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The Contrasting Responses of Mycorrhizal Fungal Mycelium Associated with Woody Plants to Multiple Environmental Factors

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Abstract: *Research Highlights:* Extraradical mycorrhizal fungal mycelium (MFM) plays critical roles in nutrient absorption and carbon cycling in forest ecosystems. However, it is often ignored or treated as a root uptake apparatus in existing biogeochemical models. *Methods:* We conducted a meta-analysis to reveal how MFM responds to various, coinciding environmental factors and their interactions. *Results:* Nitrogen (N) addition and N-phosphorus (P)-potassium (K) combination significantly decreased MFM. However, elevated CO_2, organic matter addition, P addition, and CO_2-N combination significantly increased MFM. In contrast, warming, K addition, N-P combination, and P-K combination did not affect MFM. Mycorrhizal fungal levels (individual vs. community), mycorrhizal type (ectomycorrhizal fungi vs. arbuscular mycorrhizal fungi), treatment time (<1 year vs. >1 year), and mycelium estimation/sampling method (biomarker vs. non-biomarker; ingrowth mesh bag vs. soil core) significantly affected the responses of MFM to elevated CO_2 and N addition. The effect sizes of N addition significantly increased with mean annual precipitation, but decreased with soil pH and host tree age. The effect sizes of P addition significantly increased with N concentration in host plant leaves. *Conclusions:* The differential responses revealed emphasize the importance of incorporating MFM in existing biogeochemical models to precisely assess and predict the impacts of global changes on forest ecosystem functions.

Keywords: carbon allocation; global change; mycorrhizal fungal community; mycorrhizal fungal mycelium; nitrogen addition

1. Introduction

Globally, mycorrhizal symbioses are common and extensive in terrestrial vegetation [1], and play a key role in biogeochemical cycles of forest ecosystems [2–4]. Mycorrhizal fungi transfer nutrients directly to their host plants in exchange for photosynthetically derived carbon [5]. Extraradical mycorrhizal fungal mycelium (MFM), which had been traditionally ignored in field assessments, constitutes the vast majority (about 60%–95% for ectomycorrhizas, EM) of the total mycorrhizal fungal biomass, while the Hartig net, mantle, and sporocarps constitute 5%–40% only [6]. In terms of root foraging, MFM of EM may permit a more thorough exploitation of soil mineral and organic nutrients [1,7]. In terms of absorptive efficiency, MFM of EM and arbuscular mycorrhizas (AM) is clearly more effective for obtaining greater absorption area per unit of carbon allocated to the mycelium than to the root [8]. Therefore, mycorrhizal fungi can greatly enhance the nutrient absorption capacity of the plant root system [9]. For instance, some EM fungi are known to colonize organic matter, wood ash, and leaf litter by forming a dense mycelium [5,7], and to acquire nutrients from organic

matter in the soil [10]. MFM of EM and AM accounts for 10%–50% of the belowground allocated carbon [11,12]. Furthermore, about 50%–62% of the soil organic carbon pool may be a result of fungal residues remaining in the soil after the death of the mycelium of EM [13]. This probably exceeds the input via aboveground leaf litter and belowground fine root turnover [14,15]. Overall, MFM plays an important role in the mobilization and acquisition of nutrients from the soil, and is intimately involved in the sequestration and partitioning of carbon into the soil [16,17].

Multiple important aspects of the global environment greatly influence functioning of mycorrhizal plants and their fungal associates with potential implications for forest ecosystem carbon cycling [18,19]. The atmospheric concentration of CO_2 is predicted to reach 550 ppm by 2100, and is likely to be accompanied by an increase in global average annual temperature of as much as 4.4 ± 0.5 °C for the period 2070–2099 [20]. More carbon is likely to be allocated to tree root systems under future elevated CO_2 scenarios, leading to increased belowground forest productivity [21]. An eventual consequence may be increased carbon availability to fungi for the development of the mycorrhizal mycelium [8]. Moreover, temperature is a key factor that regulates the growth and metabolic activity of mycorrhizal fungi [22]. For example, AM colonization intensity is tightly related to the temperature on the global scale, with peak colonization under warm temperature during growing season [22]. In a climate-changing world, changes in both atmospheric CO_2 concentration and temperature will influence mycorrhizal fungi and their host plant at various scales [23]. Yet, we have no information about whether relationships of MFM with temperature at the global scale are the same as those at smaller scales. As a result of greenhouse gas-induced global warming, wildfires may deeply influence soil organic carbon dynamics through heating and wildfire-deposited charcoal [24], leading to dramatic changes in soil physicochemical properties and microbial activity [25]. On the other hand, the addition of organic matter or wood ash is a common forest management practice to improve soil nutrient availability and soil structure [26]. In both laboratory and field experiments, the application of organic matter or wood ash stimulates the growth of certain EM fungi [27,28]. Colonization of mycorrhizal mycelium can enhance opportunities to acquire nutrients from the organic matter and wood ash [29]. However, we know little about mechanisms underlying the colonization of organic matter or wood ash by some mycorrhizal fungi [7].

There is an increasing interest in mycorrhizal fungal behavior to elevated atmospheric nitrogen (N) deposition, another major driver of global change. It is reported that N fertilization may negatively impact the respiration of MFM, leading to a reduced flux of plant-derived carbon back to the atmosphere via mycorrhizal fungi [30]. N addition, on the one hand, may decrease MFM amount, and may also lead to changes in the growth of MFM and the composition of the fungus community [31]. On the other hand, any extrinsic factors (e.g., N fertilization) that influence host plant's carbon production, belowground carbon allocation, and thus the carbon availability will affect the MFM, since mycorrhizal fungi are fed by carbon from their host plants [21]. The host plants allocate less carbon to mycorrhizal symbionts and thus limit the mycorrhizal growth in the case of insufficient nutrient supply [32]. The two most common groups of mycorrhizas, EM and AM fungi, employ different nutrient acquisition and carbon utilization strategies [33,34], thus, factors influencing the intrinsic properties of mycorrhizal fungal carbon utilization can also bring about large variations of MFM [18,35]. Hence, one of the significant challenges to ecologists is to realize how mycorrhizal fungi respond to the various, coinciding environmental factors and their interactions. In addition, phosphorus (P) is known to accumulate in the fungal mantle of mycorrhizal roots when supplied in excess amounts, but this P resource stored will rapidly be transferred to the host under conditions of low P availability [36]. However, we still lack the information about the impact of altered P availability on the growth of MFM, especially during the early stages of fungal development, in most forest ecosystems [37,38].

The external mycorrhizal mycelium, as the most dynamic component of the mycorrhizal symbiosis, is likely to be the fastest indicator of how mycorrhizal fungi react to the wide variation in environments [39,40]. A number of methods have been developed to collect (e.g., ingrowth mesh bag, soil core, etc.) and quantify (e.g., biomarker, visual method, etc.) the production and standing biomass

of MFM (Table 1). However, the strengths and weaknesses of the currently applied methods also potentially influence the accuracy of estimation of MFM [17]. At present, we know that MFM is critical in precisely assessing and predicting the responses of forest ecosystem carbon flow and nutrient cycle dynamics to global climate change. However, the majority of previous meta-analyses mainly focus on the responses of mycorrhizal abundance, root colonization, mycorrhizal composition, and mycorrhizal activity to global change [18,41]. Consequently, we still lack a thorough understanding in the direction and size of MFM responses to multiple environmental factors. Here, we present a meta-analysis on the effects and effect range of multiple environmental factors on the MFM. Separate meta-analyses were conducted for elevated CO_2, N addition, P addition, K addition, organic matter addition, warming, CO_2-N combination, N-P combination, P-K combination, and N-P-K combination. Our hypothesis is that MFM positively responds to CO_2, organic matter, and warming, but it negatively responds to N, P, and K availability. We aimed to answer the questions of (a) how MFM responds to multiple environmental factors and their combination, and (b) how mycorrhizal hierarchical and experimental aspects affect the responses of MFM to different environmental factors.

Table 1. Mycorrhizal fungal mycelium (MFM) estimation methods and experimental treatment settings in this meta-analysis.

Mycorrhizal Fungal Mycelium Estimation Methods and Treatment Settings		
Estimation methods		Measurement index
Biomarker	Chitin; ergosterol; glomalin; PLFA 16: 1ω5c; PLFA 18: 2ω6, 9	Fungal mycelium biomass
Non-biomarker	Agar film; fan-like manner; gridline intersection; membrane-filter	Fungal mycelium length
Experimental treatment settings		
Organic matter addition	Compost; litter; organic fertilizer; wood ash	
Elevated CO_2	Experimental soil plant atmosphere system; free air CO_2 enrichment (FACE); microcosm; open top chamber (OTC)	
Nitrogen (N) addition	Ammonium nitrate; ammonium sulfate; sodium nitrate; urea	
Phosphorus (P) addition	Apatitle; sodium dihydrogen phosphate; superphosphate; triple superphosphate	
Potassium (K) addition	Biotite; Osmocote	
Warming	Greenhouse; heating cable; infrared heating	

PLFA, phospholipid fatty acid.

2. Materials and Methods

2.1. Data Collection

We searched published peer-reviewed papers using Web of Science, Google Scholar, China National Knowledge Infrastructure and VIP Information Network (the list of the data sources shown in Appendix A). The key words used were mycorrhizal fungal mycelium, extrametrical/external mycelium/hyphae, fungal mycelia, mycorrhizal fungi, mycorrhizal fungal growth, belowground responses, elevated CO_2/atmospheric carbon dioxide, atmospheric CO_2 enrichment, nitrogen/phosphorus/potassium addition/deposition/fertilization/amendment/availability, warming, temperature manipulation, wood ash/organic matter/compost addition, woody plant/tree/shrub (Table 1). Different combinations of these words connected with AND or OR were repeatedly used to find publications. Only published woody (trees, shrubs) plant-related studies satisfying all the following criteria were selected for the present meta-analysis: (a) studies with control and treatment under the same abiotic and biotic conditions; (b) the means, standard errors/standard deviations, and sample sizes were reported; (c) both field manipulation studies and laboratory incubation studies were selected; (d) MFM was estimated by biomarker (such as ergosterol, phospholipid fatty acid, etc. for measuring the biomass of MFM) and non-biomarker (such as gridline

intersection, agar film, etc. for measuring the length of MFM) analysis from ingrowth mesh bag and soil core methods (Table 1). If a particular study reported data from several mycorrhizal fungal species or host plant species, they were considered as independent data points. When parameters were measured several times in a study, only the last sampling date was used for our meta-analysis. We identified a total of 53 studies (the list of the data sources can be found in the Appendix A), which yielded 46 observations for elevated CO_2, 11 for K addition, 37 for N addition, 23 for organic matter addition, 30 for P addition, 12 for warming, six for CO_2-N combination, 18 for N-P combination, eight for P-K combination, and 14 for N-P-K combination.

Data collection consisted of obtaining the means of the experimental and control group with their standard deviations (SD) and replicate numbers (N). Standard errors (SE) were transformed to SD according to the equation of SD = SE × sqrt (N). As the calculated response ratio is dimensionless, the units of parameters were not considered. When graphical data was presented in a paper, values were extracted from figures using GetData Graph Digitizer 2.24 (http://getdata-graph-digitizer.com). We also recorded data on experimental settings (such as field site location, experiment date, sampling method, experimental levels, and treatment duration), biotic factors (such as tree age, leaf nutrient content, and mycorrhizal type), climatic factors (such as mean annual temperature, mean annual precipitation) and edaphic factors (such as soil carbon/nitrogen ratio and pH).

2.2. Meta-Analysis

We selected the natural log-transformed response ratio (LRR) to calculate the "effect size" of different global change factors on MFM [42]. We defined LRR as the ratio of the value of a parameter in the treatment group to that in the control group. Confidence intervals (CI) of the effect size were generated using bootstrapping [43]. The treatment effects of global change factors were considered to be significant if the 95% confidence interval of LRR did not overlap with zero [43]. We implemented hierarchical mixed effects meta-analyses using the *rma.mv* function in the package *metaphor* 2.10, which allows the specification of nesting groups [44]. Hierarchical mixed effects meta-analyses include fixed and random effects to account for differences across studies [45]. The mixed effects model was used to analyze the effects of elevated CO_2 on MFM, with mycorrhizal fungal level (individual vs. community), mycorrhizal type (EM vs. AM), duration time of elevated CO_2 treatment (<1 year vs. >1 year), and MFM estimation method (biomarkers vs. non-biomarker) as main fixed factors. The mixed effects model was used to detect the effects of N addition on MFM, with sampling method (ingrowth vs. soil core), mycorrhizal type (EM vs. AM), incubation time of ingrowth method (<1 year vs. >1 year), and MFM estimation method (biomarkers vs. non-biomarker) as main fixed factors. For all mixed effects, we included study identity as a random effect to account for multiple cases coming from the same study. Host phylogeny strongly affects the plant-mycorrhizal fungus systems, it explains 75% and 20% of the variations in fungal species richness and community composition, respectively [46]. Thus, host plant phylogeny was conducted as a correlation matrix derived from the phylomatic tree (http://phylodiversity.net/phylomatic/pmws) to control for similarities [47]. We transformed phylomatic tree to the correlation matrix using the *vcv* function in the package *ape* 5.0 [48], except for studies or data in relation to K addition and P-K combination treatments due to few host plant species studied. In the Results section, we report the total heterogeneity in effect sizes (Q_t) among studies and the difference among group cumulative effect sizes (Q_m).

3. Results

3.1. Effects of Elevated CO_2 on Mycorrhizal Fungal Mycelium

The hierarchical mixed effects meta-analyses showed that woody plants' MFM significantly increased under elevated CO_2 (Q_t = 441.26, $p < 0.001$) (Figure 1). We found that the positive responses of MFM to elevated CO_2 were significantly affected by mycorrhizal fungal level (individual vs. community), mycorrhizal type (EM vs. AM), duration time of elevated CO_2 (<1 year vs. >1 year),

and mycelium estimation method (biomarker vs. non-biomarker) (Figure 2). Specifically, MFM at the individual level, EM fungal mycelium, MFM from experiments of <1 year, and MFM derived from non-biomarker method significantly increased under elevated CO_2 (Figure 2). By contrast, MFM at the community level, AM fungal mycelium, MFM from experiments of >1 year, and MFM estimated from biomarker were not altered by elevated CO_2 (Figure 2).

3.2. Effects of N Addition on Mycorrhizal Fungal Mycelium

Our meta-analysis showed that N addition (Q_t = 146.20, $p < 0.001$) significantly decreased MFM. However, we found that CO_2-N combination significantly increased MFM (Q_t = 10.72, p = 0.06) (Figure 1). The negative responses of MFM to N addition were significantly affected by sampling method (ingrowth mesh bag vs. soil core), mycorrhizal type (EM vs. AM), incubation time of ingrowth mesh bag (<1 year vs. >1 year), and mycelium estimation method (biomarker vs. non-biomarker) (Figure 3). Specifically, MFM from ingrowth mesh bag, EM fungal mycelium, MFM from incubation time of ingrowth mesh bag <1 year, and MFM estimated from biomarkers significantly decreased under N addition (Figure 3). In comparison, MFM from soil core, AM fungal mycelium, MFM from incubation time of ingrowth mesh bag >1 year, and MFM estimated from non-biomarker were unchanged under N addition (Figure 3). Furthermore, we found that the effect sizes of N addition on MFM increased with mean annual precipitation (Q_m = 8.98, $p < 0.01$) but it decreased with soil pH (Q_m = 3.85, $p < 0.05$) and host plant age (Q_m = 5.09, $p < 0.05$; Figure 4a–c). The effect size of N addition on MFM was not correlated with latitude of experimental site location (Q_m = 2.40, p = 0.12), mean annual temperature (Q_m = 3.72, p = 0.05), soil carbon/nitrogen ratio (Q_m = 0.23, p = 0.63), N addition level (Q_m = 3.84, p = 0.05), and sampling depth (Q_m = 0.00, p = 0.96).

3.3. Effects of P Addition on Mycorrhizal Fungal Mycelium

The mixed effects meta-analyses showed that MFM significantly increased in the P addition condition (Q_t = 118.70, $p < 0.001$) (Figure 1). The N-P combination (Q_t = 157.79, $p < 0.001$), and the P-K combination (Q_t = 5.07, p = 0.65) had no effects on MFM (Figure 1), but the effect sizes of P addition on MFM significantly increased with N content in host plant leaves (Q_m = 4.73, $p < 0.05$; Figure 4d). Moreover, the N-P-K combination (Q_t = 182.89, $p < 0.001$) significantly decreased MFM (Figure 1). Mycorrhizal type (Q_m = 27.25, $p < 0.001$) significantly affected the positive responses of MFM to P addition, with increases for EM fungal mycelium (RR: 0.43; 95% CI: 0.17–0.68) and for AM fungal mycelium (RR = 0.35; 95% CI: 0.14–0.55). Moreover, the response of MFM to P addition was not influenced by host plant age (Q_m = 1.02, p = 0.31), latitude of experimental site location (Q_m = 23.26, p = 0.06), sampling depth (Q_m = 0.07, p = 0.79), sampling method (Q_m = 0.23, p = 0.63), mean annual precipitation (Q_m = 0.20, p = 0.66), mean annual temperature (Q_m = 0.25, p = 0.62), P concentration in host plant leaves (Q_m = 0.32, p = 0.57), soil carbon/nitrogen ratio (Q_m = 0.00, p = 0.96), and soil pH (Q_m = 0.23, p = 0.64).

3.4. Effects of Other Environmental Factors on Mycorrhizal Fungal Mycelium

The results from the hierarchical mixed effects meta-analyses showed that MFM significantly increased under organic matter addition (Q_t = 1140.49, $p < 0.001$). In contrast, warming (Q_t = 118.94, $p < 0.001$) and K addition (Q_t = 16.61, p = 0.08) had no effect on MFM (Figure 1). Unfortunately, however, there was not sufficient information available to determine the factors that influence the responses of MFM to organic matter addition, warming, and K addition (Figure 1).

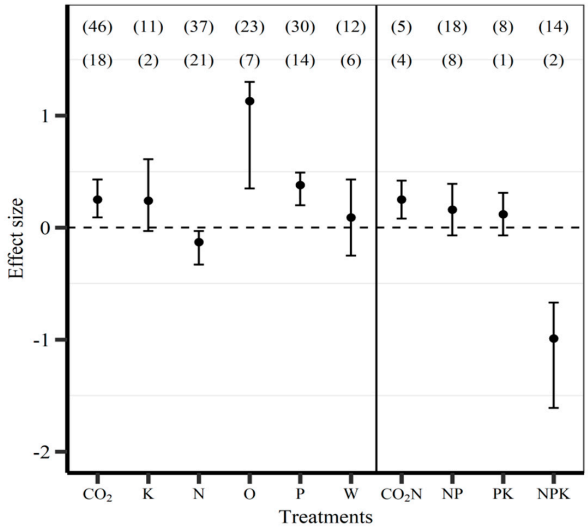

Figure 1. The mean effect size of different treatments on mycorrhizal fungal mycelium (MFM). Error bars represent 95% confidence intervals. The dashed line is drawn at a mean effect size = 0. The effect of treatments is considered significant if the 95% confidence intervals of the effect size do not cover zero. Numbers given on the top indicate the numbers of cases (above) and studies (below) for each treatment. CO_2, elevated CO_2; K, potassium addition; N, nitrogen addition; O, organic matter addition; P, phosphorus addition; W, warming; CO_2N, CO_2-N combination; NP, N-P combination; PK, P-K combination; NPK, N-P-K combination.

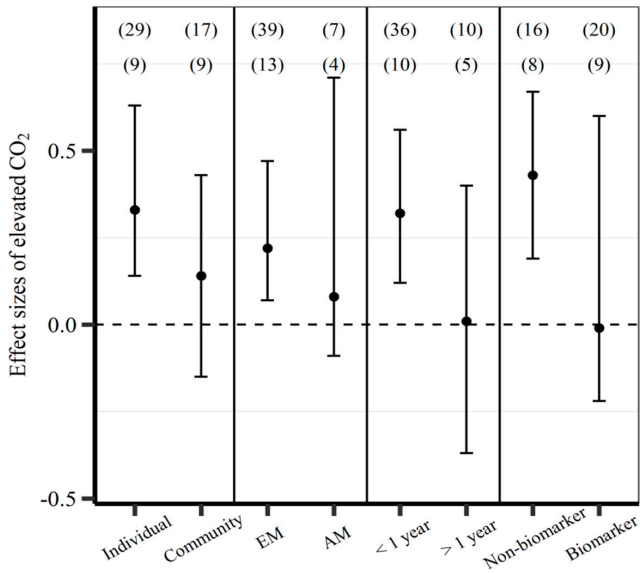

Figure 2. Mean effect size of elevated CO_2 on mycorrhizal fungal mycelium (MFM), categorized into different groups according to mycorrhizal fungal level (individual vs. community), mycorrhizal type (ecomycorrhizal fungi, EM vs. arbuscular mycorrhizal fungi, AM), duration time of elevated CO_2 treatment (<1 year vs. >1 year), and MFM estimation method (biomarkers vs. non-biomarker). The dashed line is drawn at mean effect size = 0. Error bars represent 95% confidence intervals. Numbers given on the top represent the numbers of cases (above) and studies (below).

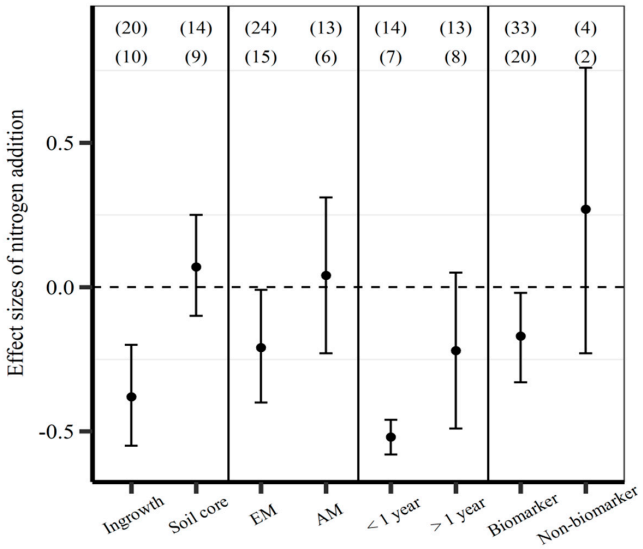

Figure 3. Mean effect size of nitrogen addition on mycorrhizal fungal mycelium (MFM), categorized into different groups according to sampling method (ingrowth vs. soil core), mycorrhizal type (EM vs. AM), incubation time of ingrowth method (<1 year vs. >1 year) and MFM estimation method (biomarkers vs. non-biomarker). The dashed line is drawn at mean effect size = 0. Error bars represent 95% confidence intervals. Numbers given on the top represent the numbers of cases (above) and studies (below).

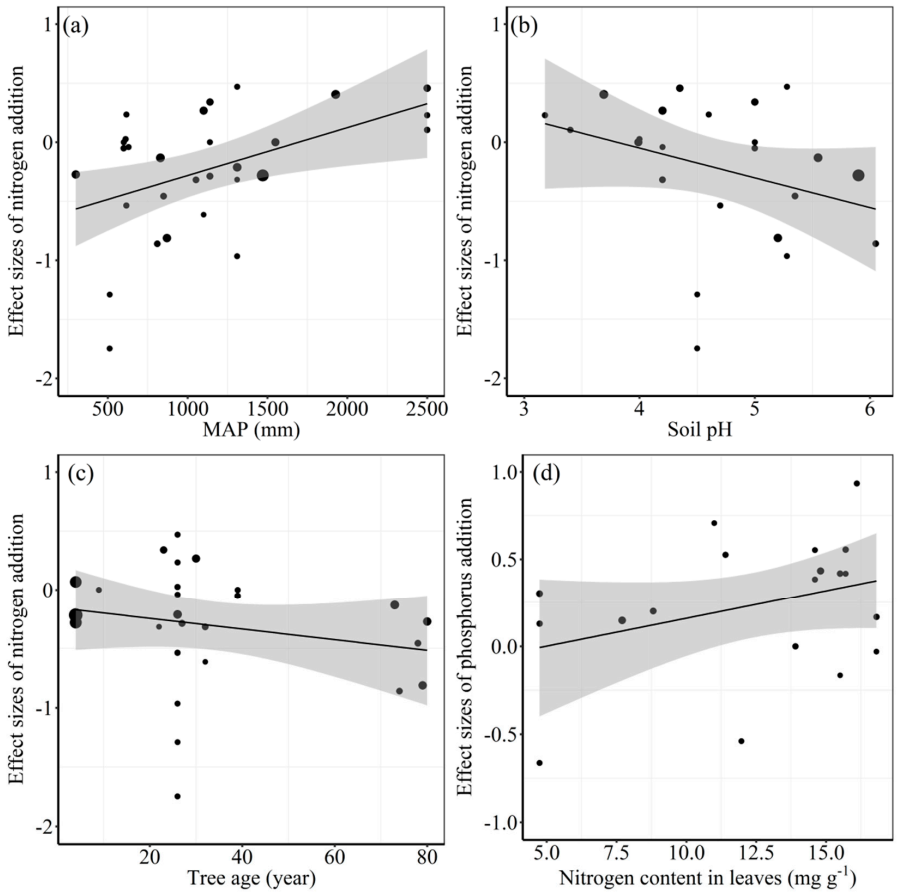

Figure 4. Relationships between effect size of nitrogen addition on mycorrhizal fungal mycelium (MFM) and mean annual precipitation (MAP, **a**), soil pH (**b**) and host plant age (**c**), and relationships between effect size of phosphorus addition on MFM and leaf nitrogen concentration in host plants (**d**). Grey bands indicate 95% confidence intervals. Sizes of dots indicate weights of observations.

4. Discussion

Our present hierarchical mixed effects meta-analyses showed that MFM significantly increased under elevated CO_2, organic matter addition, P addition, and CO_2-N combination. However, N addition and the N-P-K combination significantly decreased MFM. In contrast, warming, K addition, the N-P combination, and the P-K combination had no effects on MFM. Mycorrhizal types (AM vs. EM), treatment time (<1 year vs. >1 year), and sampling/estimation method (ingrowth mesh bag vs. soil core/biomarker vs. non-biomarker) significantly influenced the responses of MFM to elevated CO_2 and N addition. The effect size of N addition was significantly positively correlated with mean annual precipitation, but negatively with soil pH and host tree age. The effect size of P addition significantly increased with N content in host plant leaves.

4.1. The Responses of MFM to Elevated CO_2

We observed an increase in MFM under elevated CO_2, which is in agreement with our hypothesis that CO_2 had a significantly positive impact on the growth of mycorrhizal fungi. It is widely reported that elevated CO_2 proportionally increases carbon allocation to tree roots [21,34,49], and a great deal of which will be ultimately transferred to the mycorrhizal fungi to form mycelium [8,21]. However, this positive effect appeared at the individual level of mycorrhizal fungi, but disappeared at the community level. This difference may result from intrinsic properties of the two mycorrhizal fungal levels such as differences in growth rate and types of mycelia and complex interactions among different mycorrhizal fungi [16]. The constantly luxurious supply of carbon delivered by the host plant under elevated CO_2 causes a shift in mycorrhizal fungus species composition towards those with high metabolic activities and copious mycelium [50,51]. These shifts of mycorrhizal community structure and contrasting responses of different mycorrhizal fungal species will likely affect responses of MFM at community level to elevated CO_2 [52], because some mycorrhizal fungal species are not sensitive to elevated CO_2 [16,40,51]. Moreover, the positive responses of MFM to elevated CO_2 found during the first treatment year was not observed in the treatment duration group of >1 year, suggesting that the effect reduced with the prolonged experimental duration. It is considered that long-term exposure to elevated CO_2 generally reduce the initial stimulation of photosynthesis in many plant species and even frequently suppresses photosynthesis [35]. Previous studies suggest that long-term elevated CO_2 exposure leads to nutrient limitation such as N limitation [53]. Therefore, the positive growth response of MFM to elevated CO_2 may be truncated by soil nitrogen limitations.

Our meta-analysis highlights that different mycorrhizal types (AM vs. EM) exhibited the contrasting responses of MFM to elevated CO_2 and N addition. There is good evidence for greatly fundamental discrepancies between EM and AM symbioses in nutrient acquisition strategies and carbon storage properties [2]. It has been also observed that the growth patterns of EM symbioses differed from those of AM symbioses under elevated CO_2 [35]. Moreover, EM plants can allocate more carbon to their fungal partner than AM plants [54,55]. Accordingly, EM symbioses contribute more to mycorrhizal fungal growth, while AM symbioses are more favorable to plant growth under elevated CO_2 [35]. EM fungi are generally considered to be more sensitive to environmental factors than AM fungi [56,57], which is proven by our results of the significantly positive effects of elevated CO_2 and negative effects of N addition on MFM of EM. Here, we provide additional evidence that the relative dominance of EM or AM trees in a forest may partly determine the response pathways of carbon and N cycling to various global change factors. In contrast, we found the consistently positive effects of P addition on MFM of EM and AM. These results perhaps suggest the similar P utilization model, but contrasting N utilization models, between EM and AM fungi. However, our interpretation of these results is inevitably constrained by the fact that there are very few studies on AM fungi. Our analysis only included four and six studies on MFM of AM associated with woody plants under elevated CO_2 and N addition, respectively. Clearly, more studies on responses of AM fungal mycelium to global change are required to illuminate whether this difference is caused by research bias or does reflect

some underlying fundamental variations in either the biology of the mycorrhizal fungi or the host plants or the combination of both.

4.2. The Responses of MFM to N Addition

The significant reduction of MFM after N addition observed may be attributed to a greater carbon demand for aboveground plant growth, leading to relatively less carbon being allocated belowground to support nutrient assimilation [30,31]. A meta-analysis covering 31 studies notes that mycorrhizal fungal colonization of fine root tips decreases by 15% under N fertilization [41]. Plants adapt to elevated N input by weakening the associations with mycorrhizal fungi, suggesting that a higher proportion of N is taken up directly by plant roots [18]. Moreover, more carbon allocated to mycorrhizal fungi will be used for energy purposes and as carbon skeletons for amino acid assimilation in elevated N availability, consequently, less carbon will be available for mycorrhizal fungal growth [58]. It is well documented that a significant decline in ectomycorrhizal sporocarp production happens in forests subjected to elevated N deposition [6,7]. Additionally, the acidifying effect of N addition has also been found to inhibit mycorrhizal fungal activity, and thus negatively affecting mycelia growth [59]. Meanwhile, we observed significantly negative relationship between the effect size of N addition on MFM and soil pH, indicating greater suppression of mycorrhizal fungi growth in lower pH soils. Acidity stress is likely to favor mycorrhizal fungus species with slow mycelia growth [60]. In addition to soil characteristics, climate has been considered as an important driver of the intimacy of the plant-fungi relationship at global scale [22]. Our results showed that the effect size of N addition on MFM was associated with mean annual precipitation. Perhaps enhanced N leaching/export induced by higher precipitation can relieve the inhibiting effects of N addition on the MFM [61]. Moreover, it is found that the greater precipitation has a positive effect on EM fungal production [6], while drought will prevent the growth of MFM [62]. The strong and contrasting influences of N and water availabilities on mycorrhizal fungi have crucial implications for carbon and nutrient cycling dynamics in forest ecosystems [6]. Hence, the influences of changes in precipitation on the responses of mycorrhizal fungi to increasing N deposition warrants further research.

We found opposite effects of elevated CO_2 and N addition on MFM. This indicates that elevated CO_2 and increased N input have opposite effects on the relative carbon allocation of plants to belowground parts. Thus, mycorrhizas in forest ecosystems are exposed to a paradoxical situation under multiple factor changes. Indeed, global change is not a single-factor phenomenon, so the strong and contrasting effects of multiple global change factors (i.e., elevated CO_2, N deposition and changed precipitation) on MFM have important implications for carbon flow and nutrient cycling dynamics in forest ecosystems [34]. For instance, multiple soil nutrients combine to regulate fine roots and thus the association with mycorrhizal fungi [63].

Tree-age-related variation in belowground carbon allocation is vital for comprehensive understanding of complex carbon cycling during stand development [4,64]. It is evident that effect size of N addition on MFM is negatively correlated with the host plant age. Higher rates of mycorrhizal mycelia production are found in the younger stands along an age gradient of *Pinus sylvestris* stands [3]. Presumably, forest stands with different ages have different nutrient and water requirements, correspondingly, mycorrhizal fungi will vary with the age of the forest stands [65]. Stand productivity of forest ecosystems decreases at mature ages, consequently resulting in reduction of the demand for nutrient and water supply from fine roots and their associated mycorrhizal fungi [66]. Mycelia carbon use efficiency deceases significantly with increasing forest age by about 65% [4]. Thus, biomass allocation to belowground parts decreases with increasing stand age [67]. In mature forest ecosystems, elevated N input plays an additional effect on the lower carbon supply for mycorrhizal fungus growth.

Our results suggest that the incubation time of ingrowth mesh bag is an important aspect influencing the response of MFM to N addition. It is noted that a lag time, indeed, existed before mycorrhizal fungi entered ingrowth mesh bags following the insertion of bags [17]. Short-term

experiments with ingrowth mesh bags favor fast-growers and early colonizers, which first colonize the mesh bags by many fast-growing mycelia [68], while some mycorrhizal fungi specialized on nutrient utilizing may need more time to colonize the mesh bags [69]. Additionally, priority effects favor persistence of early colonizers successfully, and prevent establishment of other mycorrhizal fungus species [70]. Thus, the length of the incubation duration largely influences the composition of mycorrhizal fungi colonized into the mesh bags. Moreover, turnover rate also influences the estimates of MFM in the ingrowth mesh bags [3,71]. Therefore, mycorrhizal fungal community colonizing ingrowth mesh bags is likely to be very different from that in natural soils using soil core methods. Therefore, these differing responses of MFM to N addition from ingrowth mesh bag method vs. soil core method may be due to that some species or clades are overrepresented and some others are underrepresented or even missing in the mesh bags [17]. In addition, each of the biomarkers will bring different information about the mycorrhizal fungi. For instance, chitin and ergosterol reflect the total and the living fungal biomass in ectomycorrhizas, respectively [17,72]. Other estimation methods of MFM (e.g., agar film) cannot determine the saprotroph hyphae [40]. Accordingly, a main issue raised is how to quantify mycorrhizal hyphae. Thus, methodological effects on responses of mycorrhizal fungal mycelium to global change factors are related with mycorrhizal fungal community assemblages [73]. Given the sets of limitations of the applied methods and techniques, the combination of several techniques in the same study is a feasible way to overcome some of the limitations [17].

4.3. The Responses of MFM to P Addition, Organic Matter Addition, and Warming

We observed that P addition significantly increased the production of MFM, suggesting a further extension of the finest part of the fine root system [74]. The positive effects of P addition found in this analysis seem to be quite surprising, which were not expected by our hypotheses. Several mechanisms have been proposed to elucidate the P effects on mycorrhizal fungi. The increased soil carbon availability through increased leaf litterfall and fine root biomass caused by relieved P constraints is considered to be one reason for the positive effects of P addition on MFM [75,76]. The high P input improves soil pH and osmotic potential, which may also promote the mycorrhizal fungal growth [38]. Furthermore, plants use mycorrhizal fungi for nutrient acquisition, and improved nutrient status in the host plant is also considered as a primary factor influencing carbon allocation to MFM production [77]. Here, we found the positive effects of P addition significantly increased with increasing N content in host plant leaves. This result indicates the P gradually becomes a limiting factor for the growth of MFM with increasing N availability (higher N content in tree leaves). P addition alleviates this limitation and increases carbon input to the mycorrhizal fungi [75]. Given the tremendous discrepancy in climate, vegetation, soil types, and also P availability from tropical to boreal forest biomes in our analysis, more studies at the global scale are needed to conclude the effects of P on mycorrhizal fungi and their mycelium production. The enhanced MFM by organic matter addition is attributed to the beneficial effects of increased organic matter on water status, soil structure, CO_2 pressure, and synergistic microbial activity in the soil [78]. The higher organic matter content can improve the water-holding capacity of forest soils, leading to an increase in the mycelium growth of mycorrhizal fungi [29]. The higher organic matter content can improve soil physical properties, with increasing soil aeration and decreasing mechanical resistance to the growth of MFM [10,26]. Mineralization of organic matter results in higher concentrations of CO_2 [10], and as discussed above, elevated CO_2 concentrations can enhance mycelium growth of mycorrhizal fungi. Increased microbial enzyme activities after organic matter addition indicate a specialization of the microbial communities in favor of mycorrhizal fungi [79]. The EM fungi, in turn, invest large amounts of carbon to produce a range of hydrolytic and oxidative enzymes that break down carbon-containing compounds and mobilize nutrients from soil organic matter [80].

Unexpectedly, we failed to detect a warming effect on MFM, which does not support our hypothesis that MFM responds positively to increased temperature. This result challenges the conventional view that climate warming will advance in the spring and delay in the autumn (and thus longer

growing season length), which will have beneficial effects on mycorrhizal fungi [23,39,81]. In fact, both positive and negative effects of warming on the growth of mycorrhizal fungi have been reported [23,82]. The decrease in soil moisture with soil warming can probably limit mycorrhizal fungus activity, specifically in the dry season and dry areas [82]. Besides, mycorrhizal fungal community composition exhibits highly variable responses to elevated temperatures [83]. Consequently, we still have little information on the mechanisms of these different responses of MFM to climate warming, suggesting that much more attention should be paid to temperature effects on the mycorrhizal fungi in future studies.

5. Conclusions

Our meta-analysis demonstrates that the responses of MFM to different environmental factors depend on mycorrhizal type, mycorrhizal organization level, and experimental setting, such as duration time of treatment and sampling method. This suggests that forest nutrient and carbon cycling models should take MFM responses into account. In this meta-analysis, we also found that many other biotic and abiotic variables were not associated with effect size of environmental factors on MFM. The lack of statistically significant result does not definitely indicate that these factors do not have effects on mycorrhizal fungi, given the small number of observations or studies. Therefore, further research about the complex carbon allocation patterns between host plants and their associated mycorrhizal fungi, and a changed assemblage of mycorrhizal fungal taxa in the conditions of various global change factors covariation are urgently needed.

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Appendix A The List of the Data Sources

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