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Effects of Nitrogen Addition on Soil Aggregate Stability and Mycorrhizal Morphological Characteristics: Differential Responses of Arbuscular Mycorrhizal and Ectomycorrhizal Fungi

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Abstract: Soil aggregate stability plays a pivotal role in ecosystem functioning and carbon sequestration. Nitrogen deposition influences aggregate stability and drives differential responses in AM and ECM fungi, yet the underlying mechanisms remain unclear. This study aimed to determine how N addition influences soil aggregation, mycorrhizal morphological characteristics, and soil organic carbon (SOC) across two mycorrhizal types. A temperate forest experiment was conducted in northeastern China using 12 plots subjected to four N treatments (control, low, medium, high). Soil properties, soil aggregate traits, mycorrhizal morphological characteristics, and aggregate distributions were quantified. Relationships were examined via correlation analyses, random forest models, and structural equation modeling. N enrichment substantially increased SOC and soil water content, enhancing the proportion of large soil aggregates (4–8 mm) and elevating mean weight diameter (MWD) and geometric mean diameter (GMD), two critical indicators of soil aggregate stability. AM fungi exhibited a stronger response to N addition than ECM, reflected in greater hyphal development and carbon accumulation. SOC and water content correlated positively with aggregate stability, whereas soil pH showed a negative association. N inputs enhance soil structural stability by promoting SOC and water retention, with AM fungi demonstrating heightened sensitivity to N addition. These findings emphasize the integral role of mycorrhizal dynamics in shaping soil carbon stabilization under increasing N deposition.

Keywords: nitrogen deposition impacts; aggregate size distribution; mycorrhizal interactions; soil organic carbon (SOC); soil water content (SWC)

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1. Introduction

Soil aggregate stability plays a pivotal role in determining soil health and ecosystem functionality, influencing processes such as water infiltration, nutrient cycling, and carbon sequestration [1]. Soil aggregates, which are classified by their size and structural integrity, are strongly affected by external environmental factors such as nutrient inputs and microbial dynamics [2]. Nitrogen deposition, a widespread consequence of anthropogenic activities, has emerged as a key factor influencing soil structure and microbial activity across ecosystems globally [3]. However, its impact on the stability and size distribution of soil aggregates remains poorly understood, particularly in relation to microbial and mycorrhizal interactions [4].

Mycorrhizal fungi, particularly arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) types, are integral to soil ecosystem functioning, mediating processes such as soil structure stabilization, nutrient cycling, and carbon sequestration [5]. AM fungi, formed by symbioses with Glomeromycota, are characterized by their ability to produce glomalin, a glycoprotein that enhances soil aggregation by binding soil particles and organic matter [6]. Their extensive hyphal networks significantly increase nutrient acquisition efficiency, particularly for phosphorus, and improve soil water retention [7]. In contrast, ECM fungi, predominantly associated with trees in temperate and boreal forests, belong to the Ascomycota and Basidiomycota phyla [8]. These fungi contribute to carbon storage through the decomposition of organic matter, forming stable soil aggregates via slower nutrient cycling and carbon turnover rates [9]. The functional differences between AM and ECM fungi underscore their distinct roles in modulating soil quality, especially under changing environmental conditions such as nitrogen deposition. While AM fungi are often associated with faster nutrient dynamics and greater aggregation, ECM fungi provide long-term contributions to soil carbon storage and structural stability [10]. The differential roles of AM and ECM fungi suggest that their responses to nitrogen addition could vary, potentially leading to distinct effects on soil aggregate stability. Despite this, how nitrogen addition modulates the contributions of different mycorrhizal types to soil structure and SOC dynamics remains unclear. AM fungi and ECM fungi represent two distinct types of mycorrhizal associations formed by different fungal taxa [11]. AM fungi belong to the phylum Glomeromycota and are characterized by their ability to produce glomalin, a glycoprotein that contributes to soil aggregation by binding soil particles and organic matter [12]. In contrast, ECM fungi, primarily Ascomycota and Basidiomycota, are associated with enhanced organic matter decomposition and carbon storage through slower nutrient cycling processes [13]. These fundamental differences suggest divergent roles in soil structure stabilization, particularly under varying nitrogen inputs [14]. Understanding these distinctions is critical for elucidating their ecological functions.

A key mechanism linking nitrogen addition to soil aggregate stability lies in the interaction between SOC and SWC [15]. Nitrogen-induced changes in SOC accumulation have been shown to enhance soil aggregation by increasing the binding of organic matter to mineral particles [16]. This binding primarily occurs in the form of mineral-associated organic matter (MAOM), which results from the adsorption of organic compounds onto mineral surfaces, forming stable aggregates with a longer turnover time [17]. In contrast, particulate organic matter (POM), composed of larger and less decomposed organic fragments, contributes to aggregate formation primarily through its physical occlusion within soil aggregates [18]. While both MAOM and POM play roles in soil aggregation, the nitrogen-induced increase in SOC observed in this study likely promotes the formation of MAOM, given its stronger association with long-term carbon stabilization [19]. Moreover, shifts in fungal morphology, such as total hyphae length (THL) and total hyphae surface area (THS), may further influence SOC sequestration and soil aggregation [20]. However, the extent to which these processes vary between AM- and ECM-associated trees under nitrogen enrichment has yet to be comprehensively explored.

While significant progress has been made in understanding soil aggregation and mycorrhizal dynamics, critical knowledge gaps remain unresolved, particularly regarding the differential contributions of AM and ECM fungi to nitrogen-induced changes in soil structure and carbon dynamics [21]. First, the relative contributions of AM and ECM fungi to nitrogen-induced changes in soil aggregate size distribution and stability remain poorly quantified [22]. Second, the mechanistic linkages between SOC, SWC, and mycorrhizal morphological characteristics under nitrogen addition are inadequately understood [23]. Finally, it is unclear whether nitrogen addition amplifies the ecological functions of AM fungi more strongly than ECM fungi in stabilizing soil aggregates [24]. Addressing these gaps is essential for developing predictive frameworks for soil carbon stabilization under increasing nitrogen deposition [25].

Based on this context, we hypothesized the following: (1) Nitrogen addition alters soil aggregate size pattern by increasing the proportion of large aggregates and reducing small aggregates, thereby enhancing soil aggregate stability, with AM types exhibiting stronger responses [26]. (2) Nitrogen addition promotes SOC accumulation by altering mycorrhizal morphological characteristics, such as THL and THS [27]. (3) The effects of nitrogen addition on soil aggregate stability are mediated by SOC and SWC, with AM fungi responding more strongly than ECM fungi [28].

This study aims to elucidate the effects of nitrogen addition on soil aggregate stability and mycorrhizal morphological dynamics. Although previous studies have demonstrated the role of mycorrhizal fungi in soil aggregation, the differential responses of AM and ECM fungi to nitrogen deposition remain unclear. This study is among the first to quantify the contrasting effects of AM and ECM fungi on soil aggregation stability and carbon sequestration under nitrogen enrichment, providing novel insights into their distinct ecological roles. Additionally, by integrating structural equation modeling and machine learning-based random forest analysis, we provide mechanistic insights into the mediating roles of SOC and SWC in soil aggregation dynamics, which is crucial for understanding soil carbon stabilization under increasing nitrogen deposition.

2. Materials and Methods

2.1. Study Sites and Experiment Design

The research area is located within the Wuying National Nature Reserve in Yichun City, Heilongjiang Province, northeastern China (48°02′–48°12′ N, 128°58′–129°15′ E). It is characterized by a temperate continental monsoon climate, experiencing prolonged, frigid winters and comparatively short, warm summers. The mean annual temperature is approximately 1.8 °C, and the average yearly precipitation of about 650 mm largely occurs from June to August. Elevation in the study site extends from 230 to 560 m above sea level. The prevalent soil type is dark brown forest soil, exhibiting a pH of 6.5 and a bulk density of 1.3 g/cm^3, which supports a wide variety of shrub species.

Forest species richness in this locale is notably elevated, harboring more than 120 tree species. Approximately 85% canopy coverage furnishes suitable conditions for an understory composed of about 35 shrub species and 20 herbaceous species. The dominant tree taxa chosen for this investigation were *Pinus koraiensis, Abies nephrolepis, Acer tegmentosum, Tilia amurensis, and Fraxinus mandshurica.* Among these, *Pinus koraiensis, Abies nephrolepis,* and *Acer tegmentosum* are associated with ECM, whereas *Tilia amurensis* and *Fraxinus mandshurica* form AM.

A total of 12 uniformly sized square plots (each measuring 20 m × 20 m) were established in a protected area situated on the lower eastern slope of a forested mountain (Figure 1). Slope aspect, gradient, and vegetation coverage were considered during plot placement. A 10 m buffer zone was maintained between adjacent plots to control nitrogen transfer and preserve discrete micro-ecological settings. Four nitrogen addition treatments—low-N (25 kg $N \cdot ha^{-1} \cdot yr^{-1}$), medium-N (50 kg $N \cdot ha^{-1} \cdot yr^{-1}$), high-N (75 kg $N \cdot ha^{-1} \cdot yr^{-1}$), and an unfertilized control—were applied, each replicated three times to form the 12 plots. These supplementation levels were selected based on regional deposition patterns. Monthly applications of NH_4NO_3, dissolved in 32 L of deionized water per plot, were made from May through October using a backpack sprayer for uniform coverage. Control plots received an equivalent volume of water only. This standardized protocol preserved consistent soil moisture, minimized extraneous influences, and established a controlled environment for examining nitrogen deposition effects and underlying mechanisms. It also facilitated systematic data collection, analysis, and extrapolation on ecosystem responses under different nitrogen inputs.

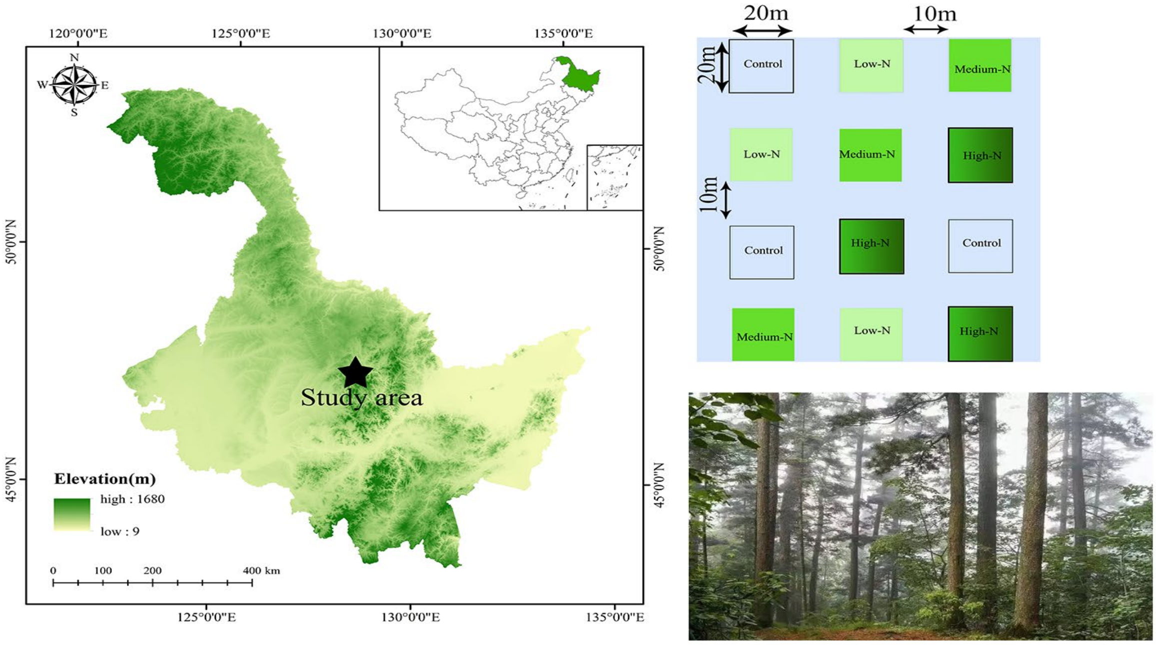

Figure 1. Sample plot and diagram of the experimental design in Wuying National Nature Reserve, Yichun City, Heilongjiang Province, northeastern China.

2.2. Sample Collection

Soil and root samples were obtained in late July 2023. Rhizosphere soils corresponding to *Pinus koraiensis*, *Abies nephrolepis*, *Acer tegmentosum*, *Tilia amurensis*, and *Fraxinus mandshurica* were each sampled. Within every plot and for each tree species, three individual trees were randomly designated, around which three replicate samples were collected per tree. The litter layer was cleared before sampling; then, a 0–10 cm soil section was drilled using a 5 cm diameter, 10 cm length soil auger, followed by the removal of rocks and plant roots. The rhizosphere soils of *Pinus koraiensis*, *Abies nephrolepis*, and *Acer tegmentosum* were mixed together, as these species are predominantly associated with ECM (ectomycorrhizal) fungi. Similarly, the rhizosphere soils of *Tilia amurensis* and *Fraxinus mandshurica* were mixed together, as they are primarily associated with AM (arbuscular mycorrhizal) fungi. This grouping was conducted to reflect the distinct mycorrhizal types and their respective ecological functions, enabling us to analyze the differential responses of ECM- and AM-associated soils to nitrogen addition. The soil samples of ECM and AM were each divided into two portions, and maintained at 4 °C until analyses were conducted to retain biological activity. One portion was employed for mycelium extraction and morphological trait measurements; the other portion was utilized for determining soil physicochemical parameters (Table 1). Concentrations of total nitrogen (TN) and total phosphorus (TP) were determined via H_2SO_4-H_2O_2 digestion following the protocol of Porter and subsequently analyzed using a continuous flow autoanalyzer (SKALAR SAN+, The Netherlands) [29]. Soil pH was assessed by mixing 4 g of air-dried soil with deionized water at a ratio of 1:2.5, allowing the suspension to equilibrate for 30 min, and measuring pH with a Sartorius PB-10 pH meter (Gottingen, Germany). The soil organic carbon (SOC) content was quantified using an automatic TC/TN analyzer (Analytik Jena AG, Multi N/C 3100, Jena, Germany). Furthermore, soil moisture content (SWC) was calculated by oven-drying 10 g of fresh soil at 105 °C to a constant weight and recording the mass difference before and after drying.

Table 1. Symbols.

Traits	Abbreviation	Units	Description
Soil properties			
Soil organic carbon	SOC	g/kg	The number of grams of organic carbon contained in each kilogram of soil
Soil water content	SWC	%	The moisture contained in fresh soil
Total nitrogen	TN	g/kg	The content of total nitrogen in the soil
Total phosphorus	TP	g/kg	The content of total phosphorus in the soil
pH	pH		Soil pH
Soil aggregate traits			
Mean weight diameter	MWD	mm	The mean weight diameter of the aggregates contained in the soil
Geometric mean diameter	GMD	mm	The geometric mean diameter of the aggregates contained in the soil
Mycorrhizal morphological characteristics			
Total hyphae length	THL	cm/g	Total length of hyphae measured within the sampled area
Total hyphae surface area	THS	cm^{-3}/g	Total surface area of hyphae measured within the sampled area
Mean hyphae diameter	MHD	mm	Averaged diameter of hyphae measured within the sampled area
Mycorrhizal root colonization	MRC	%	Percentage of root colonized by mycorrhizal fungi

2.3. Separation of Soil Aggregates

A wet sieving method was applied to separate soil aggregates [30]. Since soil aggregates generally remain moist under natural conditions, wet sieving effectively preserves their structural integrity, yielding more reliable data regarding both size and distribution [31]. Soil samples designated for aggregate analysis were gently broken apart and sieved using an 8 mm mesh to eliminate visible plant roots and larger biological materials. The samples were then air-dried at room temperature. Subsequently, around 50 g of the dried soil was placed atop a stack of sieves with apertures of 4 mm, 2 mm, 1 mm, 0.25 mm, and 0.053 mm, and immersed in water overnight. Sieving took place by consistently raising and lowering the stack through a 5 cm distance at a rate of 30 strokes per minute for 10 min, yielding five particle-size classes: 4–8 mm, 2–4 mm, 1–2 mm, 0.25–1 mm, and 0.053–0.25 mm. To assess aggregate stability, MWD and GMD (Table 1) were calculated. In these equations, the mean diameter of each aggregate fraction is denoted in millimeters, and its percentage relative to the total is indicated accordingly.

$$MWD = \sum_{i=1}^{n} x_i \omega_i \tag{1}$$

$$GMD = \exp[(\sum_{i=1}^{\mathrm{n}} \omega_i \lg x_i)/(\sum_{i=1}^{n} \omega_i)] \tag{2}$$

In this formula, x_i is the mean diameter of the soil size and the unit name is mm, and ω_{i} is the proportion of different soil granular aggregates to the total aggregates.

2.4. Mycelium Extraction and Mycelium Morphological Trait Measurements

Primary root branches were sampled from each tree species within every plot to evaluate mycorrhizal infection. Approximately 200–300 root tips per sample were randomly collected. Initial observations included macroscopic signs of fungal colonization, such as swelling at the root tips. Under a dissecting microscope, the quantity of infected root tips was recorded, and the total mycorrhizal infection rate was calculated as (number of infected root tips × 100)/(total number of root tips).

Mycorrhizal fungal hyphae were extracted according to the hyphal length measurement protocol from Shen, Q et al. [32]. Specifically, 5 g of fresh soil were placed in an electric blender (model DW-3A-90W) containing 500 mL of water and blended for 30 s. Then, 1 mL of the resulting suspension was transferred onto a 24 mm diameter filter membrane (pore size 0.4 μm) using a pipette, and vacuum filtration was performed.

After filtration, a drop of lactoglycerol solution with 0.05% trypan blue was added onto the membrane to stain the hyphae. The hyphae were visualized with a compound microscope (BX-51, Olympus, Tokyo, Japan), and images were captured for analysis. With the aid of WinRHIZO TronMF 2012 software (Regent Instrument Inc., Quebec, QC, Canada), the total hyphal length, total hyphal surface area, and mean hyphal diameter were quantified. Each sample underwent the above procedure three times, and average hyphal characteristics were recorded (Table 1).

2.5. Statistical Analysis

Data normality and homoscedasticity were examined using the Kolmogorov–Smirnov and Levene tests, respectively. One-way ANOVA was employed to explore significant differences in soil TN, TP, SWC, SOC, and pH among different treatments. Two-way ANOVA was used to investigate the interactive effects of mycorrhizal type and nitrogen addition treatments on MWD, GMD, THL, THS, MHD, and MRC. Tukey's post hoc test was then applied to determine differences among treatments at a significance level of $p < 0.05$. To elucidate potential linkages among measured variables, a correlation matrix heatmap was developed based on Pearson's correlation coefficient. Regression analyses were subsequently performed to assess the relationships between SOC and both soil aggregate traits and mycorrhizal morphological characteristics. A random forest model was additionally employed to identify the principal determinants of stability in mycorrhizal morphology and soil aggregates. Finally, to connect morphological characteristics under distinct treatments with aggregate stability, structural equation models (SEMs) were constructed using the lavaan package in R. All statistical analyses were performed using R 3.2.3.

3. Results

3.1. Soil Properties Under Different Nitrogen Treatments

There were statistically significant effects of different nitrogen treatments on soil properties under the ECM and AM types ($p < 0.05$) (Table 2). For the ECM type, the soil pH in the CK treatment was significantly higher than that in the TH treatment (Table 2). SWC was significantly higher in the TM treatment compared to the CK treatment, while and SOC were also significantly increased in the TH treatment compared to the CK treatment (Table 2). Similarly, for the AM type, the soil pH in the CK treatment was significantly higher than that in the TH treatment, and TP and SOC were significantly increased in the TH treatment compared to the CK treatment (Table 2).

Table 2. Soil properties under different treatments (means ± SE).

Mycorrhizal Type	Type	CK	TL	TM	TH
ECM	pH	6.29 ± 0.05^{a}	6.19 ± 0.04^{a}	6.12 ± 0.04^{a}	6.00 ± 0.04^{a}
	SWC (%)	34.38 ± 1.20^{b}	35.28 ± 1.29^{b}	36.43 ± 1.39^{a}	35.94 ± 1.25^{a}
	TN (g/kg)	1.72 ± 0.05^{b}	1.91 ± 0.06^{a}	2.11 ± 0.07^{a}	2.22 ± 0.06^{a}
	TP (g/kg)	0.56 ± 0.02^{a}	0.57 ± 0.01^{a}	0.59 ± 0.03^{a}	0.58 ± 0.02^{a}
	SOC (g/kg)	22.96 ± 0.29^{b}	24.11 ± 0.33^{a}	25.32 ± 0.40^{a}	25.28 ± 0.36^{a}

Table 2. *Cont.*

Mycorrhizal Type	Type	CK	TL	TM	TH
AM	pH	6.22 ± 0.04 ^{a}	6.15 ± 0.04 ^{a}	6.08 ± 0.04 ^{a}	5.80 ± 0.04 ^{b}
	SWC (%)	33.01 ± 1.02 ^{b}	34.24 ± 1.13 ^{a}	34.82 ± 1.23 ^{a}	35.23 ± 1.14 ^{a}
	TN (g/kg)	1.61 ± 0.04 ^{b}	1.81 ± 0.06 ^{a}	2.01 ± 0.06 ^{a}	2.12 ± 0.06 ^{a}
	TP (g/kg)	0.53 ± 0.05 ^{a}	0.54 ± 0.02 ^{a}	0.55 ± 0.04 ^{a}	0.57 ± 0.06 ^{a}
	SOC (g/kg)	21.55 ± 0.27 ^{b}	22.83 ± 0.30 ^{b}	24.00 ± 0.34 ^{a}	24.17 ± 0.34 ^{a}

Note: CK represents the control, TL represents the low-N treatment (25 kg N·ha^{-1}·yr^{-1}), TM represents the medium-N treatment (50 kg N·ha^{-1}·yr^{-1}), TH represents the high-N treatment (75 kg N·ha^{-1}·yr^{-1}), SWC represents soil water content, TN represents total nitrogen, TP represents total phosphorus, and SOC represents soil organic carbon. Significant differences between treatments are indicated by different lowercase letters ($p < 0.05$).

3.2. MWD and GMD Under Different Nitrogen Treatments and Their Correlations with Environmental Factors

There were statistically significant differences in MWD and GMD across treatments and mycorrhizal types (Figure 2A,B). For MWD, in the ECM mycorrhizal type, the CK treatment was significantly higher than the TH treatment, as shown in Figure 2A. In the AM type, the CK treatment was significantly higher than both the TL and TH treatments (Figure 2A). Regarding GMD, within the ECM type, the CK treatment was significantly higher than the TH treatment, whereas in the AM type, the CK treatment was significantly higher than the TL treatment (Figure 2B). Within the same treatment, MWD and GMD in ECM were significantly higher than in AM ($p < 0.05$) (Figure 2A,B). Correlation analysis revealed that MWD was significantly positively correlated with SOC, SWC, TN, and TP but negatively correlated with pH (Figure 3A). Similarly, GMD showed significant positive correlations with SOC, SWC, TN, and TP and a significant negative correlation with pH (Figure 3A). A strong positive correlation was observed between MWD and GMD ($p < 0.05$) (Figure 3A).

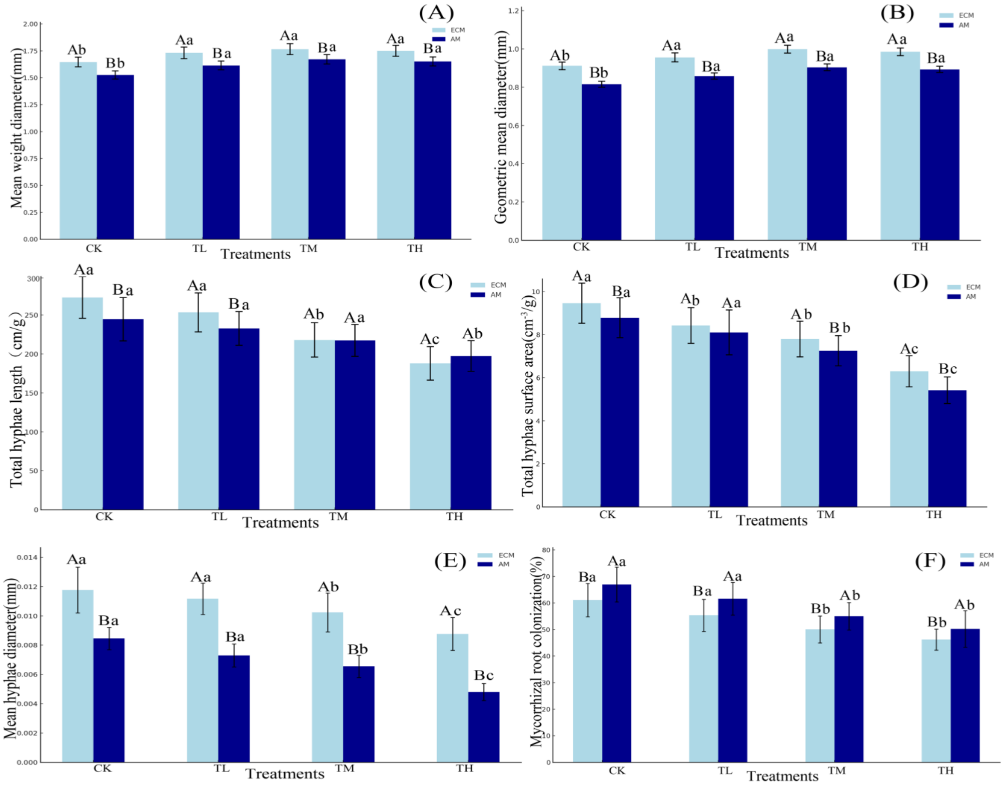

Figure 2. Changes in mean weight diameter (**A**), geometric mean diameter (**B**), total hyphae length (**C**), total hyphae surface area (**D**), mean hyphae diameter (**E**), and mycorrhizal root colonization (**F**) across different treatments. CK represents the control, TL represents the low-N treatment (25 kg N·ha^{-1}·yr^{-1}), TM represents the medium-N treatment (50 kg N·ha^{-1}·yr^{-1}), and TH represents the high-N treatment (75 kg N·ha^{-1}·yr^{-1}).

Different lowercase letters indicate significant differences between different treatments, while different capital letters denote statistically significant differences within the same treatment across different mycorrhizal types (ECM and AM). A significant difference is indicated when $p < 0.05$.

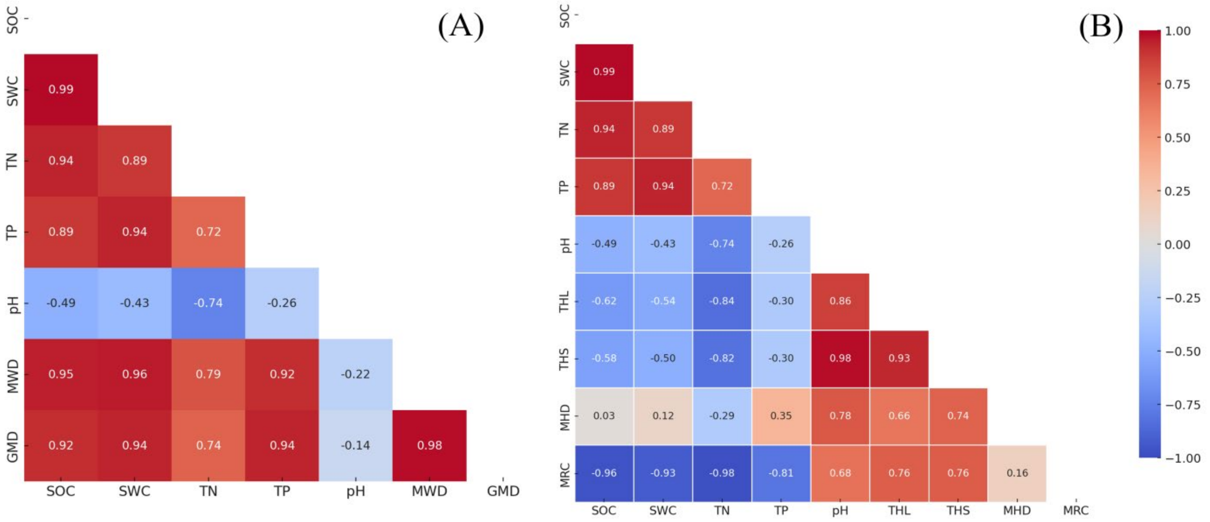

Figure 3. Heatmap of environmental factors with MWD and GMD (**A**); heatmap of environmental factors and mycorrhizal morphological characteristics (**B**). All data were log-transformed to meet the normality assumption. The color intensity in the heatmap represents the strength of the correlation, with the legend on the right side displaying the corresponding correlation coefficient values. Strong positive correlations are shown in shades of red, while weaker correlations are shown in shades of blue. The closer the value is to 1 or −1, the stronger the linear relationship between the variables. Symbols and acronyms of traits: SOC represents soil organic carbon, SWC represents soil water content, TN represents total nitrogen, TP represents total phosphorus, MWD represents mean weight diameter, GMD represents geometric mean diameter, THL represents total hyphae length, THS represents total hyphae surface area, MHD represents mean hyphae diameter, and MRC represents mycorrhizal root colonization.

3.3. Mycorrhizal Morphological Characteristics Under Different Nitrogen Treatments

There were statistically significant differences in mycorrhizal morphological characteristics across treatments and mycorrhizal types ($p < 0.05$) (Figure 2C–F). For THL, in the ECM type, the CK treatment was significantly higher than the TH treatment, while in the AM type, the CK treatment was significantly higher than both the TL and TH treatments (Figure 2C). THS in the ECM type was significantly higher in the CK treatment compared to the TH treatment, whereas in the AM type, the CK treatment was significantly higher than the TL treatment (Figure 2D). Regarding MHD, the CK treatment in the ECM type was significantly higher than the TH treatment, and in the AM type, the CK treatment was significantly higher than both the TL and TH treatments (Figure 2E). For MRC, the CK treatment in the ECM type was significantly higher than the TH treatment, while in the AM type, the CK treatment was significantly higher than the TL treatment (Figure 2F). Correlation analysis showed that THL and THS were significantly positively correlated with SOC, SWC, TN, and TP and negatively correlated with pH ($p < 0.05$) (Figure 3B). MHD was significantly positively correlated with THL and THS as well as with SOC, SWC, TN, and TP (Figure 3B). MRC also exhibited significant positive correlations with THL and THS and a significant negative correlation with pH ($p < 0.05$) (Figure 3B).

3.4. Relationships Between SOC, Soil Aggregate Traits, and Mycorrhizal Morphological Characteristics

There were statistically significant relationships between SOC and various mycorrhizal morphological characteristics, including MWD, GMD, THL, THS, MHD, and MRC, across different mycorrhizal types ($p < 0.05$) (Figure 4). For MWD, a significant positive correlation with SOC was observed in both ECM and AM types ($p < 0.01$) (Figure 4A). Similarly, GMD exhibited a significant positive correlation with SOC in both ECM and AM types (Figure 4B). In contrast, THL did not show a significant correlation with SOC in either ECM or AM types (Figure 4C). For THS, a significant negative correlation with SOC was observed in both ECM and AM types ($p < 0.01$) (Figure 4D). MHD was significantly negatively correlated with SOC in both ECM and AM types ($p < 0.01$) (Figure 4E). Lastly, MRC exhibited a significant negative correlation with SOC in both ECM and AM ($p < 0.01$) (Figure 4F).

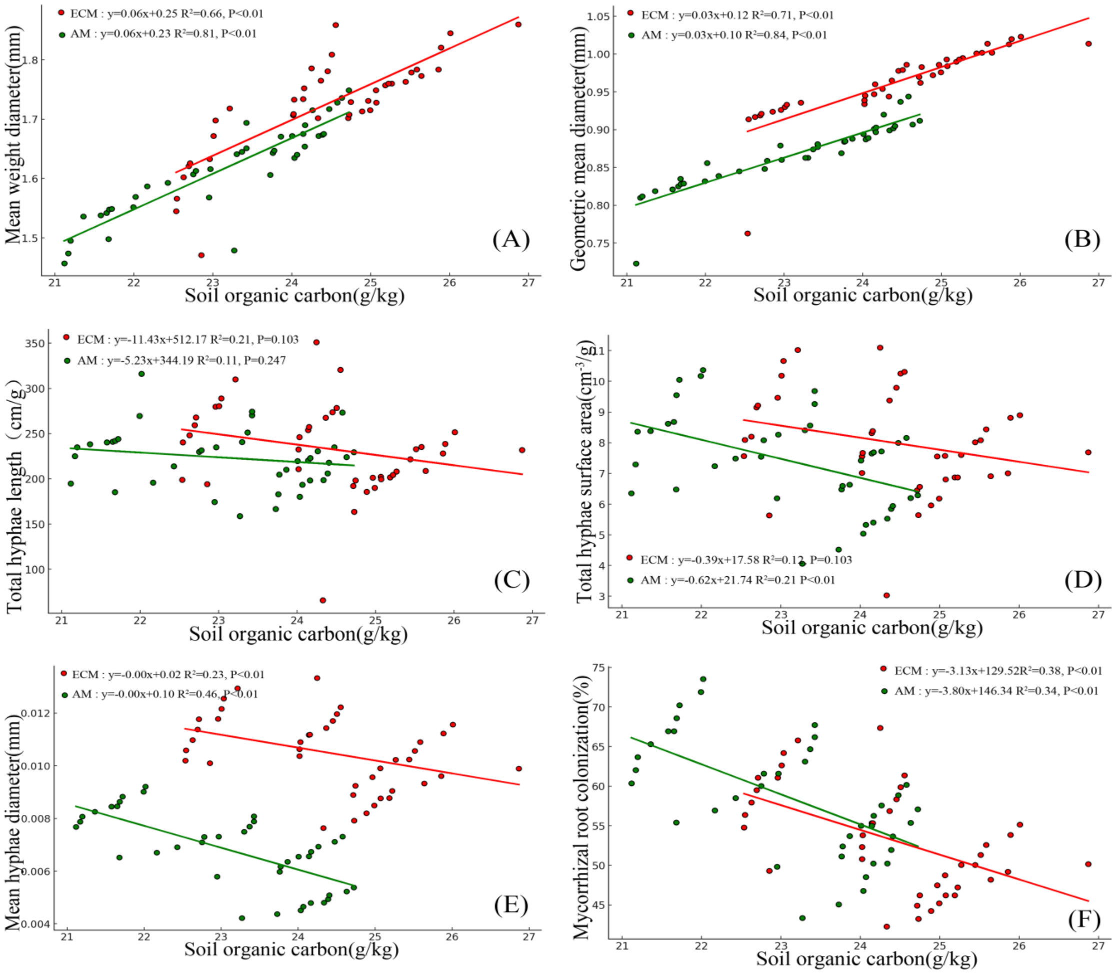

Figure 4. Relationships between soil organic carbon and mean weight diameter (**A**), geometric mean diameter (**B**), total hyphae length (**C**), total hyphae surface area (**D**), mean hyphae diameter (**E**), and mycorrhizal root colonization (**F**).

3.5. Soil Aggregate Distribution Under Different Nitrogen Treatments

Soil aggregate distribution exhibited significant differences across treatments and mycorrhizal types ($p < 0.05$) (Figure 5). In the AM type, the proportion of 4–8 mm and 2–4 mm soil aggregates in the CK treatment was significantly higher than in the TH treatment, while the proportion of 0.25–1 mm and 0.053–0.25 mm soil aggregates was significantly lower in the CK treatment compared to the TH treatment (Figure 5). Similarly, in the ECM type, the proportion of 4–8 mm soil aggregates in the CK treatment was significantly higher than in the TH treatment, whereas the proportion of 0.25–1 mm and 0.053–0.25 mm soil aggregates was significantly lower in the CK treatment compared to the TH treatment (Figure 5). Correlation analysis revealed that the proportion of 4–8 mm soil aggregates was significantly positively correlated with SOC, MWD, and GMD and significantly negatively correlated with MRC ($p < 0.05$) (Table 3). The proportion of 2–4 mm soil aggregates was significantly positively correlated with MHD (Table 3). The proportion of 0.25–1 mm soil aggregates was significantly negatively correlated with SOC, MWD, and GMD and significantly positively correlated with MRC (Table 3). Similarly, the proportion of 0.053–0.25 mm soil aggregates was significantly negatively correlated with SOC, MWD, and GMD and significantly positively correlated with MRC ($p < 0.05$) (Table 3). Correlation analysis revealed that the proportion of soil aggregates was significantly correlated with mycorrhizal type (Table 3).

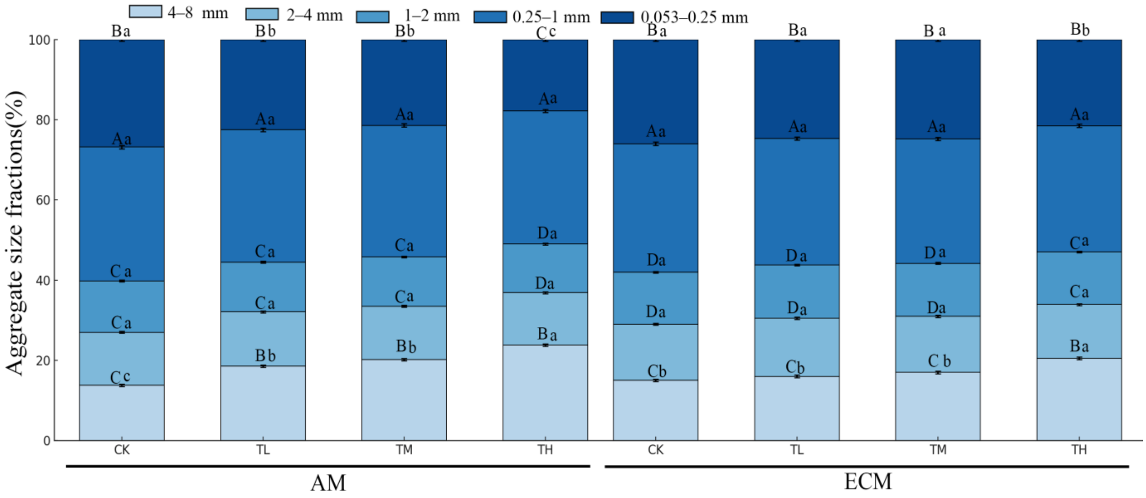

Figure 5. Soil aggregate distribution under different treatments and different mycorrhizal types. CK represents the control, TL represents the low-N treatment (25 kg N·ha^{-1}·yr^{-1}), TM represents the medium-N treatment (50 kg N·ha^{-1}·yr^{-1}), and TH represents the high-N treatment (75 kg N·ha^{-1}·yr^{-1}). Different lowercase letters indicate significant differences between aggregate sizes in different treatments in the same aggregate size at the level of 0.05. Different capital letters indicate significant differences between different soil aggregate sizes in the same treatment at the level of 0.05.

Table 3. Pearson's correlation analysis between soil aggregate size and MWD, GMD, SOC, THL, THS, MHD, and MRC.

Type	4–8 mm	2–4 mm	1–2 mm	0.25–1 mm	0.053–0.25 mm
SOC	0.913 **	0.366	0.269	−0.711 **	−0.699 **
MWD	0.973 **	0.647 *	0.4729	−0.863 **	−0.839 **
GMD	0.980 **	0.661 *	0.605 *	−0.934 **	−0.8909 **
THL	−0.399	0.488	0.375	0.003	0.0189
THS	−0.225	0.361	0.455	−0.132	−0.0608
MHD	0.403	0.852 **	0.898 **	−0.705 **	−0.697 *
MRC	−0.798 *	−0.173	−0.177	0.578 *	0.543 *
T	NS	NS	NS	NS	NS
M	*	**	**	**	**
M × T	NS	NS	NS	NS	*

Note: * $p < 0.05$; ** $p < 0.01$, NS represents not significant. The p-values of the respective variables and the model itself are shown. M represents the mycorrhizal type (ECM and AM); T represents the treatments (control, low-N (25 kg N·ha^{-1}·yr^{-1}), medium-N (50 kg N·ha^{-1}·yr^{-1}), high-N (75 kg N·ha^{-1}·yr^{-1})). SOC represents soil organic carbon, MWD represents mean weight diameter, GMD represents geometric mean diameter, THL represents total hyphae length, THS represents total hyphae surface area, MHD represents mean hyphae diameter, and MRC represents mycorrhizal root colonization.

3.6. Effects of Soil Properties and Mycorrhizal Characteristics on Aggregate Stability

The random forest variable importance analysis revealed significant differences in the relevance of various factors influencing soil aggregate stability ($p < 0.05$) (Figure 6). For MWD, TP and SOC exhibited significantly higher importance compared to other variables (Figure 6A). Similarly, for GMD, TP and SWC showed significantly higher importance (Figure 6B). In the case of THL, soil pH and TN were significantly more important (Figure 6C), while for THS, SOC and soil pH displayed significantly higher importance (Figure 6D). For MHD, GMD showed significantly higher importance than other variables (Figure 6E). Finally, for MRC, SOC had significantly higher importance than other variables (Figure 6F). The SEM results indicated significant positive correlations between soil aggregate traits and mycorrhizal morphological characteristics (Figure 7). SOC was positively correlated with both MWD and GMD (Figure 7). MWD was positively correlated with GMD (Figure 7). THL was positively correlated with GMD, while MRC was positively correlated with both THS and MHD (Figure 7). Additionally, THS was positively correlated with THL (Figure 7).

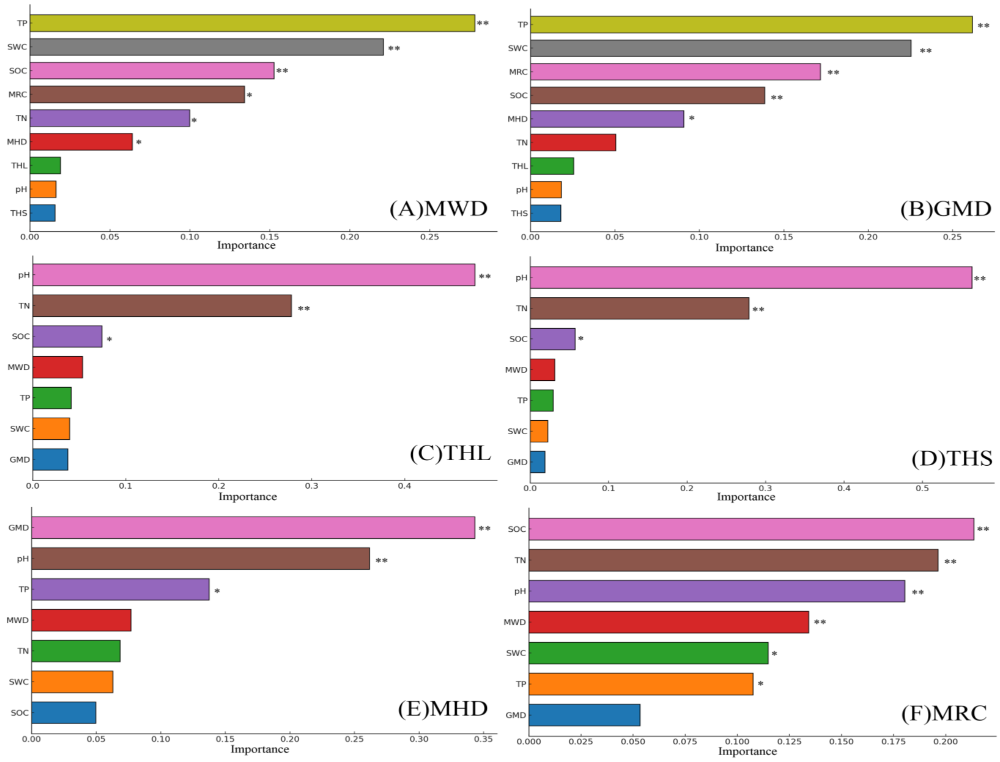

Figure 6. Random forest variable importance plot. The variables are ranked in order of relevance in predicting soil aggregate stability ((**A**) MWD, (**B**) GMD, (**C**) THL, (**D**) THS, (**E**) MHD, (**F**) MRC); colors in the graph represent the relative importance of each variable, dynamically assigned based on correlation strength. The importance measure considered for the analysis is the mean decrease in accuracy computed via the random forest classification algorithm. SOC represents soil organic carbon, SWC represents soil water content, TN represents total nitrogen, TP represents total phosphorus, MWD represents mean weight diameter, GMD represents geometric mean diameter, THL represents total hyphae length, THS represents total hyphae surface area, MHD represents mean hyphae diameter, and MRC represents mycorrhizal root colonization. A single asterisk (*) represents $p < 0.05$; double asterisks (**) represent $p < 0.01$.

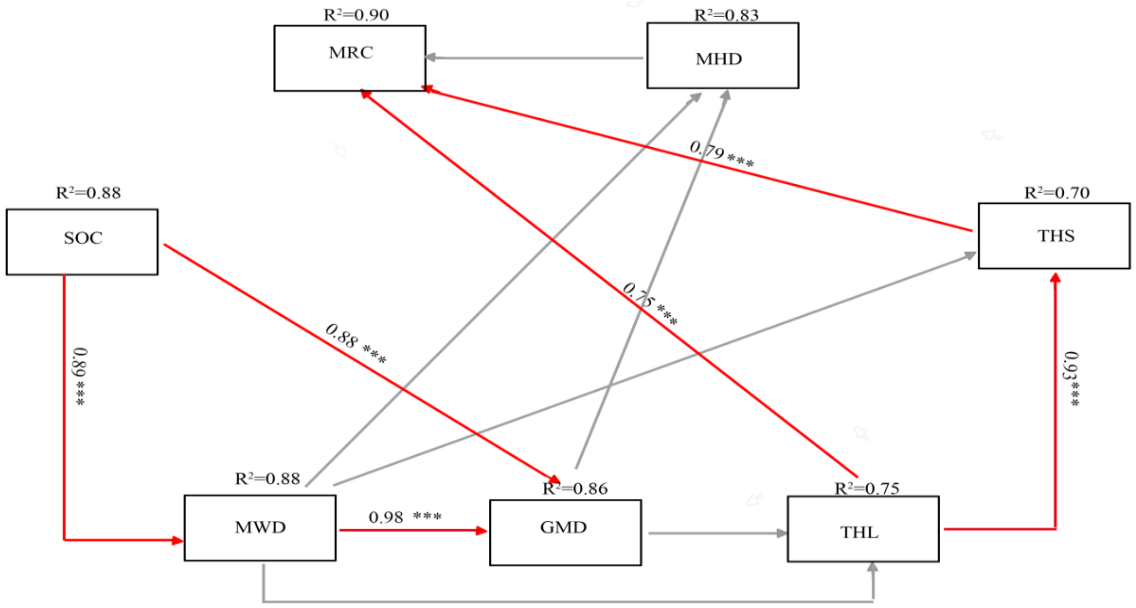

Figure 7. Structural equation model of soil aggregates traits and mycorrhizal morphological characteristics. The red arrow indicates positive correlation; the grey arrow indicates no significant correlation. SOC represents soil organic carbon, MWD represents mean weight diameter, GMD represents geometric mean diameter, THL represents total hyphae length, THS represents total hyphae surface area, MHD represents mean hyphae diameter, and MRC represents mycorrhizal root colonization. *** $p < 0.001$.

4. Discussion

4.1. Impacts of Nitrogen Addition on Soil Aggregate Stability and Mycorrhizal Type Responses

The results demonstrated that nitrogen addition significantly influenced the distribution of soil aggregates and their stability across mycorrhizal types. In the AM type, nitrogen addition increased the proportion of large aggregates (4–8 mm and 2–4 mm) while decreasing the proportion of small aggregates (0.25–1 mm and 0.053–0.25 mm) compared to the CK treatment (Figure 5). In contrast, the ECM type exhibited similar trends but with less pronounced effects. MWD and GMD were significantly higher in the AM type under nitrogen addition compared to the ECM type (Figure 2A,B). Correlation analysis revealed that MWD and GMD were positively correlated with SOC, SWC, TN, and TP, while being negatively correlated with pH (Figure 3A; Table 3).

These findings indicate that nitrogen addition enhances soil aggregate stability by promoting the formation of large aggregates. This effect was more pronounced in AM types, suggesting a greater sensitivity of AM trees to nitrogen inputs. The enhanced soil aggregation observed in AM-associated systems can be attributed not only to nitrogen-induced changes in tree nutrient uptake but also to the production of glomalin by AM fungi [33]. Glomalin acts as a binding agent, facilitating the formation of stable soil aggregates and contributing significantly to soil structure stability [34]. In contrast, ECM fungi, which lack glomalin production, may influence soil aggregation indirectly through their effects on organic matter decomposition and carbon storage [35]. These mechanistic differences between AM and ECM mycorrhizal types highlight the critical role of AM fungi in mediating soil structural responses to nitrogen deposition [36]. Future studies should further investigate the interplay between glomalin production, nitrogen inputs, and soil aggregation dynamics [37]. The positive correlations between SOC, SWC, and aggregate stability suggest that nitrogen addition-induced SOC accumulation plays a critical role in stabilizing soil aggregates. The observed trends align with the findings of Singh et al., who reported that nitrogen addition enhanced soil structural stability through increased SOC and soil moisture content [38]. The stronger response in AM types could be attributed to their higher nutrient absorption efficiency under nitrogen-enriched conditions, as suggested by HShan et al. [39]. However, discrepancies with studies such as Wu et al., which found no significant effects of nitrogen addition on ECM-associated stability, may arise from multiple factors [40]. First, differences in soil properties, such as baseline SOC levels and pH, could influence the response of ECM fungi to nitrogen inputs [41]. For example, soils with low SOC may have limited capacity for aggregate stabilization, reducing the observable effects of nitrogen enrichment [42]. Second, variations in nitrogen input rates between studies may alter fungal nutrient dynamics [43]. High nitrogen rates, as in our study, might suppress ECM fungal activity, while lower rates could yield neutral or positive effects [44]. Third, differences in experimental durations may also play a role, as short-term studies may fail to capture the delayed responses of ECM fungi to nitrogen addition [45]. These findings underscore the need for long-term and multi-scale studies to better understand the nuanced interactions between nitrogen addition and mycorrhizal-mediated soil stability [46]. Variations in soil pH across treatments may also contribute to the contrasting responses observed between mycorrhizal types [47]. Changes in pH are closely linked to nitrogen mineralization processes, particularly nitrification, which can acidify soils by producing hydrogen ions as ammonium is converted to nitrate [48]. This acidification effect is more likely to influence AM fungi, which are generally more sensitive to lower pH conditions compared to ECM fungi, whose enzymatic activity and nutrient acquisition mechanisms are better adapted to acidic soils [49]. Consequently, differences in nitrification rates among treatments could partly explain the stronger responses of AM fungi to nitrogen addition and the relatively muted responses of ECM fungi under similar conditions.

The results support the initial hypothesis that nitrogen addition enhances soil aggregate stability and that AM types exhibit a stronger response. The underlying mechanism involves SOC and SWC as key mediators, as they influence both aggregate stability and mycorrhizal morphology [50]. The increased sensitivity of AM types to nitrogen addition likely stems from their ability to rapidly utilize nitrogen for enhancing hyphal development, which subsequently improves SOC dynamics and aggregate stabilization [51].

4.2. Effects of Nitrogen Addition on Mycorrhizal Morphological Characteristics and SOC Accumulation

The results indicated that nitrogen addition significantly influenced mycorrhizal morphological characteristics across mycorrhizal types and treatments. Specifically, THL and THS in the ECM type under the CK treatment were significantly higher than those in the TH treatment, while in the AM type, THL and THS under the CK treatment were significantly higher than in both the TL and TH treatments (Figure 2C,D). Similarly, MHD and MRC followed the same trend, with the CK treatment consistently exhibiting higher values compared to nitrogen-enriched treatments in both ECM and AM types (Figure 2E,F). Correlation analysis revealed that THL and THS were significantly positively correlated with SOC, SWC, TN, and TP and negatively correlated with pH (Figure 3B). SOC was significantly positively correlated with MWD and GMD in both ECM and AM types, while MHD and MRC showed a negative correlation with SOC (Figure 4).

These findings demonstrate that nitrogen addition alters mycorrhizal morphological characteristics, which in turn significantly influence SOC accumulation. The positive correlation between THL, THS, and SOC suggests that changes in hyphal development and surface area contribute to SOC stabilization, likely through enhanced carbon input from fungal biomass. Negative correlation between MRC and SOC suggests a trade-off between root colonization and carbon allocation to fungal structures under nitrogen-enriched conditions. These results align with the observations of Parker et al., who reported that nitrogen addition promotes fungal biomass at the expense of root colonization in AM-associated systems [52]. Similarly, Thompson et al. demonstrated that increased nitrogen inputs enhance hyphal productivity and SOC accumulation, particularly in AM-dominated soils [53]. These changes in mycorrhizal morphological characteristics significantly influence SOC stabilization [54]. Changes in mycorrhizal morphological characteristics, including increased total hyphal length (THL), enhanced hyphal surface area (THS), and higher mycorrhizal root colonization (MRC), significantly influence SOC stabilization [55]. These morphological adaptations facilitate greater carbon input into the soil via fungal biomass and enhance soil aggregation by binding soil particles and organic matter [56]. For instance, the extension of hyphal networks increases soil organic carbon sequestration and improves soil structure by physically stabilizing aggregates [57]. The consistency between the current study and prior research highlights the role of nitrogen in driving fungal-mediated carbon stabilization mechanisms [58]. These findings align with previous studies highlighting the role of fungal hyphal networks in promoting SOC accumulation and soil water retention by physically stabilizing soil aggregates and increasing organic matter inputs [59]. The positive correlations with TN and TP indicate that increased fungal biomass enhances nutrient cycling efficiency, contributing to improved soil fertility [60]. In contrast, the negative correlation with pH reflects the sensitivity of fungal activity and enzymatic processes to soil acidity, which can influence nutrient availability and fungal colonization rates [61]. These results emphasize the critical role of mycorrhizal hyphal dynamics in mediating soil biogeochemical processes under varying environmental conditions [62]. However, discrepancies with studies which found no significant effect of nitrogen on ECM fungal structures, may arise due to differences in soil nutrient status, nitrogen input levels, or microbial community composition. Notably, differences in pH across treatments may also contribute to variations in fungal morphological responses [63]. Soil pH is a critical driver of mycorrhizal fungal diversity, biomass, and morphological characteristics, influencing fungal colonization rates, hyphal growth, and enzymatic activity [64]. Lower pH levels, often associated with nitrogen deposition, can inhibit AM fungal colonization and reduce hyphal length and surface area, as AM fungi are generally more sensitive to acidic conditions [65]. In contrast, ECM fungi are more tolerant of acidic soils, maintaining higher enzymatic activity and nutrient acquisition efficiency under such conditions [66]. Consequently, pH-induced shifts in fungal biomass and diversity may underlie the observed variations in fungal morphological responses across treatments, highlighting the need for further research on the interactions between soil pH and mycorrhizal function.

The findings support the hypothesis that nitrogen addition promotes SOC accumulation by altering mycorrhizal morphological characteristics. The increased THL and THS observed in AM types under nitrogen-enriched conditions likely facilitate carbon transfer from plants to fungal structures, enhancing SOC stabilization [67]. The pronounced response in AM types compared to ECM types may reflect differences in nitrogen utilization strategies, where AM fungi are more effective at adapting to high nitrogen availability [68]. These results underscore the importance of mycorrhizal dynamics in mediating SOC responses to nitrogen deposition [69].

4.3. Nitrogen Addition Enhances Soil Aggregate Stability Through SOC and SWC Regulation with Differential Responses in Mycorrhizal Types

The results demonstrated that nitrogen addition significantly impacted soil aggregate stability (MWD and GMD) across mycorrhizal types and treatments. In ECM types, the CK treatment exhibited significantly higher MWD and GMD values compared to the TH treatment, while in AM types, the CK treatment showed higher MWD and GMD values than both the TL and TH treatments (Figure 2A,B). Correlation analysis revealed that MWD and GMD were positively correlated with SOC, SWC, TN, and TP, and negatively correlated with pH (Figure 3A). Random forest and structural equation modeling further indicated that SOC, SWC, and TP were the most influential factors for MWD and GMD (Figures 6 and 7).

These findings suggest that nitrogen addition significantly enhances soil aggregate stability by promoting SOC and SWC, with AM types exhibiting a stronger response compared to ECM types. The positive correlations between SOC, SWC, and soil aggregate traits highlight the critical role of organic carbon and water content in stabilizing soil structure. The enhanced sensitivity of AM types to nitrogen addition could be attributed to their higher nutrient acquisition efficiency, which facilitates the incorporation of carbon into soil aggregates. The observed trends are consistent with previous studies. For instance, Zhu et al. reported that nitrogen addition increased SOC and improved soil aggregate stability, particularly in AM-dominated ecosystems [70]. This aligns with the stronger response of AM fungi to nitrogen enrichment observed in our study [71]. Similarly, Muneer et al. highlighted that AM fungi are more responsive to nitrogen inputs compared to ECM fungi, resulting in greater carbon deposition and improved soil structure [72]. In contrast, Corrales et al. observed limited effects of nitrogen addition on ECM-dominated soils, which could be attributed to the inherently slower nutrient cycling and carbon turnover rates associated with ECM fungi [73]. Additionally, Chen et al. found that the magnitude of SOC accumulation under nitrogen enrichment depended on soil moisture content, suggesting that hydrological conditions might mediate the observed responses [74]. These contrasting findings emphasize the importance of integrating soil physicochemical properties, fungal traits, and environmental factors when evaluating the impacts of nitrogen addition on soil structure and carbon stabilization. The positive impact of SOC and SWC on aggregate stability aligns with findings by Yu et al., who emphasized the critical role of these factors in maintaining soil integrity [75]. However, discrepancies with Karst et al., who observed limited effects of nitrogen addition on ECM-dominated soils, may be due to differences in soil nutrient baselines, experimental durations, or microbial community structures [76].

The results strongly support the hypothesis that nitrogen addition improves soil aggregate stability through SOC and SWC regulation. The differential responses observed between AM and ECM types are likely due to variations in their nutrient uptake mechanisms and carbon allocation strategies [77]. The pronounced response in AM types may be attributed to their ability to more effectively utilize nitrogen inputs to enhance carbon stabilization within soil aggregates [78]. Additionally, the significant influence of SWC on aggregate stability further underscores the importance of soil water dynamics in mediating the effects of nitrogen addition on soil structure [79]. This study highlights the necessity of integrating mycorrhizal dynamics into soil carbon stabilization frameworks to better understand the impacts of nitrogen deposition [80].

5. Conclusions

This study demonstrates that nitrogen addition significantly enhances soil aggregate stability by promoting SOC accumulation and SWC regulation, with AM types exhibiting a stronger response compared to ECM types. Across nitrogen treatments, AM displayed higher MWD and GMD, as well as a greater proportion of large aggregates, highlighting the sensitivity of AM fungi to nitrogen inputs. The strong positive correlations between SOC, SWC, and soil aggregate traits underscore the critical roles of organic carbon and water content in stabilizing soil structure. Nitrogen addition also altered mycorrhizal morphological characteristics, with THL and THS showing significant increases under nitrogen enrichment, particularly in AM types. These morphological changes enhanced SOC stabilization, likely by facilitating greater carbon input into the soil through fungal biomass. However, the observed trade-off between MRC and SOC suggests a shift in carbon allocation dynamics under nitrogen-enriched conditions. These findings align with prior studies that emphasize the role of AM fungi in nitrogen-induced soil carbon stabilization, while discrepancies with ECM-related studies highlight the potential influence of soil properties, nitrogen levels, and microbial community composition. Nitrogen addition enhanced the ecological functions of AM fungi in soil carbon dynamics, as evidenced by greater increases in soil aggregation and carbon stabilization. In contrast, ECM fungi showed relatively weaker responses under similar conditions, reflecting their distinct functional roles in soil ecosystems.

This study provides new insights into the contrasting roles of AM and ECM fungi in mediating soil aggregation under nitrogen deposition, emphasizing that AM fungi exhibit a stronger response in stabilizing soil structure. Moreover, by integrating advanced modeling techniques, we demonstrate the critical mediating roles of SOC and SWC in soil aggregate formation, offering a quantitative framework to predict soil responses under future nitrogen deposition scenarios. These findings contribute to broader discussions on soil carbon stabilization, forest ecosystem management, and climate change mitigation. Future research should further explore the long-term impacts of nitrogen addition on diverse mycorrhizal ecosystems and incorporate functional traits to better predict ecosystem responses to global nitrogen enrichment.

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