha^{-1} yr^{-1}.

Many essential factors can influence the life cycle of AMF, such as the species of host plants [16], light availability [64], temperature [65], rainfall [66], elevation [61], soil properties [67–69], and the plant community [70]. However, there is no major factor affecting AMF. In our study, AMF colonization and SR were in significant correlation with soil TOC, and AMF SD and SR were significantly correlated with soil C. AMF community structure showed a significant positive correlation with soil C, in keeping with some AMF that were motivated as the content of organic matter increased [56,71,72].

Individual abundances of AMF spores also presented interannual differences. AMF communities, well-known for efficient interannual variability of spores, and our results suggested a small number of AMF responded to N fertilization. The randomness of this species response implies that a short-time study on AMF may not be sufficient to catch variations in the response to N addition [48,73].

Even the spore community could not reflect the active colonizing AMF community composition [74], but it remains a useful indicator for when we need a reference of ecological change in replicated treatments. We can observe changes which we can measure to try to predict unmeasurable sources that are also changing, perhaps they may even predict more than the spores of AMF subsets.

5. Conclusions

We found that added N caused no significant impact to AMF colonization, hyphal length density, and classified guilds (rhizophilic, edaphophilic, and ancestral). *Septoglomus viscosum* was the predominant species in all different N addition treatments. N addition altered AMF community, and some rare species (eg. *Entrophospora infrequens*) disappeared. Our findings supported that compared with AMF structure, AMF community composition is more sensitive to short-time N deposition, and more attention should be paid to AMF community structure in future studies.

Author Contributions: Conceptualization, F.W. and X.Z.; methodology, J.J. and F.W.; software, X.G. and F.W.; validation, X.Z. and J.J.; formal analysis, J.J. and F.W.; investigation, J.J.; resources, F.W., X.Z. and M.C.; data curation, F.W.; writing—original draft preparation, F.W.; writing—review and editing, J.J. and X.Z.; visualization, F.W.; supervision, M.C. and X.G.; project administration, J.J.; funding acquisition, J.J. All authors have read and agreed to the published version of the manuscript.

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

Appendix A

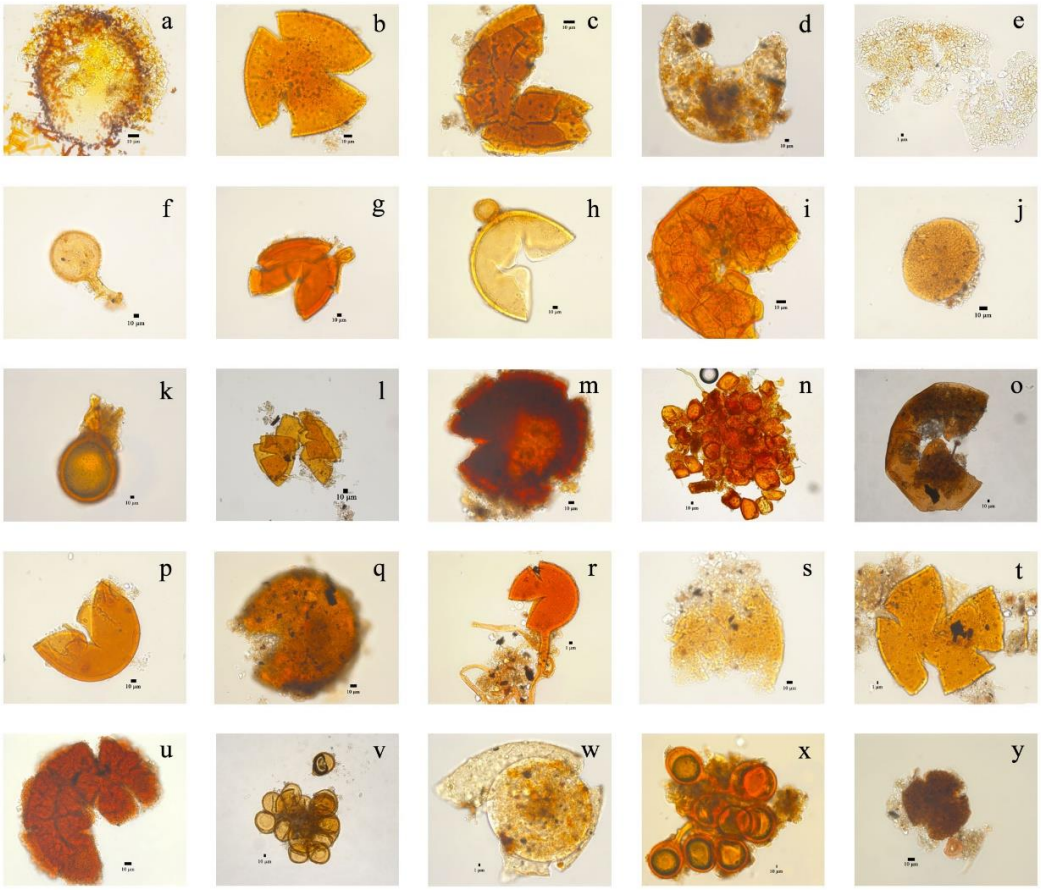

Figure A1. Identified pictures of AMF species (**a**–**z**). **a**, **i**, **m**, **n**, and **u** were mounted in PVLG + Melzer (1:1; *v/v*) and the others in PVLG.

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