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Original Article

Effects of Open-Field Experimental Extreme Climate Events on Soil Microbial Biomass and Extracellular Enzyme Activity Under *Pinus densiflora* and *Larix kaempferi* Seedlings

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ABSTRACT

Soil microbes play major roles in the terrestrial ecosystem by exchanging nutrients with plants. The present study investigated the effects of extreme climate events on the soil extracellular enzyme activities and microbial biomass in the soil covered with 0-year-old *Pinus densiflora* and *Larix kaempferi* seedlings. Open-field treatments of extreme warming (+3°C and +6°C) and the precipitation manipulation including drought (100% rainfall interception) and heavy rainfall (43.4 mm per day) were applied from April to June 2021. Soil microbial biomass carbon and nitrogen and extracellular enzyme (*acid phosphatase*, *β-glucosidase*, *N-acetyl-glucosaminidase*, and *leucine aminopeptidase*) activities were measured after the completion of all treatments. The activities of *acid phosphatase* and *N-acetyl-glucosaminidase* under the *L. kaempferi* seedlings were higher than those under *P. densiflora* seedlings by 28.0% ($p < .05$) and 75.9% ($p < .01$), respectively. It appeared that the notable enzyme activities under the *L. kaempferi* seedlings are due to the difference in carbon output from the roots and substrate provision following the deciduous layer. Compared to the precipitation control, microbial biomass carbon and nitrogen increased by 9.6% and 8.6% in the heavy rainfall treatments and decreased by 9.9% and 15.4% in the drought treatments, respectively ($p < .01$). The overall results indicate the microbial sensitivity to environmental variables as well as interactions with the planted species. Since this study confirmed only the measurements shortly after the extreme climate events manipulation, further investigation is needed to address the mechanisms of soil microbe–plant interactions in response to future climate changes.

Keywords: Extreme climate event, soil microbial biomass, soil extracellular enzyme activity

Introduction

Soil microorganisms have an important role in the terrestrial ecosystem because they impact many processes including soil fertility maintenance and biogeochemical cycle regulation, helping plant growth, organic matter decomposition, soil production, and soil carbon storage (Kennedy, 1999; Madsen, 2011; Rousk & Bengtson, 2014). In particular, the microbes in the soil have short reproduction time and small sizes that react sensitively to changes in the surrounding environment (Jansson & Hofmockel, 2020). Meanwhile, according to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC), climate change will accelerate, resulting in more frequent and severe extreme climate events (IPCC, 2022). The extreme environmental conditions related to temperature and humidity caused by extreme climate events can greatly impact soil microorganisms for a short period, causing permanent changes (GOEBEL et al., 2011). Understanding how soil microbe–plant interactions respond to climate change including extreme climate events is a research goal that will offer information on essential ecosystem processes such as soil carbon storage and net primary productivity (Berg et al. 2010; Fischer et al., 2014; Ostle et al. 2009). Therefore, there would be a need to assess how extreme climate change may affect the interaction of soil microbes with other elements in ecosystem processes to improve projections of ecological responses to different climate change scenarios.

Regarding previous studies, high temperatures, heavy rainfall, and drought conditions resulting from extreme climate events alter the structure and composition of soil microorganism in terrestrial ecosystems (Myhre et al., 2019; Wang et al., 2017). Increased soil temperature can enhance soil microbial activity as well as soil microbial biomass by accelerating metabolic rates (Allison and Treseder, 2008; Henry et al., 2005). Drought also reduces microbial activity by decreasing soil water content (Sardans et al., 2008; Sardans & Peñuelas, 2004; Schimel,

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2018), whereas increased precipitation may significantly raise microbial properties by increasing soil available substrates (Zak et al., 1994).

Representative analysis methods of soil microorganisms include soil microbial biomass and extracellular enzyme activity. The soil microbial biomass plays a major role in global nutrient cycles and provides information for monitoring the significant C, N, and P transfers in terrestrial ecosystems (Liang et al., 2017; Stockmann et al., 2013). By comparing the shifts in the amount of soil microbial biomass, we can find the factors that alter the nutrient dynamics in soil (Bardgett et al., 2008). Soil enzyme activities also provide information about the soil's ability to perform biogeochemical reactions, which are used widely by soil scientists. They can be good indicators of soil contamination or impacts of anthropogenic management, and the procedure is generally quick and simple. In particular, extracellular enzymes play an essential role in the decomposition of plant residues and organic matter. Therefore, their dynamics and relation to soil microorganisms and extracellular enzymes have been regarded as indicators of soil fertility and health (Alkorta et al., 2003; Schloter et al., 2018; Visser et al., 1992).

To investigate how extreme warming, drought, and heavy rainfall affect the soil microbial biomass and extracellular enzyme activities, we set out an open-field experimental monitoring system at a nursery planted with 0-year-old *Pinus densiflora* and *Larix kaempferi* seedlings. The hypotheses of the present study were (1) the microbial biomass and enzyme activities increase under warming, but decrease at extreme temperature (+6°C) due to the low soil water content, (2) drought will reduce soil microbial biomass and extracellular enzyme activity, whereas heavy rainfall will increase soil microbial biomass and extracellular enzyme activity, and (3) the soil microbial properties will be significantly different depending on the planted tree species.

Methods

Experimental Design

Based on the previous experimental extreme climate research, we set the criteria for the treatments using weather data of the past 112 years (1908–2020) provided by the Korea Meteorological Administration (Korea Meteorological Administration, 2021). We set the target temperature to 90th and 99th percentiles of the daily maximum temperature at the study site (Data, 2009; Mazdiyansi & AghaKouchak, 2015; World Meteorological Organization, 2016), which was confirmed to be 3°C and 6°C higher than the ambient temperature. For the number of days of the drought treatment, we considered the longest consecutive days with rainfall <1 mm during the reference period (Livada and Assimakopoulos, 2007; Tang et al., 2018; Vicente-Serrano et al., 2011), and the heavy rainfall treatment was produced using the 95th percentile of daily precipitation (Myhre et al., 2019; Pendergrass, 2018; World Meteorological Organization, 2016; Zakaria et al., 2017). Consequently, the drought treatment lasted for 31 days during the reference period, and the threshold of heavy rainfall treatment was 43.4 mm per day.

The research was carried out at an experimental tree nursery located in the Forest Technology and Management Research Center, Pocheon, South Korea (37°45′38.9″N, 127°10′13.4″E). The annual mean air temperature and precipitation in the region are 11.5°C and 1429.8 mm, respectively (1997–2021) (Korea Meteorological Administration, 2022). In April 2021, 0-year-old *P. densiflora* and *L. kaempferi* seedlings were planted in 54 experimental plots of 1.5 × 1.0 m containing a homogeneous sandy loam soil (70% sand, 20% silt, and 10% clay), following the guidelines for nursery practices (Korea Forest Service, 2020). The experimental plots were subjected to nine treatments (three temperature levels [W0: ambient, W3: warming by 3°C, W6: warming by 6°C] × three precipitation levels [P-: drought, P0: ambient, P+: heavy rainfall]), using three replicates per treatment. The 7-day simulation of the extreme warming in spring was conducted four times between April and June 2021. The experimental drought and heavy rainfall for 30 days were performed twice in the reference period, simulating the heavy rainfall treatment on the day of actual precipitation to make a difference from the drought treatment.

The warming treatment was achieved with infrared heaters (FT-1000, Mor Electronic Heating Assoc., Comstock Park, MI, USA), which were set at 90 cm above the soil surface to increase air temperature by 3°C and 6°C. A controller equipped with thermometers (SI-111, Apogee Instruments, Logan, UT, USA) connected to data loggers (CR1000X, Campbell Scientific, Inc., Logan, UT, USA) and relays (SDM-CD-16AC, Campbell Scientific, Inc.) regulated the temperature in the warming treatments to meet the target temperature by turning on the heater automatically. Precipitation was manipulated with a drip-irrigation system consisting of polycarbonate panels and an automatic pump (Kim et al., 2022). The automated rain block systems with transparent polycarbonate panels and rain detectors were installed above the P– plots and ambient rainfall was blocked to simulate drought, and the rainfall simulators consisting of two spraying nozzles (Unijet D5-35, Spraying Systems Co., Wheaton, IL, USA) were used to irrigate each P+ plots (Kim et al., 2022).

Soil Collection and Sample Preparation

After all treatments were completed in June 2021, bulk soil samples were taken by combining soil samples collected with a soil sampler (2.54 cm in diameter) from 0 to 15 cm depth of five random spots in each plot. Soil samples for physicochemical properties were air dried, whereas samples for microbial biomass and extracellular enzyme activity were maintained wet in a refrigerator at 4°C after sieving under 2 mm and removing visible plant materials. Soil microbial biomass was analyzed within 2 weeks after the soil sample collection and extracellular enzyme activities were measured within 48 hours.

Assays for Measurement of Environmental Conditions

In each plot, air temperature using an infrared temperature sensor (SI-111, Campbell Scientific, Inc.) and volumetric soil water content (vol%) at 5 cm soil depth using soil sensor probes (CS655, Campbell Scientific, Inc.) were recorded every 30 minutes connected to the data loggers (Campbell Scientific). Concentrations of soil organic carbon (SOC) in wet soil samples were quantified with a total organic C analyzer (TOC-L CPH, Shimadzu, Japan).

Soil Microbial Biomass Assay

The concentrations of soil microbial biomass carbon (MBC) and nitrogen (MBN) were measured by the chloroform fumigation-extraction method (Beck et al., 1997; Brookers et al., 1985). We prepared two sets of 10 g wet soil samples for non-fumigated and fumigated from each plot. The fumigated samples were placed with 20 mL of 99.5% chloroform in a desiccator and left in a lightless condition for 48–72 hours to be fumigated. Extracts were made for both non-fumigated and fumigated samples adding 50 mL of 0.5 M K_2SO_4 solution and filtered by Whatman No. 1 filter papers. The concentrations of water-soluble organic C and N released from the non-fumigated and fumigated soils were measured using a total organic carbon analyzer (TOC-L CPH, Shimadzu, Japan). Microbial biomass carbon and MBN concentrations were calculated by dividing the difference in the detected organic C and N concentrations

by the fumigation into the environmental factors, 0.45 and 0.54, respectively.

Soil Extracellular Enzyme Activity Assay

Soil enzyme activity was measured according to the fluorometric method (DeForest, 2009). The enzymes were *acid phosphatase* (AP), *β-glucosidase* (BG), *N-acetylglucosaminidase* (NAG), and *leucine amino-peptidase* (LAP) involved in phosphorus, carbon, and nitrogen cycle, respectively (Sinsabaugh et al., 2009). We used black polystyrene 96-well microplates (SPL Life Sciences Co. Ltd, South Korea) with fluorescent substrate analogs, and 4-methylumbelliferone and 7-amino-4-methylcoumarin (Sigma-Aldrich Co. Ltd, Yongin-si, South Korea) for standard solution. As soil extracellular enzyme activities are vulnerable to pH (A'Bear et al., 2014), we used buffers with similar pH ranges of the bulk soil samples to the enzyme–substrate solutions. Therefore, citrate-phosphate buffer solution (pH 6.8) was used for enzymes AP, BG, and NAG and trizma buffer solution (pH 7.2) was used for the LAP enzyme. Each enzyme was added to distilled water to make a substrate solution, and 125 mL of buffer was added to 1 g of a soil sample to make a sample assay solution. The plate with AP, BG, and NAG was incubated at 25°C for 2 hours, and the plate with LAP was incubated for 4 hours and 30 minutes. Then 10 µL of 1 M NaOH solution was added to each well and fluorescence intensity between 355 and 460nm was measured with a multi-detection microplate reader (HIDEX, Finland). Soil extracellular enzyme activities were evaluated in nmol substrate per hour divided into g dry soil.

Statistical Analysis

Using SAS v.9.4 (SAS Systems, Cary, NC, USA), three-way analysis of variance (three-way ANOVA) was performed to test warming, precipitation manipulation, and species' major and interactive effects on soil environment and microbial properties. In addition, an average comparison was conducted when the treatment effect was significant ($p < .05$) using a post-hoc Tukey's honest significance. Principal component analysis (PCA) was used to visualize the relationship between each value of plots and variables (e.g., pH, total carbon, total nitrogen (TN), SOC, MBC, and MBN) using R 4.0.5 software.

Results

Environmental Conditions

The average air temperature and soil moisture were significantly affected by warming ($p < .01$ and $p < .05$) and precipitation manipulation ($p < .05$ and $p < .01$; Table 1). The air temperatures increased by an average of 0.4°C under the drought condition and decreased by 0.2°C in the heavy rainfall treatment (Figure 1). Warming had a significant effect on soil moisture ($p < .01$) and promoted drier conditions producing the tendency of decreasing values as the temperature was higher in each precipitation treatment (Figure 1). Specifically, the warming treatment resulted in lower soil moisture, with averages of 0.2 vol% and 0.9 vol% less moisture in the W3 and W6 compared to the W0, respectively (Table 1 and Figure 1). Regarding the response of soil chemical properties, precipitation manipulation resulted in a significant ($p < .01$) enhancement in soil pH by the heavy rainfall treatment and the concentration of the SOC significantly increased ($p < .01$) under *L. kaempferi* seedlings (Table 1).

Soil Microbial Biomass and Extracellular Enzyme Activities

The various responses of microbial biomass were strongly influenced by soil water content following precipitation manipulation, while the extracellular enzyme activities showed little response to the drought or heavy rainfall treatments (Table 2). Compared to the precipitation

Table 1.
Results (F Values) of the three-way ANOVA Test for the Environmental Conditions (AT, SW, pH, TC, TN, and SOC) of Experimental Plots

F-Value	AT	SW	pH	TC	TN	SOC
W	189.95**	3.75*	1.81	1.12	0.29	0.07
P	4.14*	55.43**	37.58**	2.51	0.49	0.87
S	3.44	0.18	2.77	1.49	1.76	10.12*
W*P	0.69	0.21	1.02	0.26	1.44	0.63
W*S	0.44	0.12	0.06	0.14	1.89	0.41
P*S	0.84	1.47	0.05	1.65	1.36	0.52
W*P*S	0.59	1.12	0.76	1.10	1.24	0.07

Note: W = warming; P = precipitation manipulation; S = planted tree species; AT = air temperature; SW = soil water content; TC = total carbon; TN = total nitrogen; SOC = soil organic carbon.
*$p < .05$, **$p < .01$.

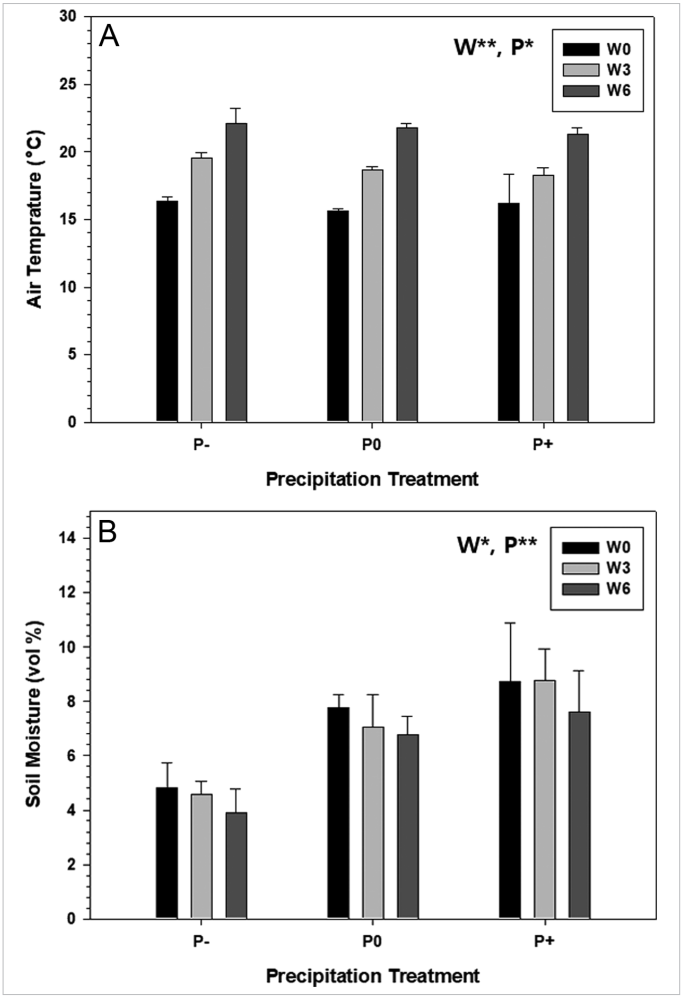

Figure 1.
(A) Mean Air Temperature (°) and (B) Mean Soil Water Content (vol %) During the Experimental Period of Extreme Climate Events. Results of three-way ANOVA Are Shown in Each Panel. Asterisks Indicate Significant Differences Between Treatments. Error Bars Indicate the Standard Deviation of the Mean. W0 = ambient temperature; W3 = +3° warming; W6 = +6° extreme warming; P- = drought; P0 = ambient precipitation; P+ = heavy rainfall.

Table 2.
Results (F Values) of the three-way ANOVA Test for the Responses of Soil Microbial Biomass and Extracellular Enzyme Activities to Warming, Precipitation Manipulation, and Their Interactions

Treatments	MBC (mg C kg^{-1} soil)	MBN (mg N kg^{-1} soil)	AP (nmol h^{-1} g^{-1} soil)	BG (nmol h^{-1} g^{-1} soil)	NAG (nmol h^{-1} g^{-1} soil)	LAP (nmol h^{-1} g^{-1} soil)
W0 × P-	53.97 ± 7.29	4.41 ± 1.14	43.64 ± 29.47	20.74 ± 11.14	21.48 ± 15.60	58.33 ± 29.33
W0 × P0	56.95 ± 9.87	4.58 ± 1.44	58.77 ± 13.23	33.55 ± 25.99	24.00 ± 7.57	61.01 ± 24.93
W0 × P+	62.62 ± 9.77	5.47 ± 0.66	68.83 ± 21.20	49.78 ± 33.21	36.53 ± 20.60	54.41 ± 14.69
W3 × P-	52.31 ± 7.45	4.05 ± 0.82	57.89 ± 28.85	31.65 ± 12.47	29.58 ± 7.83	57.19 ± 30.02
W3 × P0	60.11 ± 8.72	5.21 ± 1.11	89.91 ± 59.38	27.72 ± 15.52	40.69 ± 41.61	48.95 ± 19.81
W3 × P+	61.17 ± 2.66	4.71 ± 0.83	64.12 ± 21.05	33.73 ± 13.22	38.86 ± 25.29	52.90 ± 24.48
W6 × P-	51.97 ± 3.02	3.59 ± 0.82	66.37 ± 11.65	32.65 ± 7.89	22.82 ± 10.07	53.13 ± 22.84
W6 × P0	58.49 ± 11.19	4.44 ± 0.96	73.25 ± 20.14	26.96 ± 8.88	23.65 ± 7.05	61.30 ± 14.00
W6 × P+	68.66 ± 14.94	5.32 ± 0.44	50.17 ± 18.46	19.13 ± 6.43	24.75 ± 7.18	49.98 ± 25.09
F-value						
W	0.24	0.73.	1.13	1.22	2.79	0.15
P	6.70**	7.17**	2.12	0.66	1.27.	0.21
S	0.01	3.89	4.52*	0.11	12.72**	0.12
W*P	0.55	1.40.	1.36	2.69*	0.48	0.16
W*S	0.46	0.51	0.69	0.03.	2.10	0.94
P*S	1.91	0.43	0.34	1.09	1.08	1.17
W*P*S	0.44	1.88	1.63	2.94*	1.60	0.24

Note: Values are mean ± standard deviation.
W = warming; P = precipitation manipulation; S = planted tree species; AP = acid phosphatase; BG = β-1,4-glucosidase; NAG = N-acetyl-glucosaminidase; LAP = L-leucine aminopeptidase; MBC = microbial biomass carbon; MBN = microbial biomass nitrogen.
*$p < .05$; **$p < .01$.

control, MBC and MBN increased by 9.6% and 8.6% in the heavy rainfall treatments and decreased by 9.9% and 15.4% in the drought treatments, respectively (Figure 2). Contrary to expectations, warming did not have any effects on both soil microbial biomass and extracellular enzyme activities. The activity of BG was impacted significantly by the warming × precipitation and warming × precipitation × species interaction ($p < .05$; Table 2). In comparison to the control, the BG activity in the warming and precipitation manipulation treatments varied by –43.0–49.8% (Table 2).

The activities of AP and NAG under *L. kaempferi* seedlings were higher than those under *P. densiflora* seedlings by 28.0% and 76.0%, respectively (Figure 3). Principal component analysis explained 40.6% of the variation in soil microbial properties, as the heavy rainfall treatment had relatively higher MBC, MBN, BG, LAP, and pH than the control and drought treatment (Figure 4A), while AP, AT, and TN tended to be high under *L. kaempferi* seedlings (Figure 4B).

Discussion and Conclusions

Soil Microbial Biomass and Extracellular Enzymatic Responses to Extreme Climate Events

Our results denied the first hypothesis that microbial biomass and extracellular enzyme activity would respond significantly to increasing temperature, showing only indirect effects on BG activity. β-glucosidase changed significantly by the interaction of warming and precipitation regulation (Table 2), which is likely because extracellular enzymes that break down carbon, including BG, are highly sensitive to temperature and soil moisture (Stone et al., 2012; Zhou et al., 2013). Our results do not show a direct effect of microbial properties on warming, contradicting many studies that warming increases soil microbial biomass and extracellular enzyme activity (Barnard et al., 2020; Sistla & Schimel, 2013; Souza et al., 2017). However, according to Li et al. (2018), the warming effect depends on various environmental factors and is conditional to the annual difference in natural precipitation and the temporal variation of available soil nutrients. In addition, many climate change studies have established long-term treatment (Hargreaves et al., 2003; Rinnan et al., 2007; Sayer et al., 2017), and studies have shown that warming treatments of less than 3 years did not affect soil microbial biomass and have significant effects since 3 years of treatment initiation (Fu et al., 2012). Warming is also known to have a delayed effect on microbial biomass (Belay-Tedla et al., 2009; Fu et al., 2012). Therefore, further studies on the timing and duration of the simulation of experimental extreme climate events targeting soil microorganisms are needed in the future.

Contrary to the first hypothesis regarding the warming effect, our findings partially met the second hypothesis, showing a significant response of microbial biomass to precipitation manipulation. Our results showed that MBC and MBN decreased in drought and increased under the heavy rainfall treatment, showing a positive correlation for soil water content (Figures 2 and 4), consistent with the general results. Given that soil water content was the nearest environmental factor to MBC and MBN in the PCA results (Figure 4), it appeared to be more influential than the temperature in terms of soil microbial responses to environmental change factors. Previous research found that the strong effect of low soil moisture can be a limiting factor of enzyme activity in soils, negating any positive effect of warming (Steinweg et al., 2012). Also, soil

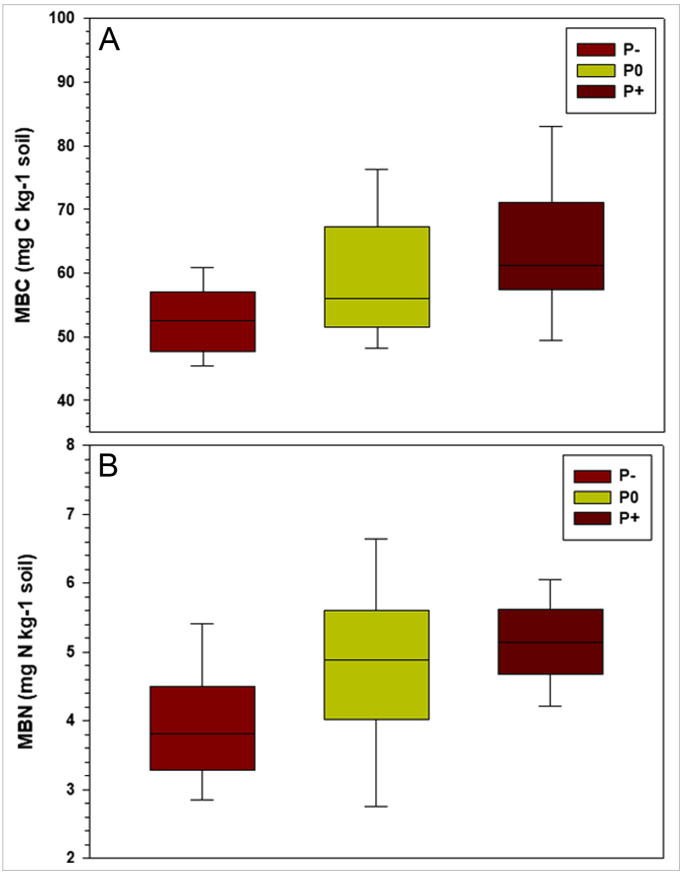

Figure 2.
Precipitation Manipulation Effects on (A) MBC and (B) MBN. Asterisks Indicate Significant Differences Between Treatments. P- = drought; P0 = ambient precipitation; P+ = heavy rainfall; box = interquartile range of data; whisker = maximum and minimum value; line in the box = medium value.

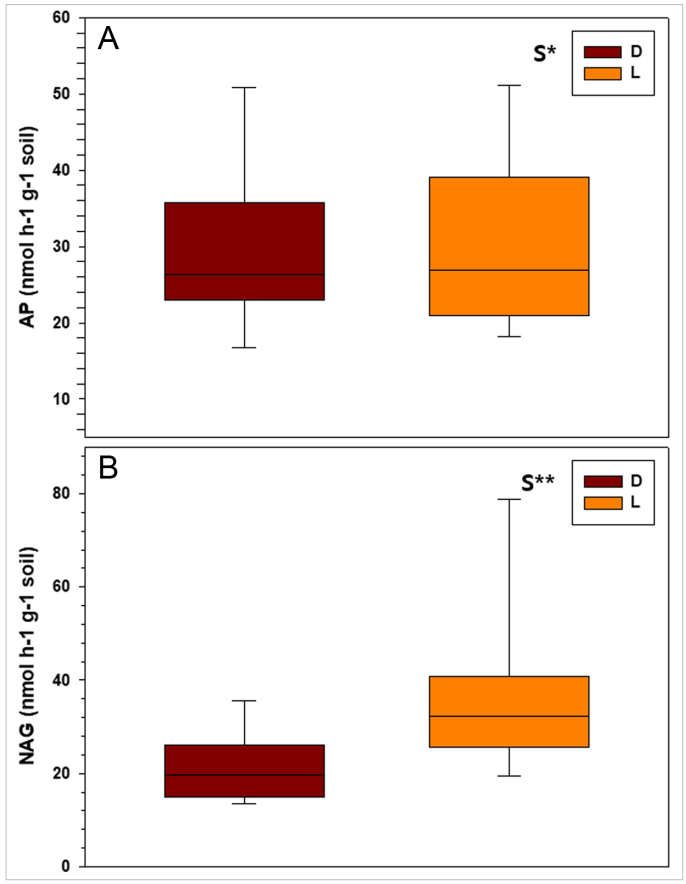

Figure 3.
Differential Effects of Tree Species on (A) AP and (B) NAG. Results of three-way ANOVA Are Shown in Each Panel. Asterisks Indicate Significant Differences Between Treatments. D = Pinus densiflora*; L* = Larix kaempferi*; box = interquartile range of data; whisker = maximum and minimum value; line in the box = medium value.*

pH was related to soil microbial and extracellular enzymatic responses to warming and precipitation manipulation (Figure 4). Stark et al. (2014) and Zhou et al. (2020) discovered that soil pH exerts a considerable impact on soil microbial acquisition and extracellular enzyme activities across ecosystems. Furthermore, previous research has indicated that hydrolytic enzymes are closely related to N clinging and N mineralization, implying the importance of inorganic N in microbial responses to soil environmental changes (Alkorta et al., 2003). Therefore, additional analysis of nutrients, especially inorganic N, seems to be needed in this study to find out the little influence of climate change factors on soil extracellular enzyme activities. Moreover, plant root exudates can alter the nutrient transport and availability in the soil and affect the interactions between plants and microbes at the plant–soil interface, thereby changing the structure and function of soil microbes (Zhao et al., 2021).

Effects of Planted Tree Species on Soil Microbial Properties

Different tree species significantly impacted soil enzyme activities, showing higher AP and NAG in soil samples beneath *L. kaempferi* seedlings (Figure 3), supporting our third hypothesis. Since SOC tends to be significantly higher under *L. kaempferi* seedlings than under *P. desiflora* seedlings (Table 1, Figure 4), we suggest that these species-specific differences are primarily due to the difference in C output from the roots and substrate provision by the deciduous layer. The deciduous layer provides C and N substrates that can promote microbial growth and support enzyme production, which increases the activity of enzymes degrading C and Nn (Sinsabaugh et al., 1993). Increasing C and N promotes phosphorus production and provides raw materials for extracellular phosphatase production, thereby increasing AP activity (Allison et al., 2006). A previous study reported that the activities of AP and NAG in the rhizosphere soil of deciduous species showed a significant increase compared to evergreen species (Ving, 2020), which is because deciduous species release more C from the root during growth compared to evergreen species, promoting microbial activity and extracellular enzyme production and inducing N conversion to improve the availability of N in the soil.

Our results appear to have a low correlation between any extracellular enzyme activities and microbial biomass C and N (Figure 4), contrary to the previous study showing a significant interaction of AP and microbial biomass (Baum et al., 2003). Additional microbial community data are needed for a more accurate analysis of these results and detailed information on soil microbial differences according to the planted tree species. The composition of soil microbial communities varies depending on the planted species, which also affects the amount of microbial biomass (Kang et al., 2018; Loranger-Merciris et al., 2006). Therefore, further analysis of soil microbial communities in future extreme weather

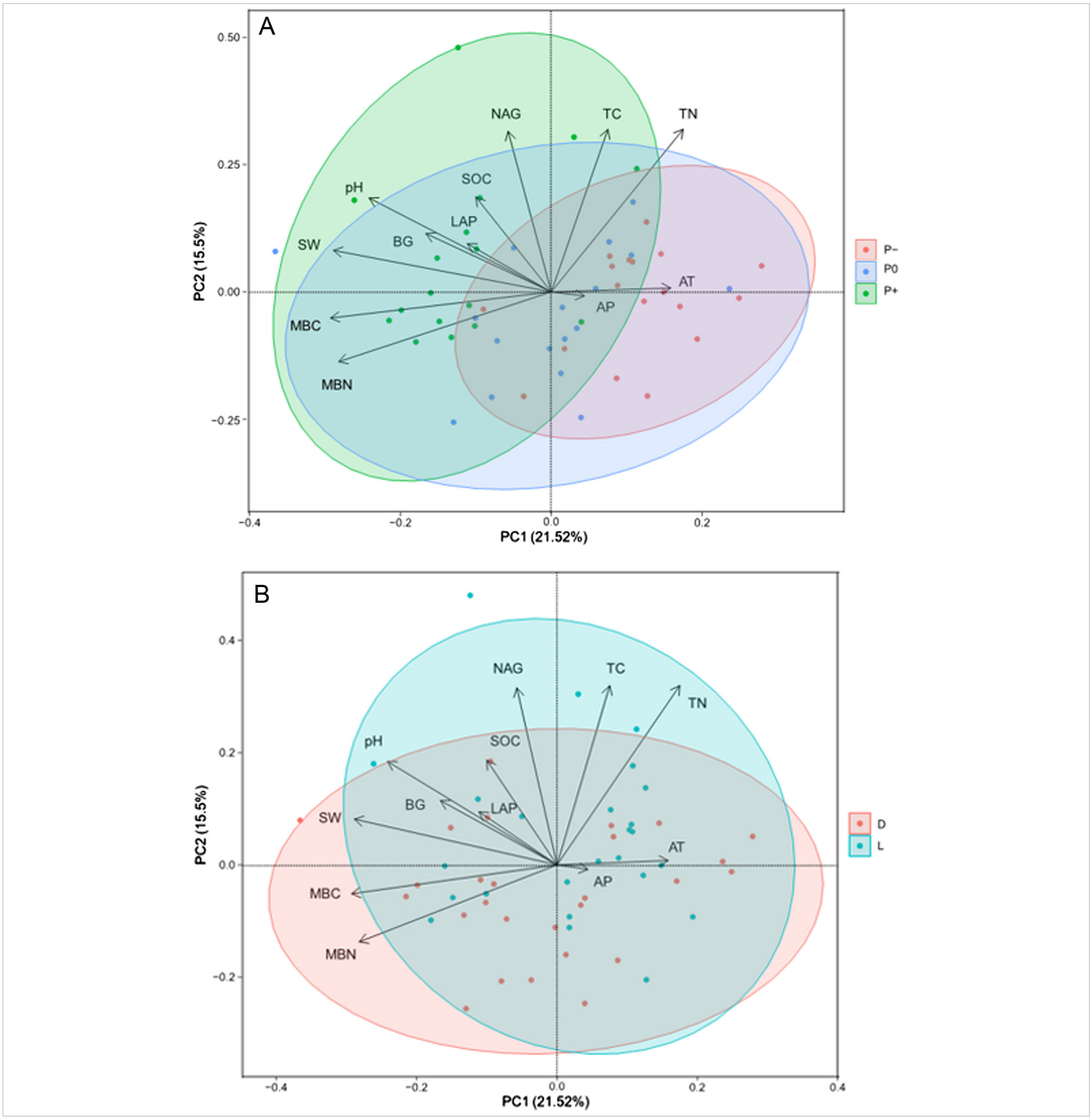

Figure 4.
Results of Principal Component Analysis Sort by (A) Precipitation Manipulation and (B) Planted Tree Species Based on Soil Microbial Biomass, Extracellular Enzyme Activities, and Soil Properties. AT = air temperature; SW = soil water content; TC = total carbon; TN = total nitrogen; SOC = soil organic carbon, P- = drought, P0 = ambient precipitation, P+ = heavy rainfall, D = Pinus densiflora, *L* = Larix kaempferi.

studies will be needed to determine the reason for the low correlation between extracellular enzyme activities, especially AP, and microbial biomass. In addition, since this study was conducted using 0-year-old seedlings, it is highly likely that root-extracted C, rather than litterfall, is the leading cause of the apparent difference in enzyme activities in this result. It is necessary to analyze the enzyme activity and soil microbial community between the root-soil and the seedling surface layer to distinguish in future studies.

Overall, the results show that microbes are sensitive to environmental variables and interact with tree species. We found that microbial properties varied highly dependent on soil water content and organic

matter, except that microbes were generally unresponsive to warming. Furthermore, the lack of a correlation between changes in microbial biomass and extracellular enzyme activities supports the idea that assessing microbial responses to environmental change requires an integrated approach. Substrate availability, in particular, may be needed to properly assess changes in enzymes in the context of soil C and N cycling responses to climate change. Because this study only confirmed measurements taken shortly after the manipulation of extreme climate events, more research is needed to address the mechanisms of soil microbe–plant interactions in response to future climate changes. Since this study confirmed only the measurements shortly after the extreme climate events manipulation, further investigation is needed to address the mechanisms of soil microbe–plant interactions in response to future climate changes.

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