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Context Dependence Of Warming Induced Shifts In Alpine Soil Microbial Functions

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Abstract

Atmospheric warming is occurring due to anthropogenic release of carbon dioxide. Climate change has the potential to increase microbial activity in soil, where a significant amount of terrestrial carbon is stored, which may lead to release of this soil carbon into the atmosphere, positively feeding back to global temperature rise. Understanding how the indirect impacts of climate warming, like shifts in plant community composition, affect soil microbes can improve predictions of ecosystem functions and services under climate change. This project examined direct and indirect consequences of warming on microbial processes using independent and combined treatments of experimental warming and dominant plant species removal along an elevation gradient in the alpine Rocky Mountains, Colorado, throughout the summer growing season. We analyzed multiple soil microbial responses to our treatments including respiration, metabolic functional diversity, microbial biomass carbon and nitrogen, and extracellular enzyme potential activity. There were few direct responses to either warming or removal treatments, and for variables that did respond to either warming or removal, it was typically also in a higher order interaction with another factor. When warming and removal interacted with each other, as they did for microbial biomass carbon and the potential activity of the enzymes β-glucosidase, Cellobiohydrolase, and Phosphodiesterase, it was because there was a negative effect of warming only when the dominant plant species was removed. We also observed that effects of both treatments vary throughout the growing season, and also differ across elevation, with higher elevations seeing stronger effects of warming and removal. Our results emphasize the need to further investigate changing plant community structure as an additional driving force when considering soil microbial responses to warming and predicting carbon dynamics under future global change.

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Introduction

Anthropogenic release of carbon dioxide (CO_2), a greenhouse gas, from fossil fuel combustion into the atmosphere has caused an increase in average global surface temperatures (IPCC, 2014). However, the impacts of global warming will be disproportionately felt in colder regions (IPCC, 2014), including high elevation montane systems, that are considered to be more vulnerable to climate change (Grabherr et al., 2010; Parmesan, 2006), likely because of the complex habitats of varying microclimates and elevation-related stressors that define montane systems (Engler et al., 2011). Climate warming has the potential to alter ecosystem carbon (C) fluxes, an important component to ecosystem productivity, by increasing soil respiration rates and therefore breakdown of soil C and release of CO_2 (Song et al., 2019). Most global terrestrial C is found in soil in the form of soil organic and inorganic matter (Lal, 2004), and montane ecosystems are thought to store significant amounts of soil C (Sundqvist et al., 2013). Further, conditions in high elevation systems can be representative of ecosystem conditions found at high latitudes, which also store significant soil C. In systems of high C storage, soil C release could positively feedback to atmospheric CO_2 levels and climate changes (Cox et al., 2000; Friedlingstein et al., 2006). However, the mechanisms that will determine the amount of soil C that may be released from terrestrial ecosystems under climate change remains uncertain, necessitating a call for investigation of soil C stock responses (Broadbent et al., 2021).

One of the first steps in predicting soil C storage fluxes under climate change is understanding how soil microbes and their functioning will respond to warming. Soil microbes regulate a number of key ecosystem processes including nutrient cycling and C retention in soil (Bardgett & Van Der Putten, 2014) and their interactions shape plant- and animal diversity, composition, and abundance in ecosystems (Classen et al., 2015). Critical for effects on soil C and

nitrogen (N) fluxes (Singh et al., 2010; van der Heijden et al., 2008), one of the processes the microbial community performs is decomposition, where microbes chemically attack C-rich organic matter in the soil, sometimes using extracellular enzymes (Schimel & Bennett, 2004; Trivedi et al., 2016), releasing plant-available nutrients. As microbes expel energy to decompose organic matter, they respire CO_2, contributing to heterotrophic soil respiration and further influencing ecosystem C dynamics (Bardgett et al., 2008). Soil microbes and their functioning will determine the soil C response to climate warming, therefore understanding how warming impacts the microbial community and factoring them into ecosystem C dynamic predictions and modeling is essential (Wieder et al., 2013).

Atmospheric warming has the potential to influence soil microbial activity and function both directly and indirectly (Bardgett et al., 2008). Soil microbial activity typically increases directly with temperature, resulting in higher rates of decomposition and soil respiration (Curiel Yuste et al., 2007; Jonasson et al., 2004; Keiser et al., 2019; Kirschbaum, 1995). Although microbial activity increases with temperature, a recent alpine study found that microbial biomass is typically lower in warmer summer temperatures (Broadbent et al., 2021), suggesting that the observed increases in activity may be attributed at least partially to increases in activity rather than an increase in abundance of microbes. Microbial functions that may be affected by warming include soil organic matter breakdown via the production of extracellular enzymes, but the effect of warming on enzyme production and activity remains elusive. Meta-analyses of enzyme activity under experimental warming show varying responses across study sites and duration and also the magnitude of warming (Fanin et al., 2022; Meng et al., 2020). Responses of enzymes to warming can also be influenced by season (summer vs winter; Machmuller et al., 2016; Sistla & Schimel, 2013), and type of enzyme including specific nutrient-acquiring (N, P, and C) enzymes (Meng et

al., 2020; Stark et al., 2018) or hydrolytic vs oxidative (Meng et al., 2020; Sistla & Schimel, 2013), while other studies found no significant changes in enzyme activity under warming (Machmuller et al., 2016; McDaniel et al., 2013). In addition to these direct effects, warming temperatures change environmental conditions, which may indirectly influence the relationship between microbial activity and temperature. For example, experimental warming can decrease soil moisture due to increased evapotranspiration (Xu et al., 2013) which could result in decreased soil microbial activity due to water limitations (Curiel Yuste et al., 2007).

Microbial responses to warming could also be altered by the changes in plant community composition and plant traits that are co-occurring with warming climates (Classen et al., 2015; Weintraub & Schimel, 2005). Ecosystems across the globe are experiencing shifts in plant community composition as an effect of warming temperatures (Parmesan & Yohe, 2003) because temperature changes can alter species range (Zhang et al., 2014), competition (Gilman et al., 2010), and interactions (Blois et al., 2013) within an ecosystem. Some systems may see shifts in preexisting species; like arctic systems noting significant increase in abundance of shrubs (Elmendorf et al., 2012) and north-western Europe observing increases in abundance of thermophilic species (Jol et al., 2009). However, others may see a change in plant community composition through the loss of species, as seen by Foden et al. (2007) in the Namib Desert, or the immigration of new species into the system, as is expected in boreal regions (Thuiller et al., 2005). Species in mountaintop systems specifically are vulnerable to climate changes because of their unique range-restriction (Parmesan, 2006; Thuiller et al., 2005). Effects of changing plant community composition can be particularly important if the dominant species is lost or changes, as some studies show that dominant plant species more strongly regulate system stability (Sasaki & Lauenroth, 2011) and function (Avolio et al., 2019). Shifts in plant composition can affect

physical soil conditions and nutrient content (Aguirre et al., 2021; Crofts et al., 2018), including through changes in litter composition and abundance, which can alter decomposition rates (Jonasson et al., 2004; Mclaren et al., 2017), ultimately influence the soil environment experienced by the microbial community. . In addition to responses to changing plant composition, microbes may respond to changes in plant traits with warming. Rising temperatures are predicted to increase photosynthetic activity in plants and consequently increase the presence of root exudates in soil (Bengtson et al., 2012) which provide energy for microbes and increase microbial activity (Bardgett et al., 2013), resulting in an increase in plant available nutrients and the breakdown of soil organic matter (Keiser et al., 2019). Microbial responses to warming are likely affected by changes in the plant community and thus both changes in plant community and microbial responses to warming must be considered in concert.

Montane ecosystems experience large seasonal changes which is likely to affect the interaction between plants and microbes. In systems which experience a cold, snow-covered winter, including high elevations and high latitude ecosystems, early in the summer there may be an annual switch from a system dominated by microbial processes to one where plants control system processes once temperatures warm and soils thaw (Edwards & Jefferies, 2013). However, atmospheric warming is altering the timing of seasonal changes, potentially indirectly influencing the timing and duration of soil microbial dominance, as seasonal transitions in functioning are likely to occur earlier and alter annual carbon fluxes (Broadbent et al., 2021; Ernakovich et al., 2014). These seasonal changes become more extreme with increasing latitudes and elevations, and high montane systems are thought to be especially sensitive to climate change (Rustad et al., 2001). Consequently, conducting warming experiments along elevational gradients provides the

opportunity to extrapolate results across ecosystem variation and further improve C dynamics predictions (Classen et al., 2015; Margesin et al., 2009; Sundqvist et al., 2013).

Understanding how current warming trends and concurrent plant composition changes will alter soil microbial functions can strengthen predictions of climate warming feedbacks and future global C dynamics. This is the basis of a multiyear, international network experiment designed to examine the effects of warming and dominant plant species removal in montane ecosystems (WaRM – Warming and Removal in Mountains). The WaRM experimental design presents a unique opportunity to examine the independent and combined effects of *in situ* experimental warming and manipulated plant community composition. At each WaRM location, the experiment is repeated at one low and one high elevation site, taking advantage of the systematic ecological changes that occur along elevational gradients (Sundqvist et al., 2013). To examine how warming and a changing plant community may be influencing microbial functions, we sampled soils and collected environmental data throughout summer 2021 from the WaRM sites in Colorado, USA. To represent microbial function, we analyzed a suite of variables including soil respiration, microbial functional diversity via community-level physiological profile (CLPP), microbial biomass carbon (MBC) and nitrogen (MBN), and soil exoenzyme potential activity. We also sampled soil at three times throughout the summer season to capture intra-annual changes: pre-growing season, peak-growing season, and post-growing season.

Our objective is to determine if warming will shift soil microbial functions *in situ*, whether plant community composition will mediate the soil microbial responses to warming, and whether these responses will be affected by elevation or time of year. Hypotheses for our project were:

1. Warming will have positive effects on microbial function. The effect may be stronger at the high elevation site where temperature is likely more limiting than at the low site.

2. Microbial variable responses to warming will be mediated by plant community composition because of the complex relationships between plants, soil, and microbes. 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.
3. In temperate ecosystems with snow-covered winters, processes are often described as microbially dominated during cold months, therefore we may see that microbial functions will be more sensitive to warming in the pre-growing season and less sensitive later in the growing season when they are instead limited by competition with plant communities.

Methods

Site Description

We leveraged experimental infrastructure from the WaRM Network Experiment. Specifically, we conducted our sampling 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 sites average between 355 – 679 mm precipitation per year and the temperatures average between 14.3 – 8.3 °C yearly, with a noted decrease in temperature and increase in precipitation as you move from low to high elevation sites (Prager et al., 2021). Both low and high elevation sites can be described as open alpine mountain meadow, with near continuous cover of forbs, grasses, and shrubs and with little to no tree cover. The low elevation site is at 2740 m elevation (38.715, -106.823) with the dominant plant species a flowering forb, *Wyethia amplexicaulis*. The high elevation site (3460 m, 38.991, -107.066) is dominated by *Juncus drummondii,* a monocot, grass-like herb.

Treatments

The WaRM experimental design is a 2 × 2 factorial warming (ambient vs. warmed) × dominant plant species removal (control vs. removed) experiment deployed at one high elevation site and one low elevation site, separated by ca. 500 m in elevation. Each of the four treatments are replicated 8 times, for total of 32 plots (2 × 2 m) at each elevation. We accomplish experimental warming with transparent hexagonal open-top chambers (OTCs), 1.5 m in diameter, in the center of each plot. Field-based warming experiments using OTCs are a well-accepted way to examine ecosystem responses to climate changes (Elmendorf, Henry, Hollister, Björk, Bjorkman, et al., 2012). We manually perform dominant species removal at the beginning of each growing season

by clipping the relative dominant species at soil level within and around the plots. Treatments at this site have been deployed each summer (June-August) of the study since 2014 (8 yrs total).

SAMPLING INTERVALS

We collected data at three intervals throughout the summer (June – August) growing season: pre-growing season, peak-growing season, and post-growing season. Pre-season was immediately after snowmelt before the majority of plant biomass has grown, and one week after warming chambers and removal treatments were deployed. Peak-season was at peak plant biomass growth for each site. Post-season was when approximately 50% of plant biomass at the sites had senesced.

SOIL SAMPLING

We collected soil samples from each of 32 plots at low and high elevation sites during the three sampling intervals. Three 2.5 cm diameter soil cores were collected randomly from the top 10 cm of the soil profile from each plot, homogenized, and sieved to 2 mm. Soil was partitioned for analyses described below and stored at RMBL facilities until shipped to University of Texas El Paso (UTEP) for analysis. Samples for microbial biomass analyses were stored at -20 °C, for enzyme analyses at -80 °C, and for community level physiological profile analyses at 4 °C. For each sampling interval, 5 g of sieved soil was dried in RMBL drying ovens at 60 °C for fresh weight-dry weight calculations.

ENVIRONMENTAL VARIABLES

Air temperature and soil temperature were recorded daily throughout the summer using iButton data loggers (Maxim Integrated Corp, USA) buried 5 cm below and suspended 5 cm above the soil surface. A Hydrosense soil moisture probe was used to measure soil volumetric moisture at approx. 10 cm depth at the time of the three soil sampling intervals. NDVI was also measured

at soil samplings using a RapidSCAN (Holland Scientific, USA) with 4 measurements taken from the corner of each plot and averaged together.

SOIL RESPIRATION

We measured soil respiration weekly using an EGM-5 Portable CO2 Gas Analyzer (PP Systems, USA). The analyzer was sealed against a 10 cm diameter PVC collar inserted into the soil and living plant material removed from within.

COMMUNITY LEVEL PHYSIOLOGICAL PROFILE

Microbial metabolism was analyzed for collected soils on a community level using a modified Biolog EcoPlate assay (Garland, 1996). ECO Plates contain replicates of 31 different C sources (polymers, amines, carbohydrates, carboxylic acids, amino acids, and miscellaneous) tagged with a tetrazolium redox dye (Biolog, USA), and the rate of metabolism for each microbial community creates a characteristic metabolic fingerprint for each sample analyzed. 4 g of soil was suspended with 36 mL of potassium phosphate buffer and shaken for 30 min. Soil suspensions were left to settle for 30 min before creating a 10^{-2} dilution with buffer. 150 uL of this dilution was pipetted into the 96 well ECO plates with each plate containing 3 samples. Plates were incubated at 22 °C for 72 h before being read (Synergy HT BioTek plate reader). 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).

MICROBIAL BIOMASS C AND N

We quantified MBC and MBN of collected soils using a modification of the chloroform fumigation method (Brookes et al., 1985). 5 g of recently thawed soil was incubated in a stoppered 250 mL Erlenmeyer flask with 2 mL chloroform for 24 h. The fumigated samples were extracted using 25 mL of 0.5 M K_2SO_4, shaken for 2 h, and then filtered through glass filter paper. Extracts

were analyzed for extractable organic carbon (EOC) and extractable total nitrogen (ETN) using a Schimadzu CN analyzer (Shimadzu Scientific Instruments Inc., USA). Non-fumigated extracts followed the same procedure described above minus the addition of chloroform. Microbial flushes representing MBC and MBN were calculated as the difference between EOC and ETN in fumigated and non-fumigated extracts. Corrections factors were not applied because they are unknown for this ecosystem or soil type.

POTENTIAL EXOENZYME ACTIVITY

Extracellular enzyme, or "exoenzyme", activity on collected soils was measured via microplate assays (Mclaren et al., 2017; Saiya-Cork et al., 2002). Activity of hydrolytic enzymes, including cellulose-degrading β-glucosidase (β-gluc) and cellobiohydrolase (Cello), hemicellulose-degrading β–xylosidase (β-xylo), carbohydrate-degrading α-glucosidase (α-gluc), chitin-degrading N-acetyl-glucosaminidase (NAG), an amino acid with N-terminal end degrading enzyme (LAP), phosphatase (Phos), and phosphodiesterase (PhosD), were measured using fluorescently tagged substrates. A slurry was created by blending 1 g of recently thawed soil with 125 mL of sodium acetate buffer adjusted to the site-specific soil pH (6.3 for low site, 4.4 for high site). The slurry was pipetted into black microplates, mixed with the fluorescently tagged substrates, and incubated for 3.5 h with measurements taken at approximately 30 min intervals (Synergy HT BioTek plate reader). Oxidative enzymes phenol oxidase (Phenol) and peroxidase (Perox) were quantified by looking at the degradation of a L-3, 4-dihydroxyphenylalanine substrate. Color absorbance was measured after approximately 24 h of incubation at 5 °C (Synergy HT BioTek plate reader).

Statistical Analysis

All statistical analyses were performed using R and R-Studio (R version 4.0.5). For air and soil temperature, the daily average temperature was calculated from the hourly readings from iButtons. Then, a plot average for the summer growing season was calculated using the daily averages. We ran a 2-way factorial ANOVA with warming and removal treatments as the main factors on the plot averages for soil and air temperature.

For NDVI and soil moisture measurements, as well as for each of the microbial variables analyzed, we ran a 4-way fully factorial ANOVA with the main factors (1) elevation (2) sampling interval (3) warming treatment and (4) 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.

Results

Environmental Variables

The OTCs resulted in significantly higher soil (Figure 1a) and air temperatures (Figure 1b) in warmed than ambient plots throughout the summer. For soil moisture, there was a significant elevation × sampling interaction because although moisture was highest for both sites in the pre-season sampling, the effect of elevation on soil moisture depended on the sampling period ($F_{2, 168}$ = 12.06, p = 0.000; Supplementary Figure 1). In the pre- and post- season, the low site had significantly higher soil moisture, but during the peak season the low site had significantly lower soil moisture (Supplementary Figure 1). Warming treatments significantly reduced NDVI, but only at the high elevation site (warming × elevation interaction; $F_{1, 140}$ = 9.33, p = 0.003; Supplementary Figure 2a). Removal treatments effectively reduced the cover of dominant plant species (N. Sanders and A. Classen, unpublished data), but resulted in lower NDVI in removal plots only at peak season (significant removal x sampling interaction; $F_{2, 140}$ = 4.58, p = 0.012; Supplementary Figure 2b). NDVI was consistently higher at the high than the low site throughout the growing season, although there was a significant elevation × sampling interaction ($F_{1, 140}$ = 28.10, p = 0.000), because the low site could not be sampled in the pre-season due to equipment issues (Supplementary Figure 2c).

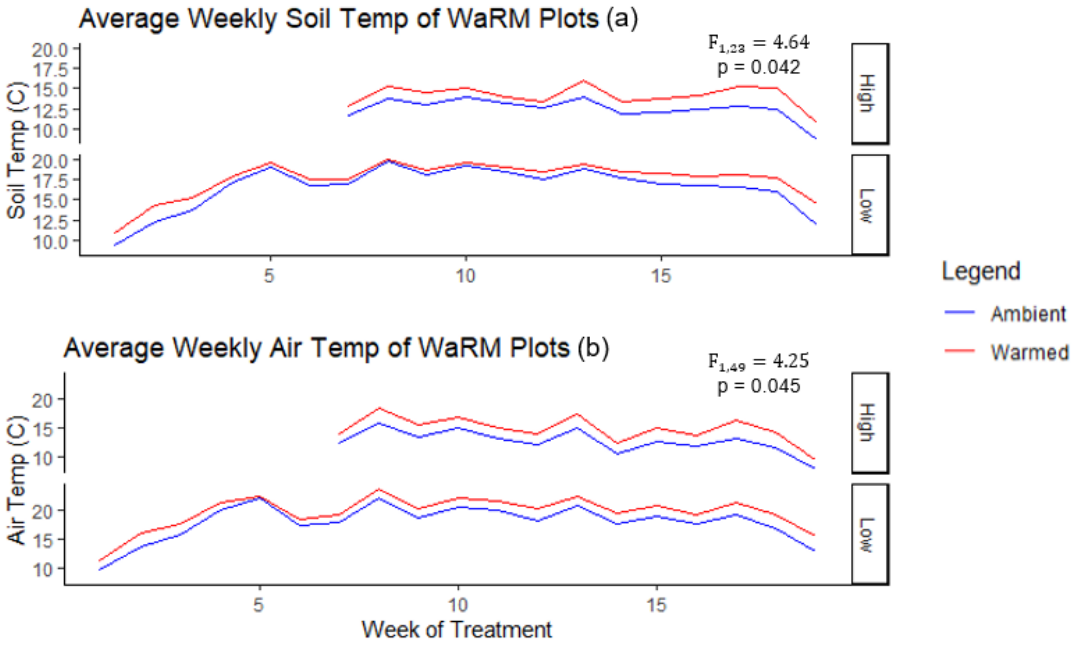

Figure 1: Soil (a) and air (b) temperature responses to warming (OTCs) at 2 elevational sites in an 8-year warming and dominant species removal experiment in alpine meadow of Colorado. Temperatures were logged approx. every hour in plots by iButtons at 5 cm depth for soil, and suspended 5cm above soil level for air. Plotted are weekly averages of the daily average soil and air temperature. Data for the high site begins at Week 7 as treatments are deployed later than the low site due to later snow melt. F- and p-values presented are effects of warming on the seasonal average of plot daily temperature averages in a 2-factor (warming and removal) ANOVA. There were no effects of removal, nor the warming by removal interaction, on temperature.

MICROBIAL RESPONSES

Across microbial response variables, in general there were strong responses to elevation and sampling interval, with fewer responses to warming or removal treatments (Table 1, Supplementary Table 1). For variables that did respond to warming or removal, it was typically also in a higher order interaction with another factor (Table 1, Supplementary Table 1). When warming and removal interacted with each other, it was often because there was only an effect of warming under removal treatment (Table 2).

Table 1: P-values from a 4-Way ANOVA on microbial response variables measured from an 8-year warming (OTCs) and dominant species removal experiment in alpine meadow of Colorado. Significant effects of treatments are highlighted dark yellow. When a treatment is involved in a significant higher order interaction (e.g., elevation x sampling) for a particular variable, only the interaction is highlighted and not the individual treatment effects. Marginally significant effects are represented by light yellow highlight. Because very few 3-factor or higher interactions were significant, they are not presented in this table and can be found in Supplementary Table 1. Similarly, 2-factor interactions with no significant results for any variable (e.g., Sampling x Warming) were also removed from the table and can be found in Supplementary Table 1.

Category	Variable	Warming	Removal	Elevation	Sampling	Warming: Removal	Warming: Elevation	Removal: Elevation	Elevation: Sampling
Microbial Activity	Soil Respiration	**0.001**	0.264	0.388	0.000	0.462	0.255	0.158	**0.000**
Community-Level Physiological Profile	Functional Diversity	0.775	0.426	0.000	0.000	0.094	0.940	0.122	**0.004**
Microbial Biomass	Microbial Biomass C	0.121	0.001	**0.000**	**0.000**	**0.023**	0.959	0.979	0.097
	Microbial Biomass N	**0.040**	**0.006**	0.080	0.000	0.077	0.894	0.784	**0.007**
Carbon-Acquiring Enzymes	β-glucosidase	0.409	0.221	0.000	0.362	**0.007**	0.090	0.938	**0.000**
	Cellobiohydrolase	0.165	0.653	0.000	0.538	**0.003**	0.180	0.945	**0.000**
	β–xylosidase	0.122	**0.031**	0.000	0.246	0.058	**0.025**	0.178	**0.000**
	α-glucosidase	0.368	0.317	**0.000**	0.132	0.551	0.124	0.248	0.424
Nitrogen-Acquiring Enzymes	N-acetyl-glucosaminidase	0.025	0.261	0.444	0.014	0.251	**0.044**	0.970	**0.000**
	LAP	0.628	0.500	0.000	0.000	0.213	0.350	0.794	**0.000**
Phosphorous-Acquiring Enzymes	Phosphatase	0.118	0.831	0.030	0.047	0.340	0.068	0.534	**0.000**
	Phosphodiesterase	0.027	0.344	0.000	0.003	**0.049**	**0.047**	0.785	**0.000**
Oxidative Enzymes	Phenol Oxidase	0.259	0.642	0.000	0.000	0.556	0.324	0.738	**0.000**
	Peroxidase	0.749	0.020	0.000	0.000	0.480	0.585	**0.011**	0.547

Table 2: Responses to the interaction between warming (OTCs) and dominant species removal treatments for multiple microbial response variables in an 8-year warming experiment in alpine meadow of Colorado. Grey shaded boxes indicate that the interaction was not significant, so independent responses to warming are presented without respect to removal treatment. When interactions between warming and removal treatments were significant, significant differences (t-test) between ambient and warmed plots within control or removal conditions are indicated with a 0 (no effect of warming), + (positive effect) or – (negative effect). In the case of Cellobiohydrolase, there was also a complex 3-factor interaction with warming x removal x elevation, but we present only the warming x removal interaction here and the 3-factor interaction is explained in the text.

Category	Variable	Control	Removed
Microbial Activity	Soil Respiration	+	+
Community-Level Physiological Profile	Functional Diversity	0	0
Microbial Biomass	Microbial Biomass C	0	- *(marginal)*
	Microbial Biomass N	-	-
Carbon-Acquiring Enzymes	β-glucosidase	0	-
	Cellobiohydrolase ***	0	-
	β–xylosidase	0	0
	α-glucosidase	0	0
Nitrogen-Acquiring Enzymes	N-acetyl-glucosaminidase	-	-
	LAP	0	0
Phosphorous-Acquiring Enzymes	Phosphatase	0	0
	Phosphodiesterase	0	-
Oxidative Enzymes	Phenol Oxidase	0	0
	Peroxidase	0	0

SOIL RESPIRATION

There was a significant influence of warming and a 2-way interaction between elevation × sampling for soil respiration (Table 1, Supplementary Table 1). Warming resulted in higher soil respiration rates (Figure 2, Table 2). The elevation × sampling interaction is because soil respiration rates were higher at the high site but only during the peak-growing season sampling (Supplementary Figure 1, Supplementary Table 2).

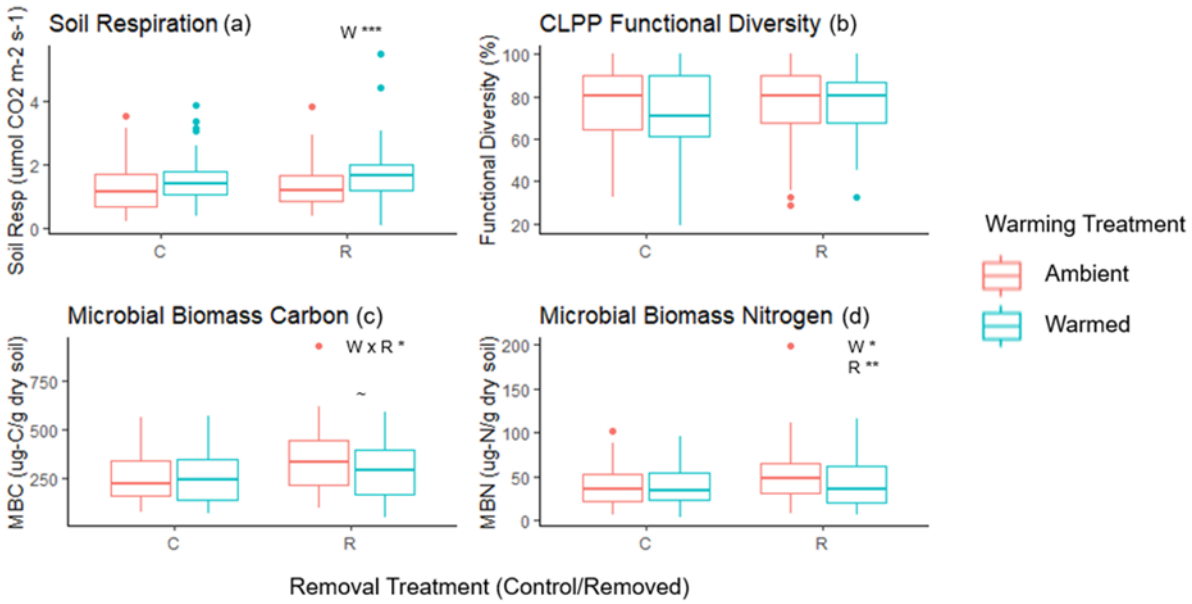

Figure 2: Responses of soil respiration (a), CLPP functional diversity (b), MBC (c), and MBN (d) to warming (OTCs) and dominant species removal treatments in an 8-year warming experiment in alpine meadow of Colorado. Significance of effects of warming (W), removal (R), or any interactions between these 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 control and removal treatments are noted with stars above the pair of ambient and warmed bars: ~ $0.1 < p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

COMMUNITY LEVEL PHYSIOLOGICAL PROFILE

For CLPP functional diversity, there were no significant effects of warming or removal, nor any interaction between the two factors (Table 1, Table 2, Supplementary Table 1). There was a 2-way interaction between elevation x sampling on functional diversity (Table 1, Supplementary Table 1) because the high elevation site had higher functional diversity than the low elevation site, with the size of the difference increasing across the growing season (Supplementary Figure 1, Supplementary Table 2).

MICROBIAL BIOMASS C AND N

There was significant influences of elevation and sampling individually and a 2-way interaction between warming × removal on MBC (Table 1, Supplementary Table 1). The high

elevation site had consistently lower MBC in comparison to the low site (Supplementary Figure 1, Supplementary Table 2). Additionally, MBC was highest at the pre-season sampling for both elevations (Supplementary Figure 1, Supplementary Table 2). The warming × removal interaction is because warming significantly lowers MBC but only under removal conditions (Figure 2, Table 2).

There were significant influences of warming and removal individually and a 2-way interaction between elevation × sampling on MBN (Table 1, Supplementary Table 1). Warming resulted in lower MBN regardless of removal treatment (Figure 2, Table 2) and removal treatments resulted in higher MBN (Figure 2). The elevation x sampling interaction is because the low elevation site had higher MBN but only during pre- and post-season sampling and not during the peak-season (Supplementary Figure 1, Supplementary Table 2).

POTENTIAL EXOENZYME ACTIVITY

Few enzymes responded to either warming or removal, and for those that did there was also usually an interaction between the two variables (Table 1, Supplementary Table 1). β-gluc and PhosD both show a negative response to warming only under removal conditions (significant Warming × Removal interaction; Figure 3, Table 1, Table 2, Supplementary Table 1). Removal increased β-xylo activity regardless of warming treatment (Table 1, Supplementary Table 1).

There was a complex 3-way interaction between elevation × warming × removal on Cello (Table 1, Supplementary Table 1) because warming caused a negative response of Cello only under removal treatment and only at the high elevations site (Table 2, Supplementary Figure 2). There was also an elevation × warming interaction for the enzymes β–xylo, NAG, and PhosD, because there was no significant difference between ambient and warmed plots at the low elevation, but a

negative effect of warming on activity at the high elevation (Figure 3, Table 1, B-xylo low site; p=0.42, high site; p=0.05).

There were a significant number of elevation × sampling interactions (Supplementary Table 1). The interactions varied between enzymes, with some showing strong differences in activity at different elevation sites consistently over growing season sampling (LAP), and for others the strongest difference in activity between elevation sites was during the pre-growing season (NAG), during the peak-growing season (β-gluc, β–xylo, Phos, PhosD), or during the post-growing season sampling (Phenol) only (Supplementary Figure 3, Supplementary Table 2).

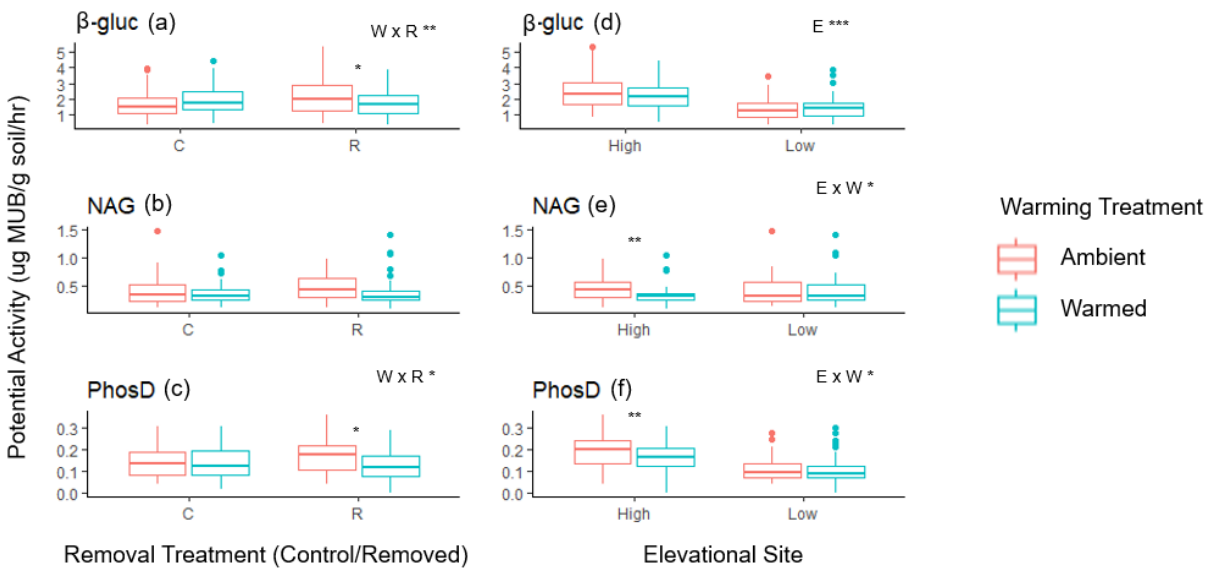

Figure 3: Potential enzyme activity responses to warming (OTCs) and dominant species removal treatments and elevation in an 8-year warming experiment in alpine meadow of Colorado. Enzymes presented include one C-acquiring enzyme (β-gluc, a and d), one N-acquiring enzyme (NAG, b and e), and one P-acquiring enzyme (PhosD, c and f; enzyme selections chosen based on Sinsabaugh and Shah 2013). 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 the control or removal treatments (a-c) or within each elevation site (d-f) are noted with stars above each pair of bars: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Discussion

The warming of the earth's atmosphere demands research to improve predictions of C cycling to determine if systems, specifically at high latitudes and altitudes, will generate positive feedback to CO_2 levels and atmospheric warming. The objectives of this study were to examine how the soil microbial community in the alpine Rocky Mountains of Colorado responded to experimental warming, if changes in plant community would mediate microbial responses to warming, and if these responses would vary by elevation or at different periods in the growing season. We deployed OTCs and dominant plant species removal treatments to analyze multiple soil microbial response variables. Although some variables responded directly to treatments, most variables responded in a context dependent way, emphasizing the importance of plant community compositional changes to warming responses in tundra soil.

Effect of warming

We hypothesized that warming would cause a positive response by microbial functions because microbial activity typically increases with temperature (Kirschbaum, 1995). For soil respiration, we did see an increase under warming regardless of any other factors (i.e., across elevation, sampling interval, and removal treatments). These results are consistent with findings of a meta-analysis of experimental warming projects across high tundra, low tundra, grasslands, and forests, where 2-9 years of experimental warming significantly increased soil respiration rates (Rustad et al., 2001) and support our first hypothesis. Likely, this increase in soil respiration rates is due to the increased availability of labile C sources under relatively short-term warming (Bradford et al., 2008; Kirschbaum, 2004) but this response could change with the magnitude and duration of warming as labile C sources 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).

Even though we saw an increase in the metabolic activity (respiration rate) of the microbial community, we saw a decrease with warming in one of the measures of microbial biomass, MBN. Jonasson et al. (2004) similarly observed a decrease in MBN with increasing temperature, and Bradford et al. (2008) noted decreases in microbial biomass in general for warmed soils. Previous studies found that alpine microbial biomass is significantly decreased from winter to summer (Broadbent et al., 2021), and if this response is due to temperature changes between seasons, increasing temperatures further in the summer could continue to decrease biomass. However, we only saw a consistent increase in response to warming for microbial biomass in our measures of MBN and decreases by MBC were context dependent, only occurring when the dominant plant species was removed in this study. Therefore, in warmed plots that had the original plant community composition, the change in microbial N with no change in microbial C means an increase in the microbial C:N ratios which could be a result of increased efficiency in N use (Mooshammer et al., 2014) or a decrease in N availability in the soil (Sistla et al., 2012).

In addition to the two variables where warming had a direct effect (soil respiration and MBN), three enzymes (β-xylo, NAG, and PhosD) also responded to warming, although these responses were dependent on elevation. For these enzymes, there was no response to warming at the low elevation site, but a negative response to warming at the high elevation site. This finding supports our hypothesis that the effects of warming will be stronger at the high elevation sites due to a hypothesized stronger temperature limitation, and is consistent with previous studies that find a stronger effect of warming on enzyme activity in colder, vulnerable environments (Meng et al., 2020). However, we predicted a positive response of enzyme activity to warming, rather than the

negative response we observed. In our experiment, we saw lower microbial biomass with warming (as measured by MBN, or a removal-dependent response by MBC), which may be the cause of decreased enzyme activity. The observed decrease in microbial biomass with warming was also hypothesized to be the cause of lower NAG activity under warming treatments in a warming experiment in harvested forest (McDaniel et al., 2013). Further, the microbial community may have been under higher N limitation with warming (as seen by decreased MBN), possibly resulting in less N available for enzyme production, which is often limited by N availability (Schimel & Weintraub, 2003; Sistla et al., 2012). Finally, the differing response to warming at different elevations may be due to shifts in microbial community structure as altitude increases (Margesin et al., 2009).

MEDIATION OF WARMING EFFECTS BY PLANT COMMUNITY COMPOSITION

Plant community composition influences physical soil conditions (Crofts et al., 2018; Mclaren & Turkington, 2010), nutrient inputs (Crofts et al., 2018) and availability (Mclaren & Turkington, 2010; Pan et al., 2016), and microbial community structure (De Long et al., 2016) and consequently plays a significant role in plant-soil-microbe interactions. As ecosystems are currently experiencing changes in plant community composition and structure (Parmesan & Yohe, 2003), we also tested how manipulating the plant community, through 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 in four did we see a significant interaction between warming and removal treatments (MBC, and three enzymes: β-gluc, Cello, and PhosD) that supported our hypothesis that plant community composition would be important in mediating warming responses. Further, for all four variables there was a warming by removal interaction because responses to warming were only significant when the dominant plant species was

removed. We know that plant species strongly influence soil microbial community composition and activity (De Long et al., 2016; Hernández-Cáceres et al., 2022), as seen by strong effects of vegetation shifts on microbial activity (D'Alò et al., 2021; Henry, 2012) and composition (Xiang et al., 2018). For our four variables, we did not see direct effects of warming but did see warming effects with removal, possibly because the dominant plant control over microbial function was stronger than any responses to warming, and removing the dominant species also removed this control. Additionally, both microbial biomass (MBC) and the three enzymes responded negatively to warming when the dominant species was removed. The decrease in microbial biomass with combined warming and removal treatments may explain the decrease in potential enzyme activity for β-gluc, Cello, and PhosD, as there were fewer microbes to produce these enzymes in treated plots. Finally, removing plants from the system also likely resulted in decreases of C inputs to the soil through plant litter and root exudates, contributing to the decrease in MBC and C-resources necessary for microbial activity.

In addition to the enzymes above which responded to warming only under removal conditions, for the enzyme Cello we also saw an interaction between warming, removal, and elevation. The Cello response was similar to warming by removal enzyme responses discussed above but was restricted to the high elevation site. This aligns with our hypothesis that the effect of warming and removal would be stronger at the high elevation site because temperature is typically a limiting factor to plant growth and diversity at that site, therefore manipulating both temperature and plant composition would invoke a stronger response in the soil microbial community at the high elevation site.

Finally, there were 2 microbial response variables that were increased by plant removal alone, with no higher order interactions (MBN, and β-xylo). β -xylo is an enzyme released by

microbes to increase C-acquisition, and its increase in activity suggests that soil microbes were C mining to increase the pool of available C in the soil when the dominant plants were removed. C and N cycles are often coupled and measurements of one can allow us to make inferences about the other. 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. This could be explained by the observed decrease in NDVI with removal at peak season, implying lower plant biomass and potentially lower C input into the soil with the removal treatment. Schmidt et al. (1999) found that microbial nutrients increased when plants were excluded due to the decrease in competition for nutrients between plants and microbes. Previous studies have found that changes in plant community composition can cause shifts belowground in microbial community structure (Fanin et al., 2022; Meng et al., 2020; Xiang et al., 2018), which could explain why we observed an increased in MBN.

INTRA-SEASONAL EFFECT OF WARMING

High latitude and elevation systems are known for their long, cold winters and short summers. The shift from winter to summer is accompanied by a change in dominance over system processes from microbes in the winter when soil is insulated by snowpack and plants are unable to grow, to plants in the summer when snow and frozen soil has thawed, and temperatures allow plant growth (Edwards & Jefferies, 2013). This seasonal shift is marked by a large crash in microbial biomass immediately after snow melt (Buckeridge et al., 2013; Edwards & Jefferies, 2013; Sistla & Schimel, 2013), which can be accompanied by a flush of available nutrients (Mclaren et al., 2018), or a shift in soil microbial community structure from fungal dominated in winter to bacterial dominated in summer (Björk et al., 2008; Buckeridge et al., 2013). In addition to large shifts in structure and function between winter and summer, studies have also shown that

soil microbial community structure and biomass (Björk et al., 2008; Edwards & Jefferies, 2013), and soil nutrient availability (Edwards & Jefferies, 2013; Mclaren et al., 2018; Weintraub & Schimel, 2005) can vary strongly across the growing season as well. Despite this, our study is one of few that combined intra-seasonal sampling with experimental warming (others include Stark et al., 2018), which complements the seasonal sampling (i.e., winter and growing season) that has also occurred with warming treatments (Sistla & Schimel, 2013). We hypothesized that warming would have the strongest impacts early in the summer season while microbes may still be dominant over ecosystem processes. 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. Although most microbial variables did respond to growing season sampling, suggesting that microbial processes vary throughout the summer season, these effects varied between low and high elevation sites (sampling by elevation interactions) for most variables, and there were no consistent trends for seasonal effects within elevations. Additionally, there were no significant interactions between sampling and warming and our hypothesis of stronger warming effects in the pre-growing season was not supported. This contrasts with a warming study by Stark et al. (2018) that also sampled multiple times across the growing season which found that warming effects on N and P pools and enzyme activity were strongest in the peak-growing season, likely due to influences of the peak plant biomass growth on soil conditions.

Conclusions & Future Directions

In this experimental warming and plant removal experiment, we aimed to determine how soil microbes respond to warming and if those responses are mediated by plant community composition. Although we found fewer responses to warming than expected most of the responses to warming were mediated by the plant community or only seen at high elevation. Our results emphasize the important role that plant community composition will play in soil responses to warming, especially in alpine mountain systems that are characterized by strict range-restriction and species that are adapted to stresses related to elevation, as multiple measures of microbial function showed a significant response to warming only when the dominant plant species was removed. Previous studies have found that microbial responses to warming are strongly related to microbial composition (Fanin et al., 2022; Meng et al., 2020), therefore future studies should investigate changes in soil community composition caused by changing plant composition to better understand the mechanisms that are causing plant community to mediate soil microbial response to warming.

High latitude and elevation systems experience strong changes throughout the winter and summer seasons in climate and concurrent shifts in microbial versus plant dominance over ecosystem processes, as well as changes in microbial community structure. However, we found that warming effects were consistent across our intra-seasonal samplings despite these seasonal shifts in soil and environmental physical conditions. Although we did not find different effects of warming at different times across the season, the significant influence of the timing of the sampling on almost all microbial response variables shows that microbial functions do vary throughout the summer growing season. As we are one of the first studies to combine warming treatments with multiple samples throughout the growing season, future studies in other ecosystems or using other

microbial response variables may find that warming effects differ at different points in the season due to shifts in microbial community structure and function. In addition to sampling warming effects intra-seasonally, 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 microbes, including not only the temperature increases and changing plant communities that we examined here, but also increased atmospheric CO_2, shifts in precipitation trends, altered soil moisture, changes in animal interactions/behavior in an ecosystem, increased N deposition, and more. Additionally, the impact of these factors may vary by season, especially in high altitude and elevation systems where warming is more intense in the winter months (IPCC 2013). We encourage more studies such as ours which combine multiple effects of climate change, which will increase their predictive ability due to possible additional interactions among other global change drivers that will influence the soil carbon response to warming (Bardgett et al., 2008; Henry, 2012).

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Supplementary Tables

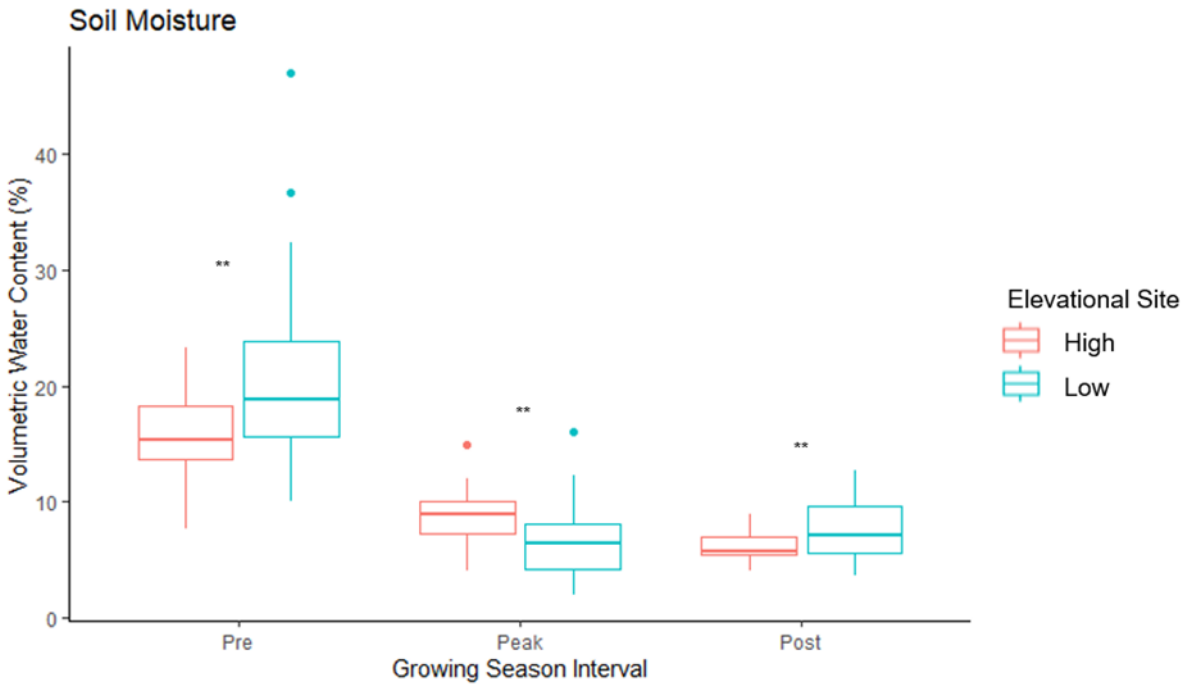
Supplementary Table 1: Results of a 4-Way ANOVA on microbial response variables measured from an 8-year warming (OTCs) and dominant species removal experiment in alpine meadow of Colorado. Degrees of freedom are presented in italics below the factor/interaction title. F-values are presented in the top line of each cell, with p-values in brackets below. Degrees of freedom numerators are presented below the treatment column titles, and degrees of freedom denominators are as follows: for Soil Respiration: 166, and for all other variables: 168. Significant effects of treatments are highlighted dark yellow. When a treatment is involved in a significant higher order interaction (e.g., elevation x sampling) for a particular variable, only the interaction is highlighted and not the individual treatment effects. Marginally significant effects are represented by light yellow highlight.

Category	Variable	Warming *df=1*	Removal *df=1*	Elevation *df=1*	Sampling *df=2*	Warming: Removal *df=1*	Warming: Elevation *df=1*	Warming: Sampling *df=2*	Removal: Elevation *df=1*	Removal: Sampling *df=2*	Elevation: Sampling *df=2*	W:R:E *df=1*	W:R:S *df=2*	W:E:S *df=2*	R:E:S *df=2*	W:R:E:S *df=2*
Microbial Activity	Soil Respiration	**10.71 [0.001]**	1.313 [0.264]	0.748 [0.388]	24.79 [0.000]	0.545 [0.462]	1.306 [0.255]	1.073 [0.344]	2.013 [0.158]	0.047 [0.954]	**8.825 [0.000]**	0.539 [0.466]	0.115 [0.891]	2.389 [0.095]	0.242 [0.785]	0.201 [0.818]
Community-Level Physiological Profile	Functional Diversity	0.082 [0.775]	0.637 [0.426]	46.17 [0.000]	28.78 [0.000]	2.842 [0.094]	0.006 [0.940]	1.129 [0.326]	2.416 [0.122]	0.226 [0.798]	**5.799 [0.004]**	3.423 [0.066]	1.169 [0.313]	1.207 [0.302]	0.835 [0.436]	1.065 [0.347]
Microbial Biomass	Microbial Biomass C	2.427 [0.121]	12.02 [0.001]	31.55 [0.000]	54.88 [0.000]	**5.258 [0.023]**	0.003 [0.959]	1.474 [0.278]	0.001 [0.979]	0.699 [0.655]	2.805 [0.097]	2.284 [0.133]	0.046 [0.991]	0.517 [0.755]	0.252 [0.598]	0.371 [0.395]
	Microbial Biomass N	**4.283 [0.040]**	**7.866 [0.006]**	3.104 [0.080]	75.75 [0.000]	3.169 [0.077]	0.018 [0.894]	2.562 [0.086]	0.075 [0.784]	0.507 [0.797]	**5.561 [0.007]**	1.307 [0.255]	0.341 [0.866]	0.793 [0.906]	0.643 [0.259]	0.076 [0.746]
Carbon-Acquiring Enzymes	β-glucosidase	0.685 [0.409]	1.506 [0.221]	66.45 [0.000]	1.022 [0.362]	**7.566 [0.007]**	2.900 [0.090]	0.306 [0.736]	0.006 [0.938]	0.963 [0.384]	**18.26 [0.000]**	1.578 [0.211]	0.278 [0.758]	0.414 [0.662]	1.972 [0.142]	0.230 [0.795]
	Cellobiohydrolase	1.948 [0.165]	0.203 [0.653]	87.40 [0.000]	0.622 [0.538]	8.998 [0.003]	1.814 [0.180]	0.580 [0.561]	0.005 [0.945]	0.469 [0.626]	8.769 [0.000]	**4.336 [0.039]**	0.155 [0.856]	0.960 [0.385]	1.958 [0.144]	0.354 [0.703]
	β–xylosidase	2.420 [0.122]	**4.750 [0.031]**	195.4 [0.000]	1.415 [0.246]	3.631 [0.058]	**5.105 [0.025]**	0.141 [0.868]	1.826 [0.178]	0.697 [0.499]	**11.68 [0.000]**	1.461 [0.228]	0.292 [0.747]	0.549 [0.579]	1.205 [0.302]	0.422 [0.656]
	α-glucosidase	0.815 [0.368]	1.006 [0.317]	**96.52 [0.000]**	2.052 [0.132]	0.357 [0.551]	2.396 [0.124]	0.599 [0.551]	1.345 [0.248]	0.738 [0.480]	0.863 [0.424]	0.373 [0.542]	1.361 [0.259]	0.359 [0.699]	1.062 [0.348]	0.037 [0.963]
Nitrogen-Acquiring Enzymes	N-acetyl-glucosaminidase	5.107 [0.025]	1.270 [0.261]	0.588 [0.444]	4.408 [0.014]	1.326 [0.251]	**4.119 [0.044]**	0.054 [0.947]	0.001 [0.970]	0.214 [0.807]	**19.89 [0.000]**	2.454 [0.119]	0.581 [0.561]	0.278 [0.757]	0.647 [0.525]	0.875 [0.419]
	LAP	0.235 [0.628]	0.457 [0.500]	1,357 [0.000]	17.13 [0.000]	1.562 [0.213]	0.877 [0.350]	0.327 [0.721]	0.068 [0.794]	1.655 [0.194]	**17.61 [0.000]**	1.518 [0.220]	0.508 [0.603]	0.561 [0.572]	1.530 [0.220]	0.606 [0.547]
Phosphorous-Acquiring Enzymes	Phosphatase	2.464 [0.118]	0.046 [0.831]	4.797 [0.030]	3.124 [0.047]	0.915 [0.340]	3.372 [0.068]	0.571 [0.566]	0.388 [0.534]	0.481 [0.619]	**14.73 [0.000]**	2.385 [0.124]	0.940 [0.392]	0.035 [0.965]	0.890 [0.413]	0.186 [0.830]
	Phosphodiesterase	4.976 [0.027]	0.899 [0.344]	60.48 [0.000]	6.096 [0.003]	**3.929 [0.049]**	**4.011 [0.047]**	0.152 [0.859]	0.075 [0.785]	0.245 [0.783]	**8.443 [0.000]**	0.000 [0.991]	1.002 [0.369]	0.347 [0.707]	0.209 [0.811]	0.947 [0.390]
Oxidative Enzymes	Phenol Oxidase	1.285 [0.259]	0.217 [0.642]	50.05 [0.000]	8.305 [0.000]	0.347 [0.556]	0.980 [0.324]	1.703 [0.185]	0.112 [0.738]	0.122 [0.886]	**8.099 [0.000]**	0.518 [0.473]	0.104 [0.901]	1.377 [0.255]	0.171 [0.843]	0.165 [0.848]
	Peroxidase	0.103 [0.749]	5.488 [0.020]	504.3 [0.000]	**13.20 [0.000]**	0.500 [0.480]	0.300 [0.585]	0.004 [0.996]	**6.577 [0.011]**	0.736 [0.481]	0.606 [0.547]	1.230 [0.269]	0.912 [0.404]	0.812 [0.446]	0.278 [0.758]	0.462 [0.631]

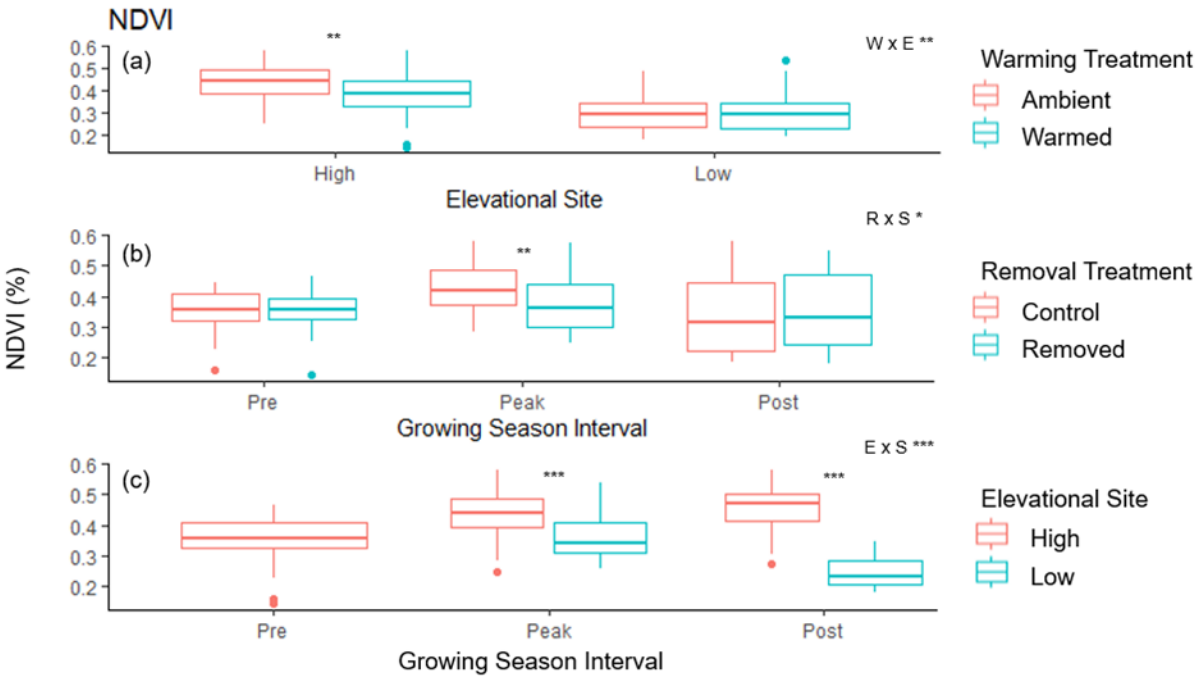
Supplementary Table 2: Responses of several microbial response variables to the interaction between elevation and growing season sampling intervals in an 8-year warming (OTCs) and dominant species removal experiment in alpine meadow of Colorado. Variable averages are presented with grey shaded boxes indicating that the interaction between elevation and sampling is significant.

Variable	Pre-Growing Season		Peak-Growing Season		Post-Growing Season	
	Low	High	Low	High	Low	High
Soil Respiration (umol CO2 m-2 s-1)	1.879	1.508	1.435	2.065	0.989	0.954
Functional Diversity (%)	81.75	90.42	64.42	73.69	58.42	82.16
Microbial Biomass C (ug-C/g dry soil)	424.2	373.4	318.9	180.0	244.4	169.6
Microbial Biomass N (ug-N/g dry soil)	67.70	70.34	34.03	34.80	38.06	19.35
β-glucosidase (ug MUB/g soil/hr)	1.939	1.906	1.109	2.615	1.104	2.359
Cellobiohydrolase (ug MUB/g soil/hr)	0.356	0.462	0.208	0.593	0.180	0.558
β–xylosidase (ug MUB/g soil/hr)	0.281	0.493	0.151	0.690	0.149	0.577
α-glucosidase (ug MUB/g soil/hr)	0.083	0.016	0.066	0.012	0.078	0.028
N-acetyl-glucosaminidase (ug MUB/g soil/hr)	0.613	0.316	0.279	0.426	0.364	0.443
LAP (ug MC/g soil/hr)	0.474	0.024	0.327	0.025	0.429	0.023
Phosphatase (ug MUB/g soil/hr)	2.611	1.871	1.127	2.423	1.725	2.180
Phosphodiesterase (ug MUB/g soil/hr)	0.155	0.173	0.078	0.179	0.090	0.177
Phenol Oxidase (nmol/g soil/h)	4.968	0.000	0.699	0.004	4.036	0.093
Peroxidase (nmol/g soil/h)	17.95	50.83	6.789	43.28	8.604	45.22

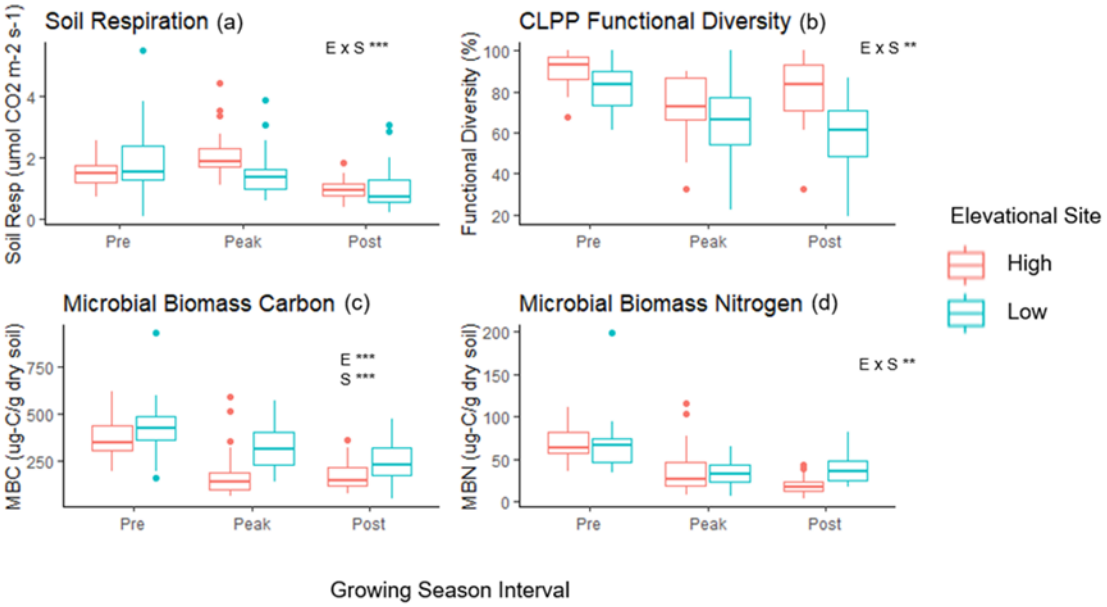
Supplementary Figures



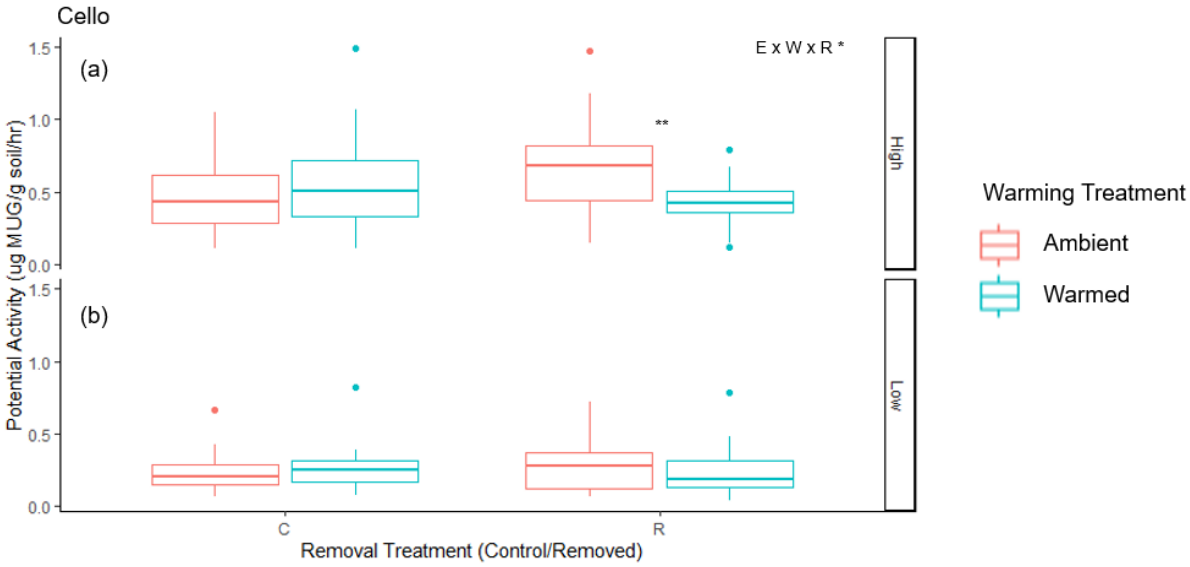
Supplementary Figure 1: Response of soil moisture to elevation and growing season sampling intervals in an 8-year warming (OTCs) and dominant species removal experiment in alpine meadow of Colorado. There was a significant interaction between elevation and growing-season sampling interval ($F_{2, 168} = 12.06$, $p = 0.000$). Significant differences (t-test) between low and high elevation at each sampling interval are noted with stars above each pair of bars: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



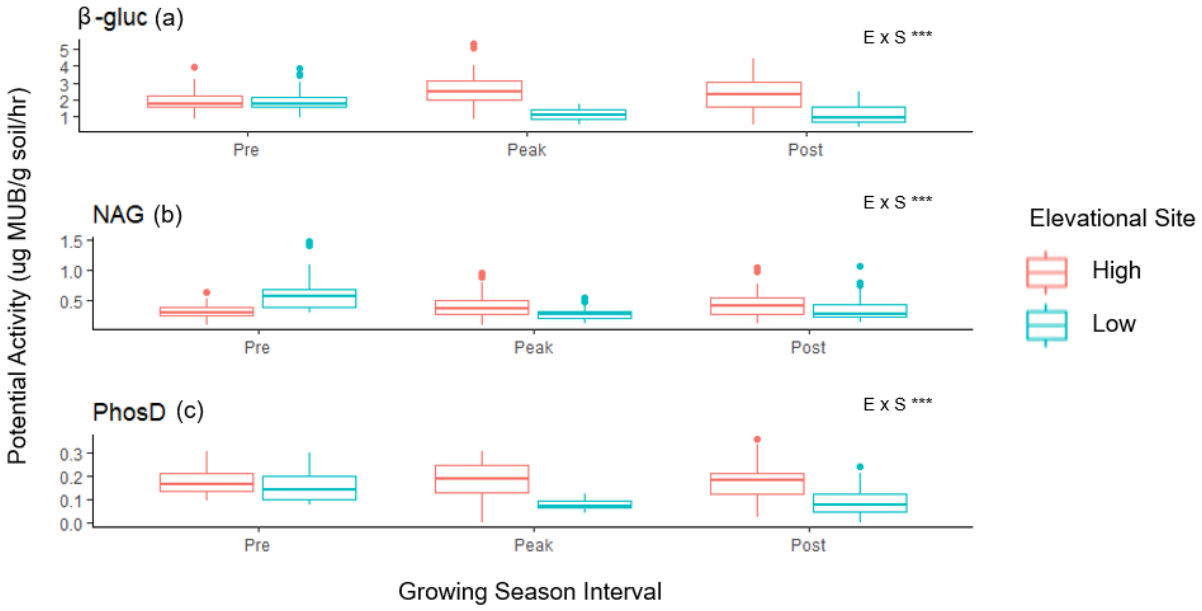
Supplementary Figure 2: Response of NDVI to experimental warming, plant removal, growing season interval, and elevation in an 8-year warming (OTCs) and dominant species removal experiment in alpine meadow of Colorado. Significant interactions between warming (W), removal (R), elevation (E), and growing-season sampling interval (S) are noted in the top right of each plot with stars. Significant differences (t-test) between bars are noted with stars above each pair of bars: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplementary Figure 3: 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 alpine meadow of Colorado. Significant effects of elevation (E), growing-season sampling interval (S), or any interactions between these treatments are noted with stars at the top right of each plot: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplementary Figure 4: Response of C- acquiring enzyme Cello potential activity to warming (OTCs) and dominant species removal at high and low elevation sites from an 8-year warming and removal experiment in alpine meadow of Colorado. Significant interaction between elevation (E), warming (W), and removal (R) is noted in the top right of the plot with stars. Significant differences (t-test) between ambient and warmed plots within the control or removal treatments at the high elevational site (a) or at the low elevational site (b) are noted with stars above the pair of ambient and warmed bars: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplementary Figure 5: Potential enzyme activity responses to elevation and growing season sampling intervals in an 8-year warming (OTCs) and dominant species removal experiment in alpine meadow of Colorado. Enzymes presented include one C-acquiring enzymes (β-gluc, a), one N-acquiring enzymes (NAG, b), and one P-acquiring enzymes (PhosD, c). Significant effects of elevation (E), growing-season sampling interval (S), or any interactions between these are noted with stars at the top right of each plot: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Vita

Sydne Spinella completed her B.Sc. in Environmental Sciences (minor in Sustainability) degree at the University of Tampa in Tampa, Florida in May 2018. Before coming to the University of Texas at El Paso (UTE), Sydne worked for the Florida Aquarium and an environmental nonprofit organization, Keep Pinellas Beautiful. She was accepted into the M.S. Environmental Science program at UTEP in the spring of 2020 and began the program in fall 2020 under the advisory of Dr. Jennie McLaren. Throughout her time as a graduate student, Sydne worked as a Graduate Research Assistant in the Plant Ecology lab and a Graduate Teaching Assistant for the following courses: Research Experience in Environmental Science 1 & 2, Special Topics in Geological Sciences – Geo-forensics. Sydne was granted a fellowship at the Rocky Mountain Biological Laboratory and received the following grants and awards: UTEP Les and Harriet Dodson Research Grant, UTEP Geology Department Scholarship, UTEP Summer Graduate Education Enhancement Award, Betty Dowse Memorial Scholarship from the Sun City chapter of the Association of Women Geoscientists. Sydne also served as the Vice President for the Biology, Environmental, and Engineering Graduate Student Group (BEE) at UTEP, whose goal is to engage with and advocate for graduate students.

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