Arbuscular mycorrhizal diversity sustains plant performance under multiple global changes with varying impacts on soil functions

Xuan Jiang[1], Xinyu Li[1], Jialing Xu[1], Cameron Wagg[2], Na Liu[1], Lianxuan Shi[1], Yingzhi Gao[1], Jixun Guo[1], and Tao Zhang[1]

[1]Northeast Normal University
[2]Fredericton Research and Development Centre

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Abstract

High biodiversity can mitigate the negative impacts of global change factors (GCFs) on differing ecosystem functions captured in multifunctionality. However, multiple GCFs occurring simultaneously may diminish the positive effects of high biodiversity on multifunctionality. Arbuscular mycorrhizal fungi (AMF) play a crucial role in determining ecosystem functionality, yet it remains unclear whether AMF alleviate the negative impact of multiple GCFs on ecosystem multifunctionality. In this study, we conducted a microcosm experiment to explore the role of AMF in maintaining ecosystem multifunctionality under multiple GCFs. We found that ecosystem multifunctionality under high AMF diversity was greater than under low AMF diversity under multiple GCFs. Notably, AMF diversity significantly improved plant growth simultaneously. While AMF inoculation significantly influenced soil functions, the diversity of AMF showed limited effect on soil functions. Our findings emphasize the importance of conserving AMF diversity to maintain ecosystem functionality.

INTRODUCTION

Ecosystem multifunctionality, which encompasses multiple aspects of efficient above-belowground carbon and nutrient cycling processes, is crucial for delivering essential services such as food production and climate regulation (Garland et al., 2021; Manning et al., 2018). However, global change factors (GCFs) negatively affect ecosystem multifunctionality in various ways. For instance, drought decreases ecosystem functionality by restricting enzyme activities that are vital for soil nutrient cycling (Lozano et al., 2021). Furthermore, the effects of fertilization on multifunctionality vary depending on the specific nutrient limitations within an ecosystem (Chen et al., 2020; Li et al., 2023a; Ma et al., 2021). While many studies have focused on the influence of single or dual GCFs on ecosystem multifunctionality, it's critical to recognize that multiple GCFs typically occur simultaneously in real-world scenarios (Rillig et al., 2021; Zandalinas and Mittler, 2022). For example, in agricultural ecosystems, besides warming and drought, anthropogenic activity formed stressors like overuse of mineral fertilizers and pesticides are also present (Seppelt et al., 2022). Recent studies have shown that the simultaneous occurrence of multiple GCFs result in a more pronounced negative impact on both biodiversity and ecosystem multifunctionality (Rillig et al., 2019; Speißer et al., 2022).

Biodiversity-ecosystem function (BEF) studies have demonstrated that biodiversity across multiple trophic levels, including both aboveground and belowground components, is crucial for maintaining ecosystem functions (Balvanera et al., 2006; Bardgett and van der Putten, 2014; Gamfeldt and Roger, 2017; Soliveres et al., 2016). It was believed that increased diversity might have positive effects on ecosystem multifunctionality even under multiple GCFs (Benkwitt et al., 2020). This may be because that with an increasing number

of species there is a greater insurance that some species may be unaffected by particular GCF, or particularly efficient at supporting an ecosystem function under a GCF perturbation, such that the functioning of the community is maintained (Loreau and Hector, 2001). Greater species diversity may enhance ecosystem function through complementary, allowing different species to support various functions under different conditions, thus maintaining ecosystem multifunctionality (Isbell et al., 2011, Wagg et al., 2021). Such a division of labor among species can result in a 'transgressive overyielding' or 'complementarity' effect where the more diverse community results in a greater effect than the best performing individual species (Loreau and Hector, 2001; Xu et al., 2024). This means that the response of an ecosystem function when faced with GCFs could be due to either the diversity of species or the presence of a particularly important species for that function.

Recent findings suggest that the negative impacts of multiple GCFs on ecosystem multifunctionality are exacerbated in communities with high plant diversity (Xu et al., 2024). This effect is attributed to the increased abundance of soil fungal pathogens under multiple GCFs thereby eliminating the positive effects of soil biodiversity on ecosystem functions. It was indicated that an increasing number of GCFs may reduce the beneficial effects of high soil microbial diversity on ecosystem multifunctionality by decreasing fungal abundance and altering fungal community composition (Yang et al., 2022). These results suggest that soil fungi play a crucial role in determining ecosystem multifunctionality under multiple GCFs. However, it is unclear whether soil fungi can alleviate the negative impacts and regulatory mechanisms of multiple GCFs on ecosystem multifunctionality.

Arbuscular mycorrhizal fungi (AMF), one of the most important components of soil fungi, can form mycorrhizal symbiosis with 72% of flowering plants (Brundrett and Tedersoo, 2018), serving as a crucial link between plants and soil. Specifically, AMF have been found to significantly improve plant performance under various stresses, including salt, drought, warming, nitrogen deposition, and elevated CO_2levels (Zhang et al., 2018; Tang et al., 2023). Previous studies have demonstrated the benefits of AMF for various ecosystem functions. For instance, AMF can reduce soil N_2O emissions and partly mitigate global warming potential (Bender et al., 2014; Cui et al., 2021). AMF can also affect ecosystem functions like primary productivity, nutrient cycling, soil carbon sequestration, and soil pathogen defense (Powell and Rillig, 2018; Wang and Rengel, 2023). Additionally, the diversity of AMF plays a vital role in determining ecosystem stability and multifunctionality (van der Heijden et al., 1998; Ma et al., 2021). Higher AMF diversity leads to faster nutrient cycling and transport, which in turn improves soil health and plant growth (Zhang et al., 2024).

However, it remains unclear whether high AMF diversity could continue to benefit ecosystem multifunctionality or not under multiple GCFs. High AMF diversity could enhance ecosystem resistance and stability to GCFs (Jia et al., 2021; Yang et al., 2016, 2014), and thus the high AMF diversity treatment may exhibit a flatter slope compared to lower diversity (Figure 1a). On the other hand, functionally redundant species could become important in a changing environment (Fetzer et al., 2015). This may lead to a opposite performance of low and high diversity treatments (Figure 1b). Previous studies have also found no significant correlation between soil biodiversity and ecosystem function (Wang et al., 2022), or between AMF diversity and ecosystem function (Jing et al., 2015). In this case, the effect of GCF number could be the same for high or low diversity treatment (Figure 1c), or no effect (Figure 1d).

In this study, we conducted a microcosm experiment to address the following two questions: (1) does greater AM fungal diversity exhibit greater multifunctionality under multiple GCFs? (2) Which ecosystem functions can AM fungal diversity support under various GCFs? We grew *Triticum aestivum* (wheat) in a controlled glasshouse experiment. Three AMF diversity treatments based on species richness: 0 (no-AMF), 1 (low diversity), and 4 (high diversity), and three GCF treatments: no GCF control, one of six GCF, and a combination of all six GCF were included. Each treatment combination allowed us to explore the interactive effects of AMF diversity and GCFs on ecosystem functions. We measured ten functions, including net photosynthetic rate, primary productivity, soil nutrient content, soil enzyme activity, and soil greenhouse gas emission to calculate multifunctionality (Garland et al., 2021), as we focused on short-term plant and soil functions. We hypothesized that the negative effect of multiple GCFs on multifunctionality would be lower under high

AMF diversity treatment (Figure 1a).

MATERIALS AND METHODS

Experimental design

The experiment was conducted in a glasshouse at the Northeast Institute of Geography and Agroecology of the Chinese Academy of Sciences (125°24'30"E, 43°59'49"N), from June to August 2023. The glasshouse maintained a controlled temperature range of 24 to 30°C, with natural light conditions. The soil used was collected from the Jilin Songnen grassland ecosystem national observation and research station, Northeast Normal University, China (123°37'2"E, 44°34'47"N). The soil was sterilized using 25 kGy of $^{60}Co_{\gamma}$irradiation at the CNNC Tongfu Radiation Technology Co., Ltd (Changchun, China). PVC pots (internal diameter 10 cm, depth 20cm) were used for the experiment. The experiment employed a fully factorial design with eight GCF treatments and six AM fungal treatments with five replicates of each combination for a total of 240 pots.

The six AM fungal treatments were: no AMF inoculation (control), inoculation one (low diversity) of the AMF species: *Gigaspora rosea* (Nicolson and Schenck, 1979), *Glomus etunicatum* (Becker and Gerdemann, 1977), *Rhizophagus irregularis* (Tisserant et al., 2013, formerly named *Glomus intraradices*), and*Funneliformis mosseae* (Nicolson and Gerdemann, 1968). These AM fungi are common in the farming–pastoral ecotone of northern China (Xiang et al., 2014). The high AMF diversity treatment was a mix of all four of the above AMF fungi. This totals six AM fungal treatments: no-AMF control, four single AMF species, and one mixture of all four AMF species.

AM fungal inoculants were prepared by culturing the AMF species on the host plant *Trifolium repens* for 4 months. Inoculation was done by first filling the pots with sterilized soil three quarters full and then added 20g of AM fungi inoculum (about 100 spores) to each tube. The mixed-inoculum treatment was created by mixing each of the four AM inoculants together in equal mass (5g) to total the 20g. A 3 cm layer of sterile soil was placed on top to avoid cross contamination and ensure the soil weight of all pots were consistent (all contained 2 kg of substrate).

Six seeds of *Triticum aestivum* (germination rate: 98%) were evenly planted in each pot. Seeds were sterilized with 1.25% sodium hypochlorite for 10 minutes and thoroughly washed with distilled water (dH_2O). All seeds successfully germinated and survived throughout the duration of the experiment. Plants were watered three times a week to maintain soil water content at 60% of soil water holding capacity (WHC). Pots were randomly rearranged every two weeks to minimize spatial variability. Each tube received a single application of 20 ml of Hoagland nutrient solution 50 days after seed germination. After 80 days, plant and soil samples were harvested.

The eight GCF treatments included: 1) a control, 2) nitrogen addition (NH_4NO_3, 10 g N $m^{-2}year^{-1}$), 3) antibiotic pollution (ciprofloxacin, 100 μg kg^{-1} soil), 4) microplastic pollution (4 g polyethylene kg^{-1} soil), 5) pesticide pollution (triadimefon wettable powder, 50 g ha^{-1}), 6) soil saline-alkali (NaCl: Na_2SO_4: $NaHCO_3$: Na_2CO_3 = 4:1:6:4, pH 8.80 ± 0.05), 7) drought (30% of WHC) and 8) a treatment of all six GCFs together. The global change treatments 3 – 6 were applied once to the soil just prior to planting. For the nitrogen addition treatment, we added NH_4NO_3 twice during the experiment on the 20^{th} and 40^{th}day after sowing separately. The drought was imposed throughout the experiment by maintaining a 30% WHC, with other treatments maintaining 60% WHC. The details on global changes treatment can be found in the supporting information.

Ecosystem functions

At the time of harvest (80 days after planting) a total of 10 responses were measured that reflect plant and soil functioning of efficient nutrient cycling and enhanced plant performance: four soil enzyme activities 1) cellobiohydrolase (CBH), 2) β-1,4-glucosidase (βG), 3) β-1,4-N-acetylglucosaminidase (NAG), and 4) alkaline phosphatase (ALP). CBH and βG are related to soil carbon that contribute to the degradation of cellulose (Ljungdahl and Eriksson, 1985). NAG contributes to the degradation of chitin and plays a role in soil

nitrogen cycling (Sinsabaugh et al., 2008). ALP can release soil phosphate, and related to AMF phosphorus uptake and transport (Larsen et al., 1996; Turner et al., 2002). Two greenhouse gases emission, 5) CH_4 and 6) N_2O that are two potent long-lived greenhouse gases (Mar et al., 2022; Thompson et al., 2019). We included four soil and plant responses indicating plant nutrient use efficiency which were 7) soil NO_3^--N and 8) soil NH_4^+-N contents, 9) net photosynthetic rate, which represents the carbon fixation capacity of the ecosystem and is the basis of primary productivity and 10) above-ground biomass, an important indicator to reflect primary production (Garland et al., 2021). In our study, higher emissions of N_2O and CH_4 from the soil were considered negative because they indicate a greater greenhouse effect and more soil nutrient loss. Similarly, higher residual plant-available soil NO3–N and NH4+-N were considered dysfunctional, as they indicate less efficient nitrogen use. Therefore, these four values were inverted by 1-x. We calculated ecosystem multifunctionality using the average method (Maestre et al., 2012; Xu et al., 2024). This was done by the '*getStdAndMeanFunctions* ' function in R package 'multifunc' (Byrnes et al., 2014).

Arbuscular mycorrhizal fungi indicators

The AMF spores were isolated through the process of wet sieving and sucrose centrifugation method (McKenney and Lindsey, 1987). The extraradical hyphae were extracted and stained with trypan blue (Jakobsen et al., 1992), and hyphal length density was quantified using a microscope with a gridded reticule at 250x magnification (Zhang et al., 2016). The root samples were cleared with 10% (w/v) KOH at 90 °C for 30 min then stained with trypan blue (Phillips and Hayman, 1970). And the degree of AMF colonization was estimated according to the method used a previous study (Mei et al., 2019).

To quantify the effect of GCF on AMF, we calculated the response index:

$$Response\ index = \ln\left(X_{\text{with global change factor}}\right) - ln(X_{\text{without global change factor}})$$

Where X was AM fungal root colonization, soil hyphal length density, and spore density. The response index of AM fungal indicators under each GCF treatment was calculated separately. If the index > 0, the treatment has a positive effect on AM fungi. On the contrary, if the response index < 0, the GCF has a negative effect on AM fungi (Xu et al., 2024).

Statistical analysis

All statistical analyses were conducted in R, version 4.3.3 (R Development Core Team, 2024). A two-way ANOVA was performed to examine the effects of AMF diversity (low vs high), GCF number (0, 1, and 6), and their interactions on ecosystem multifunctionality and the ten previously mentioned ecosystem functions. Additionally, a one-way ANOVA was used to assess the effect of AMF inoculation (absence vs. presence) on these indicators. Statistical significance was determined at $p < 0.05$, with Tukey's test employed for post hoc comparisons. Pearson correlation analysis was conducted to investigate the relationships between soil pH and root colonization, soil pH and hyphal length density, soil pH and spore density, respectively.

RESULTS

Effect of AMF diversity on ecosystem multifunctionality

Ecosystem multifunctionality under high AMF diversity was significantly greater than that under low AMF diversity ($p < 0.001$, Table 1, Figure 2), no matter under 0, 1, or 6 GCFs. Under the N addition treatment, ecosystem multifunctionality was highest in the absence of AMF, with no significant differences between low and high AMF diversity treatments. Under pesticide and saline-alkali treatments, no significant differences were observed ecosystem multifunctionality among the three AMF treatments. Under the control, microplastic, drought, and multiple GCFs treatments, ecosystem multifunctionality in high AMF diversity treatment was much higher than that in low AMF diversity treatment. The number of GCFs had a significant effect on ecosystem multifunctionality ($p < 0.001$, Table 1, Figure 2). Multiple GCFs significantly affected ecosystem multifunctionality under low AMF diversity, reducing it by 6.88% compared to a single GCF, while no AMF addition resulted in a 4.23% reduction. The smallest impact occurred with high AMF diversity, showing a

3.31% reduction compared to a single GCF. AMF diversity and the number of GCFs did not interactively affect ecosystem multifunctionality.

AMF contribution

Compared to no AMF inoculation, AMF inoculation significantly increased plant net photosynthetic rate ($p < 0.001$, Table 1), and significant differences in plant net photosynthetic rates among the different AMF diversity treatments were observed ($p < 0.001$, Table 1, Figure 3a). The net photosynthetic rate under high AMF diversity was consistently higher than that under low AMF diversity, except under microplastic and saline-alkali conditions, where the differences were not significant. Under control, antibiotic, pesticide, drought, and multiple GCFs, the net photosynthetic rate with high AMF diversity was higher than that with any single AMF species. Compared to single GCF treatments, multiple GCFs significantly reduced the net photosynthetic rate of plants inoculated with single AMF species ($p < 0.001$) but had no impact under mixed AMF or no AMF inoculation treatments. AMF inoculation also significantly increased aboveground biomass compared to no AMF inoculation ($p < 0.001$, Table 1), and significant main effect of AMF diversity on aboveground biomass was detected ($p < 0.001$, Table 1, Figure 3b). Within the same GCF treatment, aboveground biomass under high AMF diversity was much higher than low AMF diversity and no AMF inoculation except under microplastic and drought treatments. GCF numbers significantly affected aboveground biomass ($p < 0.001$, Table 1), multiple GCFs significantly reduced aboveground biomass compared to single GCF treatments.

The effect of AMF diversity on soil functions was not always significant. Significant differences in soil N_2O emissions between AMF inoculated and non-inoculated treatments were observed ($p < 0.01$, Table 1). Under multiple GCFs, low AMF diversity treatment increased the emissions of N_2O, but high AMF diversity did not affect it. A significant main effect of the number of GCFs on N_2O emissions was detected ($p < 0.001$, Table 1). Under the inoculation of a single AMF, multiple GCFs significantly increased N_2O emissions compared to single GCF and the control ($p < 0.01$, Figure 4a). AMF diversity and the numbers of GCF did not interactively affect CH_4 emissions ($p > 0.05$, Table 1). Both AMF inoculation ($p < 0.01$) and GCF numbers ($p < 0.001$) significantly affected soil NO3–N content (Table 1, Figure 4c), but the effect of AMF diversity was not significant ($p = 0.121$). The effect of GCF numbers on soil NH4+-N content was highly significant ($p < 0.001$, Table 1). When AMF was inoculated, NH4+-N content decreased with increasing GCF numbers (Figure 4d). AMF inoculation significantly affected βG activity ($p < 0.001$, Table 1). AMF inoculation significantly reduced βG activity under control, antibiotic, and pesticide treatments (Figure 4e). The effect of GCF numbers was also significant ($p < 0.05$, Table 1). Soil CBH activity ranged from 6.84 ± 0.90, with lower activity of 2.54 ± 1.37 observed under antibiotic pollution with no AMF inoculation. Both AMF inoculation ($p < 0.001$) and GCF numbers ($p < 0.01$) significantly affected CBH activity, with AMF inoculation significantly increasing CBH activity under antibiotic, microplastic, pesticide pollution, and drought conditions (Figure 4f). No significant difference in CBH activity between low and high AMF diversity was observed. For low AMF diversity, CBH activity under multiple GCFs was significantly higher than under single GCF or no GCF treatments ($p < 0.01$). AMF inoculation showed a significant effect on NAG activity ($p < 0.01$, Table 1). AMF inoculation significantly reduced NAG activity under nitrogen addition but increased it under antibiotic, microplastic and drought conditions compared to no AMF inoculation (Figure 4g). AMF diversity, GCF numbers, and their interaction significantly affected ALP activity (all $p < 0.01$, Table 1). For high AMF diversity, ALP activity under no GCF was significantly higher than that under single and multiple GCF treatments ($p < 0.001$, Figure 4h). For low AMF diversity, ALP activity under multiple GCFs was significantly higher than that under no GCF and single GCF treatments ($p < 0.001$, Figure 4h).

Compared to the effects of a single AMF species inoculation on ecosystem multifunctionality, and particularly plant growth, the results showed that high AMF diversity exhibited notable complementarity effect, where the AMF mixture exhibited a greater performance than any of the single inoculated AMF, under no GCF, multiple GCFs, and some single GCF treatments. However, such advantages and complementarity effects were not evident for soil functions.

Effect of global change factors on AM fungi

Most GCF treatments decreased AM fungal root colonization except inoculation *R. irregularis* under microplastic treatment (Figure S2d). Multiple GCFs treatment caused a 72.38% reduction in mycorrhizal colonization ($p < 0.001$, Table S2, Figure S2a). The soil hyphal length density (HLD) in high AMF diversity treatment was higher than that in low AMF diversity under the same GCF condition, except in case of saline-alkali stress ($p < 0.001$, Table S2, Figure S2b). Most GCF treatments declined HLD, but drought enhanced HLD when inoculated with *G. etunicatum* ($p < 0.001$) and in mixed inoculation scenarios ($p < 0.05$, Figure S2e). A slight increase in HLD was also observed with *R. irregularis* under antibiotic stress and with*G. etunicatum* under microplastic stress (Figure S2e). GCFs treatment significantly affected AMF spore density ($p < 0.001$, Table S2, Figure S2c), while the response of each AMF inoculation differed (Figure S2f). The negative impact of multiple GCFs on spore density in high AM fungal diversity treatment was significantly greater than that in single GCF treatments ($p < 0.001$, Figure S2f).

In our findings, we observed that soil saline-alkali stress exerted a significantly greater negative effect on AM fungi compared to the other five GCFs, except when considering multiple GCFs. Regression analysis showed that soil pH was negatively correlated with plant root mycorrhizal colonization, HLD, and spore density, notably, these correlations exhibited steeper slopes with high AMF diversity treatment than low AMF diversity ($p < 0.05$, Figure S3).

DISCUSSION

Through a two-factor greenhouse-controlled experiment, we demonstrated that high AMF diversity increased ecosystem multifunctionality. When subjected to multiple GCFs, ecosystem multifunctionality under high AMF diversity was significantly higher than that under low AMF diversity and any mono-AMF treatment, indicating complementarity among different AMF species might help maintain ecosystem multifunctionality under the increasing GCF numbers. In addition, the advantages of high AMF diversity in promoting plant photosynthesis and biomass remained consistent across all experimental conditions. This suggests that while ecosystem multifunctionality may be influenced by an interplay of factors, the positive impact of AMF diversity on plant productivity is robust and enduring.

High AMF diversity benefits ecosystem multifunctionality

Our results confirmed that high AMF diversity enhanced ecosystem multifunctionality, aligning with our expectations. Previous studies have already demonstrated the benefits of AMF diversity on ecosystem primary net productivity (Wagg et al., 2011) and nutrient reserve (Köhl and van der Heijden, 2016). In our study, the increase of ecosystem multifunctionality through high AMF diversity was primarily mediated by improved plant growth, particularly in net photosynthetic rates and aboveground biomass (Figure 3). Contrary to our expectations, high AMF diversity had no significant effect on most soil functions, compared to low AMF diversity (Figure 4, Table 1). This may stem from our use of sterilized soil, which was only inoculated with AMF without introducing other fungi or bacteria.

Increasing evidence suggests that the nutrient cycling functions of AMF are not performed in isolation but require collaboration with other soil microorganisms (Zhang et al., 2022; Sun et al., 2023; Wang et al., 2024). For instance, previous results have linked soil functions, such as organic nitrogen availability (Rozmoš et al., 2022) and soil N_2O emissions (Li et al., 2023b) to the hyphosphere core microbiome. In our experiment, the absence of soil microbial communities likely prevented AMF from recruiting distinct microbes into their hyphosphere, thereby diminishing the contribution of AMF diversity to soil functions. A global meta-analysis of field experiments highlighted a positive correlation between ecosystem multifunctionality and AMF richness (Ma et al., 2021). However, this correlation may not be directly due to the AMF alone but rather influenced by the hyphosphere microbiomes.

Thus, we speculate that the high plant productivity in high plant diversity communities may not be solely due to plant diversity itself. A study by Wilsey et al. (2023) found that biodiversity in native plant-dominated communities was significantly positively correlated with net primary productivity. However, this

positive correlation is eliminated in communities dominated by exotic species, which may be due to the presence of more pathogenic microorganisms in exotic plant-dominated communities. This highlights the importance of multiple trophic levels BEF study. Previous results have shown that multi-trophic diversity can better explain and predict ecosystem multifunctionality than single-trophic level diversity (Soliveres et al., 2016; Li et al., 2024). Li et al. (2024) found that different trophic groups exhibit varying correlations with ecosystem functions. For example, in grasslands, AMF diversity showed a significant correlation with provisioning multifunctionality (including plant productivity and community stability), rather than with multifunctionality (Li et al., 2024), which is consistent with our findings.

Multiple global change factors weaken the benefits of AMF diversity

Our results showed that ecosystem multifunctionality decreased in both high and low AMF diversity treatments, under multiple GCFs (Fig. 1a). And the multifunctionality was often greater with the high AMF diversity than the average of the AMF inoculated individually, and greater than the best performing single AMF inoculated under the control, microplastics, drought and multiple GCFs. This indicates that there is complementary among AMF in supporting multifunctionality under some GCF scenarios. Our findings align with previous biodiversity-ecosystem functioning (BEF) studies, which generally suggest that ecosystems with high biodiversity exhibit greater niche differentiation and positive complementarity, thereby enhancing ecosystem multifunctionality and resilience to environmental changes (Craven et al., 2016; Godoy et al., 2020).

However, ecosystem multifunctionality under high AMF diversity was not the highest under nitrogen addition, pesticide pollution, and saline-alkali treatment. Some studies also reported that communities with higher diversity may exhibit lower productivity (Polley et al., 2003) or ecosystem functions (Becker et al., 2012; Jousset et al., 2011), which may be related to competition among species. In this study, under non-GCF conditions, plant root mycorrhizal colonization rate, HLD, and spore density were higher in high AMF diversity treatments compared to any single AMF inoculation treatment (Figure S2a-c). However, under conditions with multiple GCFs, the negative effects of GCFs on mixed AMF treatments were more sever compared to individual AMF like *R. irregularis* and G. *etunicatum* (Figure S2d-f). These observations suggest that the presence of multiple GCFs may intensify the competition among AMF. It has been indicated that the addition of nitrogen and phosphorus can intensify the competition among AMF (Ma et al., 2021; Qin et al., 2022; Tang et al., 2023).

Plant-derived carbon is the primary resource for competition among AMF species. Research has shown that host plants allocate more carbon to AMF species that supply more phosphorus in return (Kiers et al., 2011). Beyond resource competition, species can also release allelochemicals to directly affect competitors. In high-diversity communities, while positive functional complementarity exists, complementarity in allelopathic toxins can lead to a 'negative complementarity effect' (Becker et al., 2012). AMF can also release hyphosphere exudates, while current research primarily focuses on their role in recruiting hyphosphere microbes to explain AMF ecological functions. Studies on AMF allelopathy are still limited.

The diminished effect of AMF diversity on ecosystem multifunctionality under multiple GCFs, might also relate to specific GCF factor. For example, there was no significant difference in ecosystem multifunctionality among different AMF treatments under saline-alkali and pesticide pollution conditions. Plant root mycorrhizal colonization rates and HLD showed the most negative responses to saline-alkali treatment (Figure S2d, e). Davison et al. (2021) analyzed global soil samples and found that AMF virtual taxa are often associated with a particularly narrow range of pH and temperature conditions. Previous studies have investigated the mechanisms through which AMF mitigate plant saline-alkali stress; however, there is limited research on how pH influences the growth of AMF.

AMF diversity provides stable benefits for plant growth

Although the positive effects of high AMF diversity on multifunctionality are no longer significant under multiple GCFs, its contribution to plant aboveground biomass has become more pronounced. Several studies suggest that the benefits of AMF diversity become more pronounced when host plants are subjected to

multiple stressors (Crossay et al., 2019; Gosling et al., 2016; Tang et al., 2023). This aligns with our findings regarding aboveground biomass, where under single GCF conditions, plants in the high AMF diversity treatment exhibited 8.36% greater aboveground biomass than those in the low diversity treatment. Remarkably, this difference increased to 31.60% under multiple GCFs condition (Figure 3b). The differential performance may be related to the selection preferences of host plants in varying environmental contexts. AMF selection is influenced by both the environment and the host plant itself. Under single GCF conditions, plants tend to favor specific AMF taxa that are better equipped to mitigate environmental stress and enhance carbon resources acquisition (Tang et al., 2023; Vályi et al., 2016). Although multiple GCFs intensified competition among AMF, they also strengthened the host plants' selection for AMF species, allowing high AMF diversity still exert a positive effect on plant performance under multiple GCFs.

High AMF diversity consistently enhanced plant net photosynthetic rate and aboveground biomass under all GCF scenarios, exceeding both the average effect of individual AMF inoculations and, in most cases, the effect of the single most effective AMF (Figure 3). This illustrates the complementarity among AMF species when inoculated as a mixture. These findings contrast with previous meta-analysis indicating that multi-species AMF inoculation does not significantly enhance plant performance compared to single-species inoculation under single GCF (Tang et al., 2023). However, it has been shown that multi-family AMF assemblages can boost plant performance indicating that it is not the number of AMF species that enhances plant performance, but rather the phylogenetic diversity within the AMF community (Gosling et al., 2016; Tang et al., 2023). Thus, the functional complementarity among different AMF families could become increasingly crucial when multiple GCFs occur simultaneously (Gosling et al., 2016; Horsch et al., 2023; Tang et al., 2023).

CONCLUSION

Overall, our results demonstrate that high AMF diversity enhanced ecosystem multifunctionality. However, under multiple GCFs, the competition among AMF likely intensified, leading to no significant differences in ecosystem multifunctionality between high and low fungal diversity treatments. These multiple GCFs also may have enabled plants to select more efficient mycorrhizal partners, ensuring that high AMF diversity consistently benefited plant photosynthesis and growth. Our findings suggest that BEF studies should integrate both aboveground and belowground multi-trophic levels biodiversity within a unified framework. Additionally, our research emphasizes the importance of preserving AMF diversity for plant growth, particularly in agricultural ecosystems that are suffering from multiple global changes.

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Table 1. ANOVA models testing the effects of AMF and number of global change factors on ecosystem functions. AM-NM: with vs without AMF addition; A: AMF diversity levels L vs H; G: numbers GCFs levels 0, 1, or 6. Significant effects ($P < 0.05$) are in bold; marginal significant effects ($0.05 < P < 0.1$) are underlined.

	Variable	DF	F-value	p-value
Ecosystem multifunctionality	AM-NM	1	0.021	0.884
	A	**1**	**24.43**	**< 0.001**
	G	**2**	**12.91**	**< 0.001**
	A:G	2	1.59	0.205
Net photosynthetic rate	**AM-NM**	**1**	**26.27**	**< 0.001**
	A	**1**	**107.90**	**< 0.001**
	G	**2**	**12.38**	**< 0.001**
	A:G	2	0.85	0.490
Aboveground biomass	**AM-NM**	**1**	**12.62**	**< 0.001**
	A	**1**	**28.30**	**< 0.001**
	G	**2**	**107.26**	**< 0.001**
	A:G	2	2.62	0.075
Soil N_2O emissions	**AM-NM**	**1**	**11.02**	**0.001**
	A	1	0	0.988
	G	**2**	**19.99**	**< 0.001**
	A:G	2	1.23	0.293
Soil CH_4 emissions	**AM-NM**	**1**	**13.05**	**< 0.001**
	A	1	0.24	0.624
	G	2	0.94	0.393
	A:G	2	0.08	0.926
Soil NO_3^--N content	**AM-NM**	**1**	**9.63**	**0.002**
	A	1	2.43	0.121
	G	**2**	**25.00**	**< 0.001**
	A:G	2	2.66	0.072
Soil NH_4^+-N content	**AM-NM**	**1**	**16.27**	**< 0.001**
	A	1	0.10	0.754
	G	**2**	**17.03**	**< 0.001**
	A:G	2	0.01	0.989
βG activity	**AM-NM**	**1**	**64.02**	**< 0.001**
	A	1	1.37	0.244
	G	**2**	**3.69**	**0.026**
	A:G	2	2.07	0.129
CBH activity	**AM-NM**	**1**	**53.56**	**< 0.001**
	A	1	0.50	0.479
	G	**2**	**6.76**	**0.001**
	A:G	2	0.42	0.661
NAG activity	**AM-NM**	**1**	**7.78**	**0.006**
	A	1	0.07	0.795
	G	**2**	**3.04**	**0.050**
	A:G	2	0.97	0.379
ALP activity	AM-NM	1	0.01	0.949
	A	**1**	**15.89**	**< 0.001**

	Variable	DF	F-value	p-value
	G	**2**	**9.41**	**< 0.001**
	A:G	**2**	**5.58**	**0.004**