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RESEARCH ARTICLE

Context dependence of warming-induced shifts in montane soil microbial functions

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Abstract

1. High elevation and latitude ecosystems are experiencing high levels of anthropogenic atmospheric warming. Climate warming may directly change soil microbial activity and alter ecosystem carbon dynamics and productivity, but increasing evidence suggests these responses may depend on other biotic factors such as plant community composition and abiotic factors such as moisture.
2. We examined how abiotic (warming) and biotic (presence of dominant plant species) factors interact to affect soil microbial processes. Our experiment deployed the independent and combined treatments of experimental warming and dominant plant species removal in a high and low elevation montane meadows. We analysed multiple soil microbial responses to warming and the presence of a dominant plant at three times throughout the growing season, including soil respiration, microbial metabolic functional diversity, microbial biomass carbon and nitrogen, and extracellular enzyme potential activity.
3. Overall, there were few independent microbial responses to either the warming or the removal treatments. There was a significant interaction between warming and the removal of a dominant plant species, where microbial biomass and the activity of some microbial enzymes were lower in warmed plots where the dominant species was removed relative to control plots.
4. The effect of warming on extracellular enzyme activity was typically observed only at the high-elevation site. In contrast, we found that effects of warming were consistent across the growing season, despite strong temporal variation in microbial properties.
5. Our results emphasize the need to further consider soil microbial responses to warming under multiple environments, including shifts in both biotic and abiotic factors, to aid in predictions of carbon dynamics under future global change.

KEYWORDS
dominant species removal, elevation, extracellular enzymes, open top chamber, soil respiration

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1 | INTRODUCTION

Impacts of global warming are being disproportionately felt in colder regions, including high-elevation montane systems (Grabherr et al., 2010; Mountain Research Initiative EDW Working Group, 2015; Parmesan, 2006) that are defined by complex habitats of varying microclimates and elevation-related stressors (Engler et al., 2011). Understanding how warming will interact with biotic factors to influence microbial function can strengthen predictions of ecosystem function under climate warming because soil microbes regulate a number of key ecosystem processes including nutrient cycling and C retention (Bardgett & Van Der Putten, 2014), and this regulation can be altered by warming (e.g. Song et al., 2019). Thus far, the impact of warming on microbial biomass and activity is unclear, as some studies have found positive effects and others have found negative effects (Broadbent et al., 2021; Fanin et al., 2022; Jonasson et al., 2004; Kardol et al., 2010; Kirschbaum, 1995; Meng et al., 2020), while others have failed to detect any effects of warming (Machmuller et al., 2016; McDaniel et al., 2013). In addition to the direct effects on microbial biomass and function, warming temperatures change environmental conditions, such as soil moisture (Xu et al., 2013), which may indirectly influence the relationship between microbial activity and temperature.

Atmospheric temperature changes can alter plant species ranges (Zhang et al., 2014) consequently influencing soil microbial functions. Shifts in plant community composition can affect physical and biogeochemical soil conditions (Jonasson et al., 2004; McLaren, Darrouzet-Nardi, et al., 2017; McLaren & Turkington, 2010) and ultimately influence the soil environment experienced by the microbial community. This biotic control over microbial activity may mediate the response of soil microbes to abiotic influences such as warming (Kardol et al., 2010). The role of plant species within a system can be examined through removal experiments (Diaz et al., 2003; McLaren & Turkington, 2010), and dominant plant species regulate ecosystem stability and function more strongly than other species (Avolio et al., 2019; Rewcastle et al., 2021). Plant species influence soil microbial community composition (De Long et al., 2016; Hernández-Cáceres et al., 2022 although see Marshall et al., 2011) and may alter microbial activity as measured by enzyme activity (D'Alò et al., 2021; Henry, 2012). Because microbial responses to warming may be affected by changes in the plant community, both plant community and microbial responses to warming must be considered in concert.

Finally, the effects of warming on plant and microbial responses may vary temporally, particularly in seasonally variable montane ecosystems that are sensitive to climate changes (Rustad et al., 2001). Microbial biomass and community structure varies across seasons, with spring snowmelt, typical in montane systems, instigating a crash in microbial biomass, and the associated microbial dominance over ecosystem processes such as nutrient cycling. The seasonal transition also prompts a shift in microbial community structure (Buckeridge et al., 2013; Edwards & Jefferies, 2013). Climatic warming is altering the timing of seasonal changes, potentially indirectly influencing the timing and duration of soil microbial dominance and turnover, as seasonal transitions in functioning of some montane systems are likely to occur earlier.

Here, we report on an experiment—WaRM (Warming and Removal in Mountains) which includes factorial warming and dominant species removal treatments at both high and low elevation at 10 sites internationally (Prager et al., 2022) and presents a unique opportunity to examine the independent and combined effects of these treatments in situ. In this paper, using the WaRM site in Colorado, USA, we examine the role of dominant plant species in controlling or influencing the response of soil microbial functions to warming, and whether this control varies across an elevational and temporal gradient. We measured microbial function with a suite of variables including soil respiration, microbial functional diversity via community-level physiological profiles (CLPPs), microbial biomass carbon (MBC) and nitrogen (MBN), and potential soil exoenzyme activity. We sampled soil three times during summer 2021 to capture temporal variation in: pre-growing season, peak-growing season, and post-growing season. We tested three inter-related hypotheses:

H1. Warming will positively affect microbial function. The effects will be stronger at the high-elevation site where ambient temperatures are likely more limiting to microbial activity than at the lower elevation site.

H2. Microbial responses to warming will be mediated by the dominant plant removal treatment, as a decrease in plant substrates entering the soil could decrease microbial activity. This mediation may differ at the low- and high-elevation sites, with the high site showing a stronger dependence on plant composition because of lower plant diversity.

H3. In temperate ecosystems with snow-covered winters, ecosystem processes are often described as microbially dominated during cool months (Edwards & Jefferies, 2013), thus, we hypothesize that microbial functions will be more sensitive to warming in the pre-growing season when there is less competition with plants.

2 | MATERIALS AND METHODS

2.1 | Site description

We conducted this work at the WaRM experimental sites near the Rocky Mountain Biological Laboratory (RMBL) in the West Elk range of the southern Rocky Mountains in Colorado, USA, during the summer 2021 growing season. The low-elevation site (low site) is at 2740 m elevation (38.715, −106.823) in an open meadow without tree cover, and the dominant plant species is a flowering forb, *Wyethia amplexicaulis*. The geological material at this site is

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characterized as Mayoworth loam soil (Rewcastle et al., 2021). The high-elevation site (high site) (3460 m, 38.992, −107.067) is characterized as Moran-Rubble land complex with gravelly loam/sandy loam (Rewcastle et al., 2021) and is also described as open meadow with no tree cover. This site is dominated by *Juncus drummondii*, a monocot, grass-like herb. The low- and high-elevation sites have a mean summertime temperature of 14.9 and 10.9°C, respectively, and a mean summertime precipitation of 143 and 151 mm (Hijmans et al., 2012 WorldClim data as cited in Prager et al., 2022). Research was conducted under the RMBL USFS Special Use Permit GUN1120 (OMB 0596-0082).

2.2 | Treatments

The WaRM experimental design is a 2×2 factorial warming × dominant plant species removal experiment deployed at the high-elevation site and the low-elevation site. Each of the four treatments were replicated eight times, for total of 32 plots (each of which is 2×2 m) at each elevation with warming imposed via transparent hexagonal open-top chambers (OTCs), 1.5 m in diameter, in the centre of each warming plot and the dominant plant species (listed above) removed via clipping at soil level within removal plots. Treatments at this site have been deployed each summer (June–August) since 2013.

2.3 | Sampling times and soil sampling

We collected data at three timepoints during summer 2021: pre-growing season [low site: approx. May 26; high site: approx. July 6], peak-growing season [low site; approx. June 23, high site; approx. July 21] and post-growing season [low site; approx. August 18, high site; approx. Sept 14]. Sampling occurred over 3–4 days around the dates listed. Pre-season was immediately after snowmelt before the majority of plant biomass had emerged and 1 week after warming chambers and removal treatments were implemented. Peak season was at peak biomass for each site. Post-season was when approximately 50% of plant biomass at the sites had senesced. We collected soil core samples from all plots at both sites for each timepoint. Three 2.5-cm-diameter soil cores were collected randomly from the top 10 cm of the soil profile from each plot, homogenized and then sieved to 2 mm.

2.4 | Environmental variables

Air and soil temperatures were recorded daily using iButton data loggers (Maxim Integrated Corp, San Jose, California, USA) suspended 5 cm above and buried 5 cm below the soil surface. A Hydrosense soil moisture probe was used to measure soil volumetric moisture (0–10 cm) at each soil sampling timepoint. We measured the normalized difference vegetation index (NDVI) at each soil sampling using a RapidSCAN (Holland Scientific, Lincoln, Nebraska, USA) with four measurements taken from the corner of each plot and averaged together.

2.5 | Soil respiration

We measured soil respiration weekly using a LI-COR 6400XT with a 10-cm-diameter soil respiration chamber (LI-COR Biosciences, Lincoln, Nebraska, USA) with above-ground-living plant material removed.

2.6 | Community-level physiological profile

Potential microbial metabolism was analysed on a community level using a Biolog EcoPlate assay (Garland, 1996). Briefly, 4 g of soil was suspended with 36 mL of potassium phosphate buffer and a dilution of this suspension added into 96-well ECO plates. Plates were incubated before being analysed (Synergy HT BioTek plate reader, Winooski, Vermont, USA). Functional diversity in CLPPs between soil samples was calculated by observing the richness (number of positive tests after background correction) of sample responses to all 31 carbon sources (Garland, 1996).

2.7 | Microbial biomass C and N

We quantified MBC and MBN using a modification of the chloroform fumigation method (Brookes et al., 1985). Two sets of soil extracts, one fumigated with chloroform and one not, were analysed for extractable organic carbon (EOC) and extractable total nitrogen (ETN) using a Shimadzu CN analyser (Shimadzu Scientific Instruments Inc., Columbia, Maryland, USA). Microbial flushes representing MBC and MBN were calculated as the difference between EOC and ETN in fumigated and non-fumigated extracts.

2.8 | Potential exoenzyme activity

Exoenzyme activity on collected soils was measured via microplate assays (McLaren, Buckeridge, et al., 2017; Saiya-Cork et al., 2002). Hydrolytic C-acquiring enzymes including β-glucosidase (β-gluc), cellobiohydrolase (Cello), β−xylosidase (β-xylo) and α-glucosidase (α-gluc); N-acquiring enzymes including N-acetyl-glucosaminidase (NAG) and LAP; and P-acquiring enzymes including phosphatase (Phos) and phosphodiesterase (PhosD) were analysed. Hydrolytic enzymes were measured using fluorescently tagged substrates and oxidative enzymes (phenol oxidase (phenol) and peroxidase (perox)) using L-3, 4-dihydroxyphenylalanine substrate in a slurry with soil and sodium acetate buffer adjusted to the site-specific soil pH (6.3 for low site, 4.4 for high site) following McLaren and Buckeridge (2021).

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2.9 | Scale of inference

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Spatial gradient: Plots	Elevation site (×2), warming treatment (×2), species removal (×2)	8 replicates/treatment/elevation = 64 plots, sampled at 3 times during the growing season = 192 samples

2.10 | Statistical analysis

All statistical analyses were performed using R and RStudio (R version 4.0.5, packages: ggplot2, dplyr, lubridate, gridExtra and olsrr). For air and soil temperatures, the daily average temperature was calculated from the hourly readings and the plot average for the summer season using the daily averages. We implemented a two-way factorial ANOVA with warming and removal treatments as the main factors on the plot averages for soil and air temperature.

For soil moisture measurements, as well as for each of the microbial variables analysed, we ran a four-way fully factorial ANOVA with the main factors (1) warming treatment, (2) removal treatment, (3) elevation and (4) sampling date. Because we were unable to measure NDVI at the low-site pre-season sampling, we ran a separate three-way ANOVA for each elevation with the factors (1) sampling date, (2) warming treatment and (3) removal treatment. Normality of residuals was confirmed for all variables. In the case of significant interactions identified in the ANOVA, a *t*-test was run to compare individual treatments with a corrected p-value of 0. 025 (Bonferroni correction for two factors) used to account for multiple *t*-tests.

3 | RESULTS

3.1 | Environmental variables

The OTCs significantly increased both soil (low site: 0–1°C; high site: 1–2°C) and air temperatures (low site: 1–3°C; high site: 2–3°C) in warmed plots throughout the growing season (Figure S1). For soil moisture, there was a significant elevation × sampling date interaction ($F_{2, 168} = 12.06$, $p < 0.001$; Figure S2). In the pre- and post-season, the low site had 27% and 21% higher soil moisture respectively than the high site, but in the peak season, the high site soil moisture average was 30% greater (Figure S2). At the low site, there was a significant sampling × removal interaction ($F_{1, 56} = 18.48$, $p < 0.001$) on NDVI, where removal reduced NDVI during the peak season only (Figure S3b). At the high site, NDVI was significantly influenced by sampling ($p < 0.001$) and warming ($p < 0.001$) individually, where NDVI increased across the season (Figure S3c) and warming reduced NDVI (Figure S3a).

3.2 | Microbial responses summary

There were few independent warming effects on microbial response variables, as these effects were typically in a higher order interaction with another factor. When effects of warming were dependent on elevation, generally warming effects were stronger at high, rather than low, elevations, as predicted by H1 (Figure 1a, Table S1). Also as predicted (H2), warming and removal treatments interacted with each other, and usually there was an effect of warming only when dominant species were removed (Figure 1b, Table S2). Warming effects, however, did not typically vary among sampling dates (not supporting H3), although there were strong microbial responses to elevation and sampling date independently.

3.3 | Soil respiration

Soil respiration was higher in warmed plots (Figure 2a, Table S2, H1) but this effect did not depend on the removal treatment (Figure 2a, Table S2, H2). There was no interaction between warming and sampling date (Table S2, H3). There was a significant interaction between elevation × sampling date on soil respiration (Table S1) where soil respiration rates were higher at the high site, but only at the peak-growing season sampling date (Figure S4, Table S3).

3.4 | Community-level physiological profile

For CLPP functional diversity, there were neither significant effects of warming nor a warming × elevation interaction (H1). There were also no independent effects of removal nor was there any interaction between warming and removal (H2) (Tables S1 and S2). There was no interaction between warming and sampling date (Table S2, H3) but there was a two-way interaction between elevation and sampling on functional diversity (Table S1) because the high site had higher diversity than the low site, with the size of the difference in functional diversity increasing across the growing season (Figure S4, Table S3).

3.5 | Microbial biomass C and N

There was no independent effect of warming, nor a warming × elevation interaction (H1) for MBC. There was a warming × removal interaction because warming marginally lowered MBC but only under the removal treatment (H1; Figures 1 and 2, Table S1). There was also no interaction between warming and sampling date (H3, Table S1). The high site had consistently lower MBC in comparison with the

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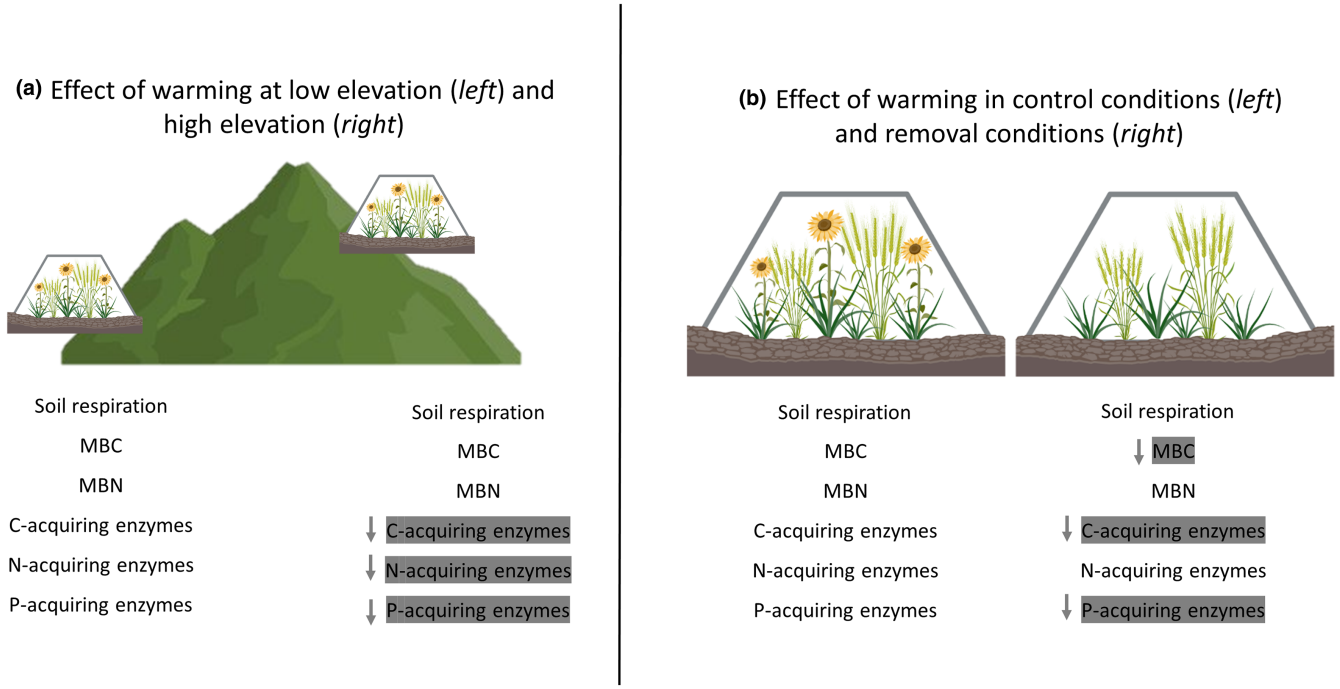


FIGURE 1 Summary of responses of microbial variables in an 8-year warming (OTCs) and dominant plant species removal experiment in a montane meadow of Colorado. Only variables for which there was an interaction between either (a) warming and elevation or (b) warming and removal are presented. Grey highlight indicates a significant effect of warming within (a) an elevation or (b) a removal treatment, and the arrow indicates the direction of the effect. There were no warming × sampling date interactions, and thus responses are presented across sampling dates. *Created with BioRender.com*.

low site (Figure S4, Tables S1 and S3). Additionally, MBC was highest at the pre-season sampling for both elevations (Figure S4, Tables S1 and S3).

Warming resulted in lower MBN regardless of elevation (H1) or removal treatment (H2; Figure 2), whereas removal treatments independently resulted in higher MBN (Figure 2). There was no warming × sampling date interaction for MBN (H3) but there was an elevation × sampling date interaction because the low site had higher MBN but only during pre- and post-season sampling and not during peak season (Figure S4, Table S3).

3.6 | Potential exoenzyme activity

Few of the enzymes assayed responded to either warming or removal, and when they did there was, generally, an interaction between warming and removal (Table S1). There was a complex three-way interaction between warming × elevation × removal on Cello (Table S1). Warming reduced Cello activity only at the high site (H1) and only under the removal treatment (H2; Table S2, Figure S5). In support of H1, there was an elevation × warming interaction for the enzymes β–xylo (Figure S5), NAG and PhosD (although, NAG and PhosD individual *t*-tests were only marginally significant after correcting for multiple tests), because there was no significant difference between ambient and warmed plots at the low site but there was a negative effect of warming on enzyme activity at the high site (Figure 3, Table S1, Figure S5).

β-gluc and PhosD both responded negatively to warming, but only under removal conditions (significant warming × removal interaction; Figures 1 and 3, Tables S1 and S2). PhosD did not show a significant pattern after correcting for multiple tests. Removal increased β-xylo activity regardless of the warming treatment (Table S1).

Warming effects were consistent across sampling dates, and thus our results do not support H3 (Table S1). There were a number of elevation × sampling interactions (Table S1), but these varied among enzymes, with some enzymes responding strongly to elevations at all three sampling dates (e.g. LAP). Other enzymes differed between elevations only during the pre-growing season (e.g. NAG), during the peak growing season (e.g. β-gluc, β–xylo, Phos, PhosD), or during the post-growing season sampling (e.g. phenol) (Figure S6, Table S3). Perox, the only oxidative enzyme that responded to treatments, had a sampling date × removal interaction (Table S1).

4 | DISCUSSION

We examined how alpine meadow soil microbial communities respond to experimental warming, if biotic factors (presence/absence of dominant plant species) mediated the microbial responses to warming, and if the microbial response to warming and dominant plant removal varied between high and low elevations or at different times during the growing season. Although

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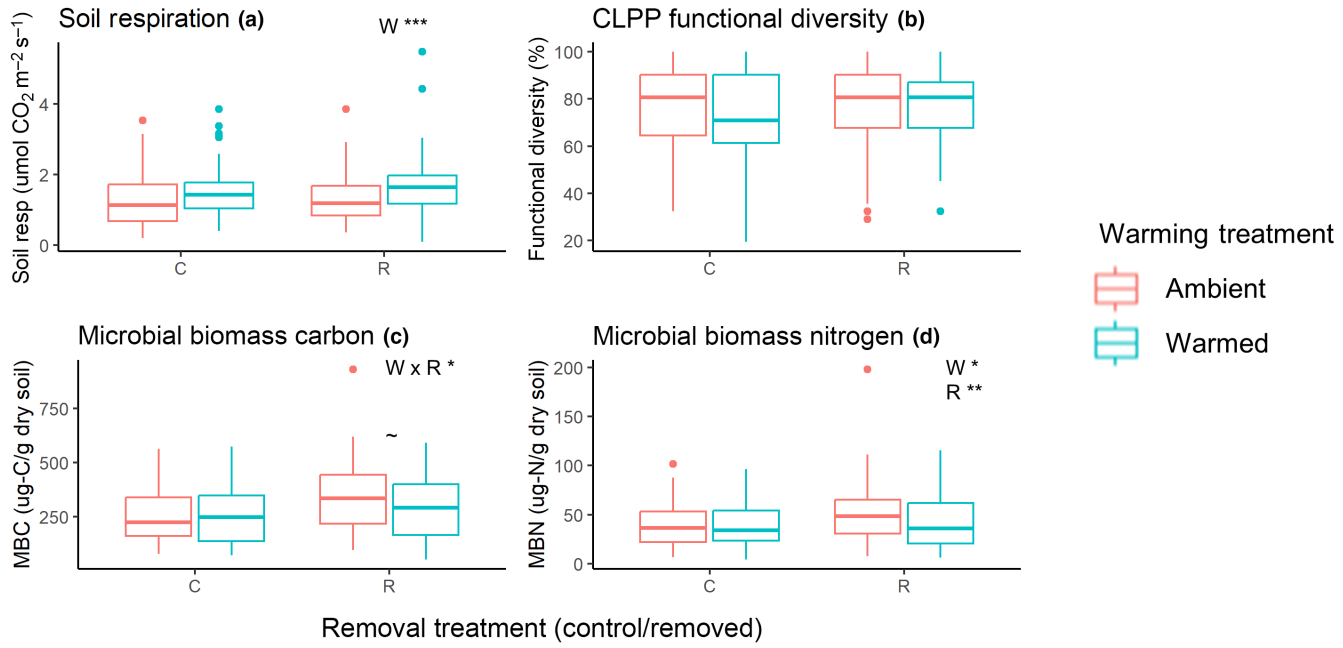


FIGURE 2 Responses of soil respiration (a), CLPP functional diversity (b), MBC (c) and MBN (d) to warming (OTCs) and dominant species removal treatments across samplings in an 8-year warming experiment in montane meadow of Colorado. Significance of effects of warming (W), removal (R) or interactions are noted with stars at the top right of each plot. When interactions between treatments were significant, significant differences (*t*-test) between ambient and warmed plots within control and removal treatments are noted with stars above the pair of ambient and warmed bars: $\sim 0.1 < p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

some microbial variables responded independently to the warming or plant removal treatments, microbial responses tended to be context dependent. Our findings highlight that ecosystems are complex and can respond to shifts in biotic and abiotic factors differently across space and time. Multiple global change factors are changing simultaneously, and the response of ecosystems to experimental multiple global change factors is often magnified when compared to ecosystem responses to single factor experiments (Komatsu et al., 2019). Thus, the context dependence of our results highlights the importance of simultaneously examining multiple global change factors to better understand ecosystem function. We note that warming treatments in this experiment resulted in moderate increases in temperature (<3°C) and long-term temperature changes may be more important for determining microbial responses to warming (Alster et al., 2023); despite this, we saw strong responses for some enzymes and detected strong interactions with removal and elevation for other enzymes.

4.1 | Warming influences soil microbial activity (H1)

Microbial activity is, in general, temperature limited, thus we hypothesized that warming would lead to an increase in microbial activity and processes (Kirschbaum, 1995). We found, in support of our first hypothesis, that soil respiration did increase under warming (i.e. across elevations, sampling dates and removal treatments). Our soil respiration result, where warming increased respiration, is consistent with those in a global-scale meta-analysis of experimental warming projects (Song et al., 2019). Increases in soil respiration under warming conditions are likely due to the increased availability of labile C sources under relatively short-term warming (Bradford et al., 2008; Kirschbaum, 2004), supported by the decrease in activity of C-acquiring enzymes with warming in this study. The increase could also be a result of a decrease in metabolic efficiency, specifically in breaking down recalcitrant substrates, with warmer temperatures (Frey et al., 2013). However, the simulation of soil respiration with warming could change with the magnitude and duration of the warming experiment, as labile C will be depleted over time and microbes may acclimate individually or shift their community structure to better adapt to warming (Romero-Olivares et al., 2017).

While microbial metabolic activity (respiration rate) increased with experimental warming, microbial biomass decreased with warming. Prior work has also found that MBN, specifically, decreased with warming (Jonasson et al., 2004) and that microbial biomass generally declines with warming (Bradford et al., 2008). Further, previous studies found that alpine microbial biomass significantly decreases from the winter to the summer (Broadbent et al., 2021); a pattern that may reflect warming summer temperatures leading to reduced microbial biomass. Because decreases in MBC with warming did not occur unless the dominant plant species was also removed, in warmed plots with the original plant community, there was a decrease in microbial N with no change in microbial C (increase in the microbial C:N ratios) which could be a result of increased efficiency

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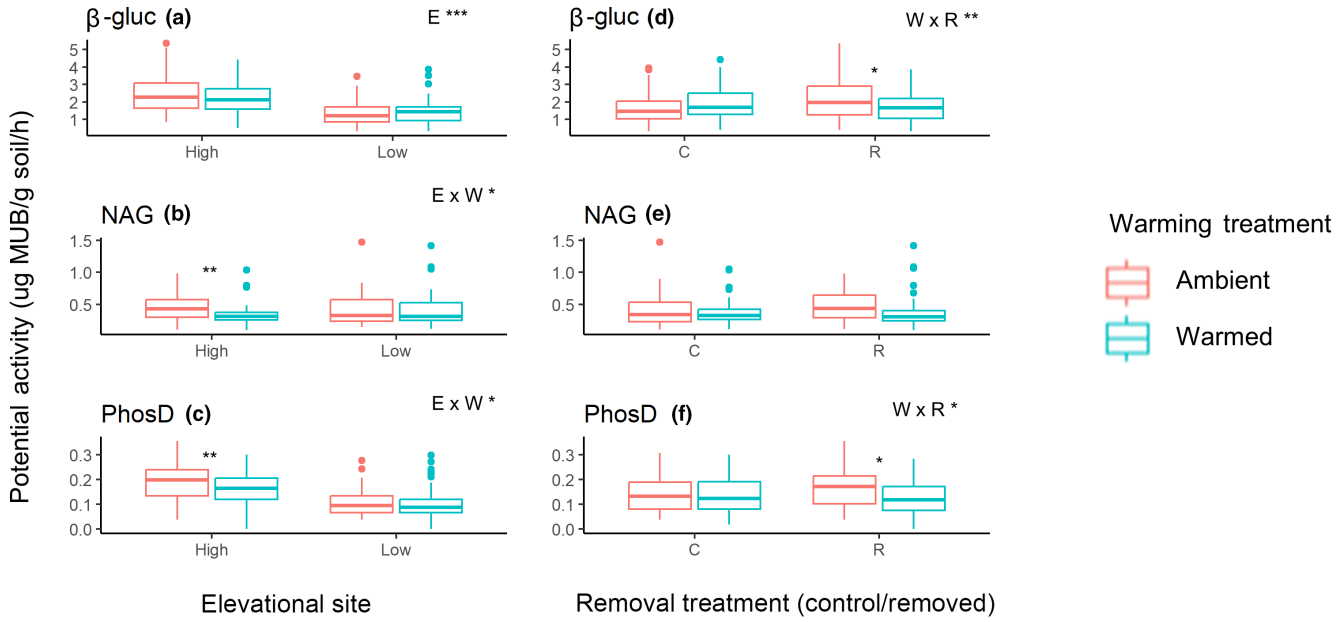


FIGURE 3 Potential enzyme activity responses to warming (OTCs) and dominant species removal treatments and elevation in an 8-year warming experiment in montane meadow of Colorado. We present a single C-acquiring enzyme (β-gluc, a and d), N-acquiring enzyme (NAG, b and e) and P-acquiring enzyme (PhosD, c and f) which showed a significant interaction between warming treatments and elevation or warming and removal treatments. Other enzymes that responded to the interaction between warming and elevation (β-xylo) or had higher level interactions with warming (Cello) are presented in the Supporting Information. Significant effects of warming (W), removal (R) and elevational site (E), as well as any interactions between these treatments, are noted with stars at the top right of each plot. When interactions between treatments were significant, significant differences (*t*-test) between ambient and warmed plots within each elevation site (a–c) or within the control or removal treatments (d–f) are noted with stars above each pair of bars: $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

in N use (Mooshammer et al., 2014) or a decrease in N availability in the soil (Sistla et al., 2012).

In support of our first hypothesis, we found that warming had a larger impact on microbial activity at the high-elevation site. In addition to the two variables where warming had an independent effect across elevations (soil respiration and MBN), three enzymes (β-xylo, NAG and PhosD) responded to warming only at the high site. These responses could not only be a result of a stronger warming treatment at the high elevation but are also consistent with previous studies that find a stronger effect of warming on enzyme activity in colder environments (Meng et al., 2020). Interestingly, we observed a negative effect of warming, rather than the predicted positive response, on enzyme activity at the high-elevation site. Decreased microbial biomass resulted in lower enzyme activity under warming in a forest ecosystem (McDaniel et al., 2013) and this pattern could also hold in our study system. Further, the microbial community may have been under higher N limitation with warming (as suggested by decreased MBN), possibly resulting in less N available for enzyme production, which is often limited by N availability (Sistla et al., 2012). Finally, the differing response to warming at different elevations may be due to shifts in microbial community structure with increasing altitude, such as the reported decrease in the soil bacterial to fungal ratio with increasing altitude (Margesin et al., 2009). Overall, our findings support our hypothesis that warming effects will be stronger at higher elevations.

4.2 | Plant community composition determines warming response (H2)

Plant community composition influences physical soil conditions, nutrient availability (McLaren & Turkington, 2010), and microbial community structure (De Long et al., 2016; although, see Marshall et al., 2011) and consequently is likely to play additional indirect roles in plant–soil–microbe interactions. As ecosystems are currently experiencing changes in plant community composition concurrently with climate change (Parmesan & Yohe, 2003), we also tested how manipulating the plant community, through the removal of the dominant species, would mediate microbial responses to warming. Of the 14 response variables we measured, which encompassed a range of microbial processes, only four were significantly impacted by the interaction between the warming and the removal treatment (MBC, and three enzymes: β-gluc, Cello [at high elevation only] and PhosD). The response of soil communities to the interaction between warming and dominant plant removal underscores our hypothesis that plant community composition would mediate how microbial communities respond to warming. Further, for all four variables where the interaction between warming and dominant plant removal treatments was significant, the warming by removal interaction was because responses to warming were only significant when the dominant plant species was removed, possibly because the dominant plant

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control over microbial function was stronger than any responses to warming (Kardol et al., 2010). Similarly, clipping studies have found that biomass removal, regardless of species, can mask the effects of warming on soil microbial properties, perhaps because effects of warming on plant-microbe interactions play a stronger role in determining soil microbial function than the direct effects of warming itself (Zhang et al., 2005).

Additional effects of plant removal were not dependent on environmental context; removal increased two microbial response variables (MBN and β-xylo) in all environments. β-Xylo is an enzyme released by microbes to increase C acquisition, and its increase suggests that soil microbes were C mining when dominant plants were removed. Concurrently, the increase in MBN indicates that microbes are immobilizing N, altering C:N ratios, further implying that soil C availability is lacking under removal conditions. Low C availability may result from reduced plant biomass (observed decrease in NDVI with removal at peak season) and potentially lower C input into the soil with the removal treatment. Schmidt et al. (1999) found that microbial biomass nutrients increased when plants were excluded due to the decrease in competition for nutrients between plants and microbes. Therefore, the decrease in biomass that resulted from our removal treatment might have influenced soil microbial enzymes. Although few of our measured variables responded to plant removal, the response of both MBC and MBN suggests that plant composition played an important role in determining microbial biomass and their nutrient acquisition, key variables in multiple ecosystem processes.

4.3 | Effects of warming vary by season (H3)

Our study is one of the few that examined the effects of warming at multiple points within a growing season (intra-seasonal sampling), which complements inter-seasonal sampling (i.e. winter and growing season) with warming in other studies (Sistla & Schimel, 2013). We hypothesized that warming would have the strongest impacts early in the summer while microbes may still be dominant over ecosystem processes, and as the season progressed the effects on microbial processes would be less as control shifted to plants in the summer (Edwards & Jefferies, 2013). However, we found no intra-seasonal variation in warming effects on microbial biomass and function. In one of the few other examples of warming studies that sampled intra-seasonally, a study by Stark et al. (2018) found that warming effects on N and P pools and enzyme activity were strongest in the peak-growing season when plant communities have strong influence on soil conditions, contrary to our hypothesis. Although the effects of warming did not vary with season, we did find significant variation in environmental variables, specifically soil moisture and soil and air temperature, during the growing season that could impact microbial function. Consequently, although the effects of warming on measured microbial processes did not vary across the season, most microbial variables responded independently to sampling date during the growing season with intra-seasonal effects on microbial variables varying between low and high sites.

5 | CONCLUSIONS

Soil microbes predominantly responded to warming in a context dependent way, with negative responses to warming both when dominant species were removed and also at high elevation. Our results emphasize the important role that biotic factors, like plant community composition, will play in soil responses to warming. Previous studies have found that microbial responses to warming are strongly related to microbial composition (Fanin et al., 2022; Meng et al., 2020), and future studies should investigate changes in soil community composition to better understand the mechanisms that are causing the interaction between these biotic and abiotic factors to influence soil microbial response.

High latitude and elevation systems experience seasonal strong changes in climate between the winter and summer seasons and there is a hypothesized concurrent shift in microbial to plant dominance over ecosystem processes (Edwards & Jefferies, 2013). However, we found that warming effects on microbes were consistent across our intra-seasonal samplings despite shifts in soil and environmental physical conditions and microbial functions. Our study is one of the first to examine warming effects at multiple points throughout the growing season, and despite our finding of temporally consistent effects of warming, we suggest that future studies in strongly seasonal ecosystems include intra-seasonal sampling. In addition, more warming studies that incorporate multiple seasons are needed, because warming is more pronounced in winter for high-latitude and high-elevation systems (Kreyling et al., 2019), but most warming experiments taking place during the growing season (Kreyling & Beier, 2013).

Climate change will include multiple driving factors that could influence soil microbial community activity, including not only temperature increases and changing plant communities we examined here but also increased atmospheric CO_2, shifts in precipitation, altered soil moisture, changes in animal interactions/behaviour, increased N deposition and more (Classen et al., 2015). A study on global change impacts on plant species composition has found that inclusion of multiple, rather than a single, global change factors increases likelihood of effects (Komatsu et al., 2019). Given the strong context dependence, we found of the effects of warming on soil microbial function, we encourage more studies which combine multiple biotic and abiotic effects of climate change, which will increase their predictive ability due to possible interactions among global change drivers (Bardgett et al., 2008; Henry, 2012; Litchman & Thomas, 2023).

AUTHOR CONTRIBUTIONS

All authors conceived the ideas and designed methodology. Sydne Spinella collected and analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

None of the authors have conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Data available from the Environmental Data Initiative Data Portal https://doi.org/10.6073/pasta/4d4ab1d167c790ae0cb2ab71cfbcd65d (McLaren & Spinella, 2024).

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REFERENCES

Alster, C. J., van de Laar, A., Goodrich, J. P., Arcus, V. L., Deslippe, J. R., Marshall, A. J., & Schipper, L. A. (2023). Qunatitying thermal adaptation of soil microbial respiration. *Nature Communications*, *14*, 5459.

Avolio, M. L., Smith, M. D., Forrestel, E. J., Chang, C. C., La Pierre, K. J., & Burghardt, K. T. (2019). Tansley review demystifying dominant species. *New Phytologist*, *223*, 1106–1126. https://doi.org/10.1111/nph.15789

Bardgett, R. D., Freeman, C., & Ostle, N. J. (2008). Microbial contributions to climate change through carbon cycle feedbacks. *The ISME Journal*, *2*(8), 805–814. https://doi.org/10.1038/ismej.2008.58

Bardgett, R. D., & Van Der Putten, W. H. (2014). Belowground biodiversity and ecosystem functioning. *Nature*, *515*(7528), 505–511. https://doi.org/10.1038/nature13855

Bradford, M. A., Davies, C. A., Frey, S. D., Maddox, T. R., Melillo, J. M., Mohan, J. E., Reynolds, J. F., Treseder, K. K., & Wallenstein, M. D. (2008). Thermal adaptation of soil microbial respiration to elevated temperature. *Ecology Letters*, *11*(12), 1316–1327. https://doi.org/10.1111/J.1461-0248.2008.01251.X

Broadbent, A. A. D., Snell, H. S. K., Michas, A., Pritchard, W. J., Newbold, L., Cordero, I., Goodall, T., Schallhart, N., Kaufmann, R., Griffiths, R. I., Schloter, M., Bahn, M., & Bardgett, R. D. (2021). Climate change alters temporal dynamics of alpine soil microbial functioning and biogeochemical cycling via earlier snowmelt. *The ISME Journal*, *15*, 2264–2275. https://doi.org/10.1038/s41396-021-00922-0

Brookes, P. C., Landman, A., Pruden, G., & Jenkinson, D. S. (1985). Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology and Biochemistry*, *17*(6), 837–842. https://doi.org/10.1016/0038-0717(85)90144-0

Buckeridge, K. M., Banerjee, S., Siciliano, S. D., & Grogan, P. (2013). The seasonal pattern of soil microbial community structure in mesic low arctic tundra. *Soil Biology and Biochemistry*, *65*, 338–347. https://doi.org/10.1016/j.soilbio.2013.06.012

Classen, A. T., Sundqvist, M. K., Henning, J. A., Newman, G. S., Moore, J. A. M., Cregger, M. A., Moorhead, L. C., & Patterson, C. M. (2015). Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? *Ecosphere*, *6*(8), art130. https://doi.org/10.1890/ES15-00217.1

D'Alò, F., Odriozola, I., Baldrian, P., Zucconi, L., Ripa, C., Cannone, N., Malfasi, F., Brancaleoni, L., & Onofri, S. (2021). Microbial activity in alpine soils under climate change. *Science of the Total Environment*, *783*, 147012. https://doi.org/10.1016/j.scitotenv.2021.147012

De Long, J. R., Dorrepaal, E., Kardol, P., Nilsson, M. C., Teuber, L. M., & Wardle, D. A. (2016). Contrasting responses of soil microbial and nematode communities to warming and plant functional group removal across a post-fire boreal Forest successional gradient. *Ecosystems*, *19*(2), 339–355. https://doi.org/10.1007/S10021-015-9935-0/FIGURES/6

Diaz, S., Symstad, A. J., Chapin, F. S., III, Wardle, D. A., & Huenneke, L. F. (2003). Functional diversity revealed by removal experiments. *Trends in Ecology & Evolution*, *18*(3), 140–146. https://doi.org/10.1016/S0169-5347(03)00007-7

Edwards, K. A., & Jefferies, R. L. (2013). Inter-annual and seasonal dynamics of soil microbial biomass and nutrients in wet and dry low-Arctic sedge meadows. *Soil Biology and Biochemistry*, *57*, 83–90. https://doi.org/10.1016/J.SOILBIO.2012.07.018

Engler, R., Randin, C. F., Thuiller, W., Dullinger, S., Zimmermann, N. E., Araújo, M. B., Pearman, P. B., Le Lay, G., Piedallu, C., Albert, C. H., Choler, P., Coldea, G., De Lamo, X., Dirnböck, T., Gégout, J. C., Gómez-García, D., Grytnes, J. A., Heegaard, E., Høistad, F., … Guisan, A. (2011). 21st century climate change threatens mountain flora unequally across Europe. *Global Change Biology*, *17*(7), 2330–2341. https://doi.org/10.1111/J.1365-2486.2010.02393.X

Fanin, N., Mooshammer, M., Sauvadet, M., Cheng, M., Alvarez, G., Bernard, L., Bertrand, I., Blagodatskaya, E., Bon, L., Fontaine, S., Niu, S., Lashermes, G., Maxwell, T. L., Weintraub, M. N., Lisa, W., Moorhead, D., & Nottingham, A. T. (2022). Soil enzymes in response to climate warming: Mechanisms and feedbacks. *Functional Ecology*, *36*, 1378–1395. https://doi.org/10.1111/1365-2435.14027

Frey, S. D., Lee, J., Melillo, J. M., & Six, J. (2013). The temperature response of soil microbial efficiency and its feedback to climate. *Nature Climate Change*, *3*, 395–398.

Garland, J. L. (1996). Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. *Soil Biology and Biochemistry*, *28*(2), 213–221. https://doi.org/10.1016/0038-0717(95)00112-3

Grabherr, G., Gottfried, M., & Pauli, H. (2010). Climate change impacts in alpine environments. *Geography Compass*, *4*(8), 1133–1153. https://doi.org/10.1111/J.1749-8198.2010.00356.X

Henry, H. A. L. (2012). Soil extracellular enzyme dynamics in a changing climate. *Soil Biology and Biochemistry*, *47*, 53–59. https://doi.org/10.1016/j.soilbio.2011.12.026

Hernández-Cáceres, D., Stokes, A., Angeles-Alvarez, G., Abadie, J., Anthelme, F., Bounous, M., Freschet, G. T., Roumet, C., Weemstra, M., Merino-Martín, L., & Reverchon, F. (2022). Vegetation creates microenvironments that influence soil microbial activity and functional diversity along an elevation gradient. *Soil Biology and Biochemistry*, *165*, 108485. https://doi.org/10.1016/J.SOILBIO.2021.108485

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2012). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, *25*, 1965–1978. (for WorldClim 2018 database citation).

Jonasson, S., Castro, J., & Michelsen, A. (2004). Litter, warming and plants affect respiration and allocation of soil microbial and plant C, N and P in arctic mesocosms. *Soil Biology and Biochemistry*, *36*(7), 1129–1139. https://doi.org/10.1016/j.soilbio.2004.02.023

Kardol, P., Cregger, M. A., Campany, C. E., & Classen, A. T. (2010). Soil ecosystem functioning under climate change: Plant species and community effects. *Ecology*, *91*(3), 767–781. https://doi.org/10.1890/09-0135.1

13652435, 2024, 5, Downloaded from https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2435.14538 by University Of Michigan Library, Wiley Online Library on [18/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Kirschbaum, M. U. F. (1995). The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. *Soil Biology and Biochemistry*, *27*(6), 753–760. https://doi.org/10.1016/0038-0717(94)00242-S

Kirschbaum, M. U. F. (2004). Soil respiration under prolonged soil warming: Are rate reductions caused by acclimation or substrate loss? *Global Change Biology*, *10*(11), 1870–1877. https://doi.org/10.1111/J.1365-2486.2004.00852.X

Komatsu, K. J., Avolio, M. L., Lemoine, N. P., Isbell, F., Grman, E., Houseman, G. R., Koerner, S. E., Johnson, D. S., Wilcox, K. R., Alatalo, J. M., Anderson, J. P., Aerts, R., Baer, S. G., Baldwin, A. H., Bates, J., Beierkuhnlein, C., Belote, R. T., Blair, J., Bloor, J. M. G., … Zhang, Y. (2019). Global change effects on plant communities are magnified by time and the number of global change factors imposed. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(36), 17867–17873. https://doi.org/10.1073/PNAS.1819027116/-/DCSUPPLEMENTAL

Kreyling, J., & Beier, C. (2013). Complexity in climate change manipulation experiments. *Bioscience*, *63*(9), 763–767. https://doi.org/10.1525/BIO.2013.63.9.12

Kreyling, J., Grant, K., Hammerl, V., Arfin-Khan, M. A. S., Malyshev, A. V., Peñuelas, J., Pritsch, K., Sardans, J., Schloter, M., Schuerings, J., Jentsch, A., & Beierkuhnlein, C. (2019). Winter warming is ecologically more relevant than summer warming in a cool-temperate grassland. *Scientific Reports*, *9*(1), 1–9. https://doi.org/10.1038/s41598-019-51221-w

Litchman, E., & Thomas, M. K. (2023). Are we underestimating the ecological and evolutionary effects of warming? Interactions with other environmental drivers may increase species vulnerability to high temperatures. *Oikos*, *2023*(2), e09155. https://doi.org/10.1111/OIK.09155

Machmuller, M. B., Mohan, J. E., Minucci, J. M., Phillips, C. A., & Wurzburger, N. (2016). Season, but not experimental warming, affects the activity and temperature sensitivity of extracellular enzymes. *Biogeochemistry*, *131*(3), 255–265. https://doi.org/10.1007/s10533-016-0277-6

Margesin, R., Jud, M., Tscherko, D., & Schinner, F. (2009). Microbial communities and activities in alpine and subalpine soils. *FEMS Microbiology Ecology*, *67*(2), 208–218. https://doi.org/10.1111/J.1574-6941.2008.00620.X

Marshall, C. B., McLaren, J. R., & Turkington, R. (2011). Soil microbial communities resistant to changes in plant functional group composition. *Soil Biology and Biochemistry*, *43*(1), 78–85. https://doi.org/10.1016/J.SOILBIO.2010.09.016

McDaniel, M. D., Kaye, J. P., & Kaye, M. W. (2013). Increased temperature and precipitation had limited effects on soil extracellular enzyme activities in a post-harvest forest. *Soil Biology and Biochemistry*, *56*, 90–98. https://doi.org/10.1016/J.SOILBIO.2012.02.026

McLaren, J. R., & Buckeridge, K. M. (2021). Enhanced plant leaf P and unchanged soil P stocks after a quarter century of warming in the arctic tundra. *Ecosphere*, *12*, e03838. https://doi.org/10.1002/ecs2.3838

McLaren, J. R., Buckeridge, K. M., Van De Weg, M. J., Shaver, G. R., Schimel, J. P., & Gough, L. (2017). Shrub encroachment in Arctic tundra: Betula nana effects on above-and belowground litter decomposition. *Ecology*, *98*(5), 1361–1376.

McLaren, J. R., Darrouzet-Nardi, A., Weintraub, M. N., Gough, L., McLaren, J. R., Darrouzet-Nardi, A., Weintraub, M. N., & Gough, L. (2017). Seasonal patterns of soil nitrogen availability in moist acidic tundra. *Arctic Science*, *4*, 98–109. https://doi.org/10.1139/AS-2017-0014

McLaren, J. R., & Spinella, S. (2024). *WARM experiment soil microbial function, RMBL Colorado, 2021 ver 1*. Environmental Data Initiative. https://doi.org/10.6073/pasta/4d4ab1d167c790ae0cb2ab71cfbcd65d

McLaren, J. R., & Turkington, R. (2010). Ecosystem properties determined by plant functional group identity. *Journal of Ecology*, *98*(2), 459–469. https://doi.org/10.1111/J.1365-2745.2009.01630.X

Meng, C., Tian, D., Zeng, H., Li, Z., Chen, H. Y. H., & Niu, S. (2020). Global meta-analysis on the responses of soil extracellular enzyme activities to warming. *Science of the Total Environment*, *705*, 135992. https://doi.org/10.1016/J.SCITOTENV.2019.135992

Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S., & Richter, A. (2014). Stoichiometric imbalances between terrestrial decomposer communities and their resources: Mechanisms and implications of microbial adaptations to their resources. *Frontiers in Microbiology*, *5*(Feb), 22. https://doi.org/10.3389/FMICB.2014.00022/BIBTEX

Mountain Research Initiative EDW Working Group. (2015). Elevation-dependent warming in mountain regions of the world. *Nature Climate Change*, *5*, 424–430. https://doi.org/10.1038/nclimate2563

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, *37*, 637–669. https://doi.org/10.1146/ANNUREV.ECOLSYS.37.091305.110100

Parmesan, C., & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, *421*, 37–42. www.nature.com/nature

Prager, C. M., Classen, A. T., Sundqvist, M. K., Barrios-Garcia, M. N., Cameron, E. K., Chen, L., Chisholm, C., Crowther, T. W., Deslippe, J. R., Grigulis, K., He, J.-S., Henning, J. A., Hovenden, M., Høye, T. T. T., Jing, X., Lavorel, S., McLaren, J. R., Metcalfe, D. B., Newman, G. S., … Sanders, N. J. (2022). Integrating natural gradients, experiments, and statistical modeling in a distributed network experiment: An example from the WaRM Network. *Ecology and Evolution*, *12*(10), e9396. https://doi.org/10.1002/ECE3.9396

Rewcastle, K. E., Henning, J. A., Read, Q. D., Irwin, R. E., Sanders, N. J., Ee, A., Classen, T., Rewcastle, C., Henning, J. A., Read, Q. D., Irwin, R. E., Sanders, N. J., & Classen, A. T. (2021). Plant removal across an elevational gradient marginally reduces rates, substantially reduces variation in mineralization. *Ecology*, *103*, e03546. https://doi.org/10.1002/ecy.3546

Romero-Olivares, A. L., Allison, S. D., & Treseder, K. K. (2017). Soil microbes and their response to experimental warming over time: A meta-analysis of field studies. *Soil Biology and Biochemistry*, *107*, 32–40. https://doi.org/10.1016/J.SOILBIO.2016.12.026

Rustad, L. E., Campbell, J. L., Marion, G. M., Norby, R. J., Mitchell, M. J., Hartley, A. E., Cornelissen, J. H. C., Gurevitch, J., Alward, R., Beier, C., Burke, I., Canadell, J., Callaghan, T., Christensen, T. R., Fahnestock, J., Fernandez, I., Harte, J., Hollister, R., John, H., … Wright, R. (2001). A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia*, *126*(4), 543–562. https://doi.org/10.1007/S004420000544

Saiya-Cork, K. R., Sinsabaugh, R. L., & Zak, D. R. (2002). The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. *Soil Biology and Biochemistry*, *34*(9), 1309–1315. https://doi.org/10.1016/S0038-0717(02)00074-3

Schmidt, I. K., Jonasson, S., & Michelsen, A. (1999). Mineralization and microbial immobilization of N and P in arctic soils in relation to season, temperature and nutrient amendment. *Applied Soil Ecology*, *11*(2–3), 147–160. https://doi.org/10.1016/S0929-1393(98)00147-4

Sistla, S. A., Asao, S., & Schimel, J. P. (2012). Detecting microbial N-limitation in tussock tundra soil: Implications for Arctic soil organic carbon cycling. *Soil Biology and Biochemistry*, *55*, 78–84. https://doi.org/10.1016/J.SOILBIO.2012.06.010

Sistla, S. A., & Schimel, J. P. (2013). Seasonal patterns of microbial extracellular enzyme activities in an arctic tundra soil: Identifying direct and indirect effects of long-term summer warming. *Soil Biology and Biochemistry*, *66*, 119–129. https://doi.org/10.1016/j.soilbio.2013.07.003

Song, J., Wan, S., Piao, S., Knapp, A. K., Classen, A. T., Vicca, S., Ciais, P., Hovenden, M. J., Leuzinger, S., Beier, C., Kardol, P., Xia, J., Liu, Q., Ru, J., Zhou, Z., Luo, Y., Guo, D., Langley, J. A., Zscheischler, J.,

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... Zheng, M. (2019). A meta-analysis of 1,119 manipulative experiments on terrestrial carbon-cycling responses to global change. *Nature Ecology & Evolution*, *3*, 1309–1320. https://doi.org/10.1038/s41559-019-0958-3

Stark, S., Ylänne, H., & Tolvanen, A. (2018). Long-term warming alters soil and enzymatic N:P stoichiometry in subarctic tundra. *Soil Biology and Biochemistry*, *124*, 184–188. https://doi.org/10.1016/J.SOILBIO.2018.06.016

Xu, W., Yuan, W., Dong, W., Xia, J., Liu, D., & Chen, Y. (2013). A meta-analysis of the response of soil moisture to experimental warming. *Environmental Research Letters*, *8*, 044027. https://doi.org/10.1088/1748-9326/8/4/044027

Zhang, M. G., Zhou, Z. K., Chen, W. Y., Cannon, C. H., Raes, N., & Slik, J. W. F. (2014). Major declines of woody plant species ranges under climate change in Yunnan, China. *Diversity and Distributions*, *20*(4), 405–415. https://doi.org/10.1111/DDI.12165

Zhang, W., Parker, K. M., Luo, Y., Wan, S., Wallace, L. L., & Hu, S. (2005). Soil microbial responses to experimental warming and clipping in a tallgrass prairie. *Global Change Biology*, *11*(2), 266–277.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Results of a 4-Way ANOVA exploring microbial response variables that were measured in an 8-year warming (OTCs) and dominant species removal experiment in montane meadows located in Colorado, USA.

Table S2. Responses to the interaction between warming (OTCs) and dominant species removal treatments for multiple microbial response variables in an 8-year warming experiment in montane meadow of Colorado.

Table S3. Average (± Standard Error) microbial response to the interaction between elevation and growing season sampling intervals in an 8-year warming (OTCs) and dominant species removal experiment in montane meadows located in Colorado, USA.

Figure S1. Soil (a) and air (b) temperature responses to warming (OTCs) at 2 elevational sites in an 8-year warming and dominant species removal experiment in a montane meadow in Colorado, USA.

Figure S2. Response of soil moisture to elevation and growing season sampling intervals in an 8-year warming (OTCs) and dominant species removal experiment in montane meadow of Colorado.

Figure S3. Response of NDVI to experimental warming, plant removal, growing season time-point, and elevation in an 8-year warming (OTCs) and dominant species removal experiment in montane meadow of Colorado.

Figure S4. Responses of soil respiration (a), CLPP functional diversity (b), MBC (c), and MBN (d) to elevation and growing season sampling intervals in an 8-year warming (OTCs) and dominant species removal experiment in montane meadow of Colorado.

Figure S5. Response of C- acquiring enzymes Cello (a, b) and β–xylo (c) potential activity to warming (OTCs) and dominant species removal at high and low elevation sites from an 8-year warming and removal experiment in montane meadow of Colorado.

Figure S6. Potential enzyme activity responses to elevation and growing season sampling intervals in an 8-year warming (OTCs) and dominant species removal experiment in montane meadow of Colorado.

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