Seasonal effects of long-term warming on ecosystem function and bacterial diversity

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1 Abstract

Across biomes, soil biodiversity promotes ecosystem functions. However, whether this relationship will be maintained under climate change is uncertain. Here, using two long-term soil warming experiments, we investigated how warming affects the relationship between ecosystem functions and bacterial diversity across seasons, soil horizons, and warming duration. Soils were sampled from these warming experiments located at the Harvard Forest Long-Term Ecological Research (LTER) site, where soils had been heated +5 °C above ambient for 13 or 28 years at the time of sampling. We assessed seven measurements representative of different ecosystem functions and nutrient pools. We also surveyed bacterial community diversity. We found that ecosystem function was significantly affected by season, with autumn samples having higher function than summer samples. The effect of warming on bacterial diversity was similarly affected by season, where warming in the summer was associated with decreased bacterial evenness in the organic horizon. Despite the decreased bacterial diversity in the warmed plots, we found that the relationship between ecosystem function and bacterial diversity was unaffected by warming or warming duration. Our findings highlight that season is a consistent driver of ecosystem function as well as a modulator of climate change effects on bacterial community diversity.

2 Introduction

Climate change is driving losses in biodiversity, and these losses threaten both ecosystem productivity (Tilman et al. 2012) and stability (Naeem et al. 1997; Rohner et al. 2024). While there is evidence that bacterial diversity promotes ecosystem function, not enough is known about how increased temperatures associated with climate change will affect this relationship. Studies examining the effects of warming have revealed decreases in both diversity and ecosystem function (Wu et al. 2022), a weaker relationship between diversity and ecosystem function (Hong et al. 2021), or even abiotic modulators of the diversity-ecosystem function relationship (Domeignoz-Horta et al. 2020). Increased temperatures also alter interactions between taxa in microbial communities (Anthony et al. 2021; Van Nuland et al. 2020), which might explain why at higher temperatures decreases in diversity have disproportionate effects on ecosystem function (García et al. 2018). Much of the basis for the current understanding of the effects of warming on the relationship between biodiversity and ecosystem function (BEF) has been gleaned from laboratory incubations or manipulated communities, which has limited our ability to accurately predict future ecosystem productivity. Clarifying whether the dual stresses of increased temperature and biodiversity loss affect ecosystem function in natural soils in the field is therefore critical to gaining a clearer understanding of the potential impacts of climate change.

Long-term global change experiments, like those at Harvard Forest Long-Term Ecological Research (LTER) site, provide a unique opportunity to examine long-term soil warming in a field setting. Given that the effects of warming on ecosystem function can change or compound over time (Hong et al. 2021; Melillo et al. 2017), maintaining and studying long-term climate change experiments is critical to the ability to predict ecosystem function. Additionally, microbial community compositional shifts resulting from climate change can take over a decade to manifest (DeAngelis et al. 2015; Frey et al. 2008). How microbial communities respond to climate change is influenced by the duration of stress, with acclimation theorized to occur in the short-term and adaptation theorized to occur in the long-term (Martiny et al. 2023). Two soil warming experiment was established in 1991 and 2006 at the Harvard Forest, where soils have been

continuously heated +5 °C above ambient along with control soils that have not been heated. Both the soil carbon quantity and quality has declined in response to heating (Melillo et al. 2017), resulting in an increase in lipid concentration in the heated mineral soils (Pold et al. 2017). The effects of warming on soil respiration at these sites has been nonlinear over decadal time scales at Harvard Forest (Knorr in review; Melillo et al. 2017), where there are years when there is a large treatment effect of heating compared to controls and years where there are no differences between heated and control plots. These changes in ecosystem function and nutrient pools have occurred in conjunction with changes to the microbial community. The abundance of Alphaproteobacteria as well as Acidobacteria have increased in the heated plots (DeAngelis et al. 2015), and the overall diversity of the fungal community has decreased in the heated plots (Anthony et al. 2021). Altogether, these changes call into question how the relationship between ecosystem function and bacterial diversity will be altered by global warming.

Climate change alters both abiotic and biotic drivers of ecosystem function, which complicates predicting the response of ecosystem function to climate stresses, such as warming. One approach to addressing and condensing the dissimilar responses of individual ecosystem functions to climate change is the use of ecosystem multifunctionality (EMF) indices, which aggregate individual ecosystem functions into a single index. Compared to single function approaches, which examine ecosystem functions separately, EMF indices are more sensitive to biodiversity changes (Gamfeldt et al. 2008) and can better represent inherent trade-offs between ecosystem functions (Giling et al. 2019). For instance in plant trait simulation study, ecosystem nitrogen use efficiency and litter quality had a negative relationship, indicating a trade-off between these two functions (Fiedler et al. 2021). An EMF meta-analysis that incorporated results from 68 different warming studies observed that warming tends to have positive effects on EMF (Zhou et al. 2020). While kinetic effects are partially driving the positive effect of warming on EMF (García-Palacios et al. 2015), warming-related changes in abiotic conditions, such as more limited substrate availability, also can increase certain ecosystem functions, like enzyme activity (Pold et al. 2017). However, warming does not always have a positive effect on EMF, and when climate change alters abiotic conditions, warming can have a negative effect on EMF. For instance, warming decreases EMF under nitrogen limitation (Dong et al. 2021) or precipitation declines (Ma et al. 2022). Warming can also shift biotic drivers of EMF, like plant community functional traits, which in turn will have negative effects on EMF (Valencia et al. 2018). Microbial diversity and community composition, two of the most studied biotic drivers of EMF, also are influenced by warming, where decreases in dominant taxa due to warming can result in decreases in EMF (Chen et al. 2023). The complex interactions between biotic and abiotic drivers of EMF have limited the integration of climate change into the existing framework of BEF relationships.

Temperate deciduous forests experience marked seasonal differences, and further complicating predictions, the effects of warming on ecosystem function and microbial diversity are also season-dependent. Long-term warming can deepen the active layer of the soil, resulting in faster upticks in soil respiration after the spring thaw (Contosta et al. 2011). Additionally, warmed soils exhibit higher temperature sensitivity of respiration in the spring and winter (Contosta et al. 2013), suggesting that with increasing temperatures, soil CO_2 efflux will increase non-linearly. Microbial biomass similarly has a seasonal cycle that tends to peak in fall (Contosta et al. 2015; Domeignoz-Horta et al. 2023), although decreases in microbial biomass due to warming might alter this cycle (Pold et al. 2017). Extracellular enzyme activity is likewise responsive to the seasonal shifts in the availability of carbon. Following or coinciding with leaf fall, the activity of peroxidase, phenol oxidase, and other oxidative enzymes involved in the decomposition of lignin tends to increase (Baldrian et al. 2013; Domeignoz-Horta et al. 2023). Microbial diversity likewise exhibits seasonal

shifts; however, the season with highest diversity varies from study to study. Summer, with its higher soil temperatures and higher availability of photosynthates as root exudates, has been observed as the season with the highest microbial diversity (Du et al. 2018; López-Mondéjar et al. 2015). Conversely winter has been noted as having the highest microbial diversity, due to the availability of products from the decomposition of fresh leaf litter in the fall (Landesman et al. 2019). Moreover, season is consistently identified as a driver of microbial community composition in forest soils (Kaiser et al. 2010; Contosta et al. 2015), where the community composition shifts along with leaf fall. While during the spring and summer, the community is dominated by organisms that can utilize root exudates, such as Proteobacteria, the fresh influx of leaf litter that occurs during the fall selects for organisms that can decompose lignin, such as Actinobacteria and *Mucilaginibacter* (Kuffner et al. 2012; Landesman et al. 2019; López-Mondéjar et al. 2015). Despite variability in microbial communities and ecosystem functions across seasons, most studies that examine the relationship between ecosystem multifunctionality and microbial diversity utilize a single snapshot of the community and ecosystem at the time of sampling.

Here, we examined how 13 or 28 years of long-term soil warming affected ecosystem multifunctionality and bacterial diversity in two different seasons. We utilized previously published ecosystem function data, as well as additional ecosystem function data paired with bacterial 16S rRNA gene amplicon sequencing to test the hypothesis that warmed soils would have a stronger relationship between bacterial diversity and ecosystem multifunctionality than the control soils.

3 Methods

3.1 Site description and sample collection

Soil samples were collected from two long-term warming soil experiments located at the Harvard Forest in Petersham, MA (42°30′30″N, 72°12′28″W), as described in Domeignoz-Horta et al. 2023. The Prospect Hill Soil Warming Study was established in 1991 (Melillo et al. 2002), and the Soil Warming x Nitrogen Addition (SWaN) Study was established in 2006 (Contosta et al. 2011). Warmed plots are heated continuously +5 °C above ambient using buried resistance cables placed 10 cm below the soil surface and spaced 20 cm apart. Soil samples were collected in 2019 on July 15th and October 19th, from Prospect Hill and SWaN, which had been warmed for 28 and 13 years, respectively, at the time of sampling. The experiments are located adjacent to one another and have the same dominant plant overstory (*Acer rubrum, Betula lenta, Betula papyrifera, Fagus grandifolia, Quercus velutina, Quercus rubra*) and soil type (coarse-loamy incepitsols). Duplicate cores were taken from each plot to 10 cm depth using a tulip bulb corer. Cores were separated into the organic and mineral horizons, duplicate soil cores were pooled by depth increment, roots and rocks were removed, and then the soil was sieved <2 mm. After sieving, samples were taken back to the lab for further analyses. The full sampling design was two sites (Prospect Hill, SWaN) x two treatments (control, heated) x two seasons (summer, fall) x two sampling depths (organic horizon, mineral soil) x five replicate plots for a total of 79 samples (one sample from the SWaN experiment had a mineral horizon that was beyond the reach of the corer for the July sampling).

3.2 Ecosystem functionality measurements and multifunctionality calculation

To assess ecosystem functionality, we utilized five soil properties or ecosystem functions that were measured as a part of Domeignoz-Horta et al. (2023), including microbial biomass carbon, respiration, and the potential

activities of four enzymes: phenol oxidase, peroxidase, β-glucosidase (BG), and N-acetyl-glucosaminidase (NAG). Additionally, we measured total soil organic carbon and total nitrogen. Altogether, there were seven different ecosystem functions or soil properties measured for this study.

To measure total C and N, soils were dried in a 65 °C oven until they reached a constant mass. Constant mass was verified by weighing the soils multiple times throughout the drying process. The drying temperature was selected so soils could be utilized for a separate analysis that was performed in Domeignoz Horta et al. (2023) but not included in this study. After the soils achieved a constant mass, they were then ground to a fine powder using a mortar and pestle. The soil was then weighed and packaged in duplicate into tins, which were run on a Perkin Elmer 2400 Series II CHN Elemental Analyzer with acetanilide as a standard at the University of New Hampshire Water Quality Analysis Lab. Total C and total N or duplicates were averaged.

For microbial biomass carbon measurements, four replicate soil samples were each split into two subsamples, with one group serving as a control and one group which was fumigated with chloroform vapors under vacuum pressure for 24 hours. Dissolved organic carbon (DOC) was then extracted from both the unfumigated and fumigated samples using 15 mls of 0.05 M K_2SO_4 and quantified on a Shimadzu TOC analyzer. Microbial biomass carbon was determined by subtracting the DOC concentration in the unfumigated subsample from the fumigated subsample.

Soil respiration was measured on triplicate subsamples (0.15 or 0.3 g for the organic horizon or mineral soil, respectively) that were placed into Hungate tubes. Tubes were then sealed and incubated at 15 °C for 24 hours. A 30 ml headspace sample was then taken and injected into an infrared gas analyzer (Quantek 906) to measure CO_2 concentrations.

Potential extracellular enzyme activity was measured using fluorescent substrates. Soil slurries were prepared with 1.25 g wet weight soil and 175 mls of 50 mM pH 4.7 sodium acetate in a Waring blender. For the BG and NAG assays, 200 µls of soil slurry was pipetted into black 96 well plates, and for the oxidative enzyme assay, 500 µls of soil slurry was pipetted into deep well plates. Plates were then placed in a 15 °C incubator for 25 minutes to allow for temperature acclimation. This temperature reflects the average air temperature between the summer sampling and the fall sampling. After temperature acclimation, either 50 µls of 4000 µM 4-methylumbelliferyl β-D-glucopyranoside, 50 µls of 2000 µM 4-methylumbellifery N-acetyl-glucosaminidase, or 500 µls of 25 mM L-DOPA + 0.03% H_2O_2 were added to each well. Each plate contained a standard curve as well as a slurry-only control. All plates were read on a SpectraMax M2 plate reader. BG and NAG plates were measured at 360/450 nm excitation/emission wavelengths after substrate addition. Oxidative enzyme plates were incubated for 4 hours after substrate addition, and then 100 µls were removed, transferred to a clear 96 well plate, and read at 460 nm. Enzyme activity was normalized for each sample by the sample's microbial biomass carbon.

Multifunctionality was calculated for the organic horizons and mineral soil in R (R Core Team 2023) using the multifunc package (Byrnes et al. 2014). We included microbial biomass carbon, soil respiration, total carbon, total nitrogen, N-acetyl glucosaminidase activity, β-glucosidase activity, and oxidative enzyme activity in the ecosystem multifunctionality index. These functions represent both process rates (respiration, potential enzyme activity) and nutrient pools (total C, total N, microbial biomass) (Garland et al. 2021). The organic horizon and mineral soil layers were analyzed separately due to documented differences in soil parameters (Pold et al. 2017) and microbiome properties (Roy Chowdhury et al. 2021; DeAngelis et al. 2015). A multifunctionality index was calculated for each sample by taking the average of the z-score transformed ecosystem functions. While there are different ways of assessing diversity-multifunctionality relationships,

we selected the averaging approach for ease of interpretation. Individual ecosystem functions' relationship with diversity was also investigated. For this approach, the raw untransformed function measurements were used.

3.3 DNA extraction and library preparation

Bacterial diversity was measured using 16S ribosomal RNA (rRNA) gene amplicon sequencing. DNA was extracted from soils using the Qiagen Powersoil kit following the manufacturer's protocol, and DNA concentration was measured using the Picogreen dsDNA kit (ThermoFisher Scientific).

We sequenced the 16S rRNA gene V4 region using the primers 515F (5' - GTG YCA GCM GCC GCG GTA A- 3') and 806R (5' - GGA CTA CNV GGG TWT CTA AT - 3') following the Earth Microbiome Project protocol (Caporaso et al. 2012), with minimal modifications. Forward primers contained the 5' Illumina adapter, 12 basepair unique Golay barcode, forward primer pad and linker, and 515F. Reverse primers contained the 3' reverse complement Illumina adapter, reverse primer pad and linker, and 806R. Templates were amplified in duplicate prior to sequencing using a 25 µl reaction containing 10 µl 2X Invitrogen Platinum Hot Start master mix, 0.5 µl 10 µM forward primer, 0.5 µl 10 µM reverse primer, 13 µl molecular grade H_2O, and 1 µl template. Samples were amplified in an Eppendorf Mastercycler Pro thermocycler with the program parameters of 94 °C for 3 min, 35 cycles of 94 °C for 45 s, 50 °C for 60 s, 72 °C for 90 s, and a final extension at 72 °C for 10 min. Technical replicate amplicons were combined and visualized on a 1% agarose gel. We quantified the amplicons with the PicoGreen Assay for dsDNA (ThermoScientific) and pooled 300 ng of each sample. We used AMPure XP magnetic beads (Beckman Coulter) to clean the pooled library. Prior to sequencing, the quality of the amplicon library was checked using Qubit Fluorometer (Thermo Fisher Scientific Inc) and 2100 Bioanalyzer DNA 7500 assay (Agilent Technologies, Inc). The library quantification was done using NEBNext Library Quant Kit for Illumina (New England Biolabs). The amplicon library was spiked with Illumina PhiX and sequenced on Illumina MiSeq platform using the v2-300 cycle kit with 156 bp paired end chemistry (Illumina Inc). Custom Read1, Read2, and Index Sequencing primers were used for sequencing. Negative controls with no template were PCR amplified and sequenced as well. Amplicon library quality assessment and sequencing was performed at the Genomics Resource Laboratory (RRID:SCR_017907), Institute for Applied Life Sciences, University of Massachusetts Amherst, MA, USA.

3.4 Sequencing processing and analysis

Raw FastQ files were demultiplexed and primers trimmed using cutadapt (Martin 2011). Bacterial 16S rRNA gene sequences were processed with the DADA2 pipeline (v.1.18.0) (Callahan et al. 2016), which generates amplicon sequence variants (ASV), in R. Forward reads were trimmed to 150 bp, and reverse reads were trimmed to 140 bp. Any reads with expected error higher than 2 or with any ambiguous nucleotides were discarded, and all PhiX sequences were also removed. Error rates for the forward and reverse reads were estimated separately using the function 'learnErrors'. ASVs were called for the forward and reverse reads using their respective error models. Denoised forward and reverse ASVs were merged using the function 'mergePairs' with default parameters. Prior to assigning taxonomy, any chimera sequences were identified *de novo* and removed. Taxonomy was assigned using SILVA (v 138.1) (Quast et al. 2012). All sequences that were classified at the family level as "mitochondria" or at the order level as "chloroplast" were filtered out from the final ASV table. To account for differences in sequencing depth between samples, raw ASV counts

were normalized with library size estimation factors calculated in DESeq2 (Love et al. 2014; McMurdie et al. 2014). Any ASVs that had 1 or 0 reads total across all samples after normalization were discarded.

Our final data set comprised 69 out of 79 possible samples due to issues with sequencing, where each combination of season, soil type, warming treatment, and warming duration had at least three replicates, except the October organic horizon control treatment from 13-year experiment, which had only two replicates. At the end of sequence processing, this data set had 4,345,430 reads, with an average depth of 62,977 reads per sample, and 8,754 total ASVs. The no template negative controls had fewer than 500 reads. After correcting with a sample specific library size estimate calculated in DESeq2 (Love et al. 2014), the total read count was 4,159,442, with an average read count of 60,281 per sample. Rarefaction curve analyses indicated that for the samples that were successfully sequenced, the sequencing efforts were adequate.

Since a variety of diversity metrics have been correlated with EMF, we calculated Simpson diversity, Shannon's H, Chao1 estimated richness, community structure (PCoA axes), and evenness (Pielou's J). Diversity metrics were calculated using the packages vegan (Oksanen et al. 2007) and phyloseq (McMurdie et al. 2013). The distance matrix for the principal coordinate analysis was calculated using Bray-Curtis dissimilarity using the function 'vegdist' in the package vegan.

3.5 Statistical analysis

All statistical analyses were done in R (R Core Team 2023). Prior to any statistical analysis, we evaluated whether warming treatment and warming duration could be combined into one variable, where both Prospect Hill and SWaN plot control samples would be combined into a single "control", Prospect Hill warmed samples would be "28 year warmed", and SWaN warmed samples would be "13 year warmed". This was done after checking for significant differences between the control plot samples from Prospect Hill and SWaN using either a Wilcoxon rank sum test or a t-test. We found no significant differences between the control plots' EMF in Prospect Hill and SWaN. However, we did find significant differences in bacterial diversity between the control plots from the two experiments ($p = 0.0260$). As a result, warming treatment and warming duration were combined into a single variable when EMF was the response variable, but not when bacterial diversity was the response variable.

We built a set of general linear models using raw or log-transformed data to investigate the effects of warming, soil type, season, and/or site on four response variables: individual ecosystem functions, nutrient pools, EMF, or bacterial diversity. When normality of model residuals was not satisfied or the response variable was not normally distributed, we constructed generalized linear models with non-Gaussian distributions. We considered both additive and interactive effects between warming, soil type, season, and/or site.

A set of candidate generalized linear models were constructed with either EMF or bacterial diversity as the response variable. First, models were built using a Gaussian distribution and model residuals were tested for normality, except for models where Chao1 estimated richness was the response variable. When Chao1 estimated richness was the response variable, models utilized a Poisson or negative binomial distribution, which are both appropriate for count data. Poisson models for Chao1 estimated richness were checked for overdispersion by looking at the ratio between residual deviance and degrees of freedom, and if overdispersion was observed (ratio > 1.1), a negative binomial distribution was used with the R package MASS (Venables et al. 2002). For the mineral soils, any model where EMF was the response variable had a log-transformation applied to EMF to make the residuals normally distributed. The results from the log-transformed models are reported without back-transformation. We found that the different diversity metrics all performed similarly

based on their ΔAICc, so we elected to utilized Shannon's H, Pielou's J, and Chao1 estimated richness as predictors since taxon richness and community evenness have both been found to be drivers of ecosystem function (Delgado-Baquerizo et al. 2020; Wittebolle et al. 2009).

We also tested whether warming treatment, warming duration, soil type, and/or season affected the relationship between the individual ecosystem functions or nutrient pools and bacterial diversity by constructing linear models. Model residuals were tested for normality using a Shapiro-Wilk test. If residuals were not normally distributed, a log-transformation was applied. If the model residuals were still not normally distributed after a log-transformation, we constructed a set of generalized linear models using a gamma or inverse Gaussian distribution. Residuals for these models were validated using the DHARMa package (Hartig 2022). Results for models where a log-transformation was applied or for models that did not use a normal distribution are reported without back-transformation.

Finally, we then investigated how warming treatment, warming duration, soil type, and/or season affected the relationship between EMF and bacterial diversity. For all of these analyses, we constructed a set of candidate linear models. In these sets of candidate models, we considered all predictors and possible interactions between warming treatment and diversity, warming treatment and horizon, warming treatment and season, or warming treatment and warming duration. All model residuals were checked for normality, and if necessary a log-transformation was applied to the response variable. Estimates and errors from the log-transformed models are reported without back-transformation.

For all of the candidate sets of models, model fit was assessed using AICc in the package (Mazerolle 2020). If models were within 2 ΔAICc units, we considered the models equally supported, and used a likelihood ratio test for the nested models to select a model. If the models with similar ΔAICc were not nested, we selected the model that explained more variance in the data. Model residual deviance was also compared to model null deviance to examine model fit. To account for multiple comparisons, a Tukey's HSD test was utilized when significant interactions between warming treatment, warming duration, and/or season were found. Otherwise, p-values had a Benjamini-Hochberg correction applied to them. We utilized a p-value cutoff of 0.05 for statistical significance. If the p-values were between 0.05 and 0.1, we report it as a trend. All estimates are reported with a 95% confidence interval.

We also conducted a *post-hoc* analysis to see whether any of the dominant ASVs in the organic horizon in the summer were differentially abundant between control and heated plots using three different methods: Analysis of Community Microbiomes with Bias Correction in the R package ANCOM-BC (v.2.0.3) (Lin et al. 2020), log2 fold change using the package DESeq2 (Love et al. 2014), and a χ^2 test between counts in the heated and control plots. Dominance was determined based on a relative abundance threshold of $\geq$ 0.001. Of the 8,629 ASVs in the final dataset, 256 ASVs were identified as dominant, which accounted for 2.967% of the total ASVs. For all three differential abundance methods, p-values were adjusted for multiple comparisons using a Benjamini-Hochberg correction.

4 Results

4.1 Bacterial diversity

The effect of warming on bacterial evenness varied by season in the organic horizon, where the best fitting model based on ΔAICc was the interaction model with warming and season. Warming had a negative impact on Pielou's J in the summer but no effect in the fall (Table 1). This pattern was not observed in the mineral

soil, where warming did not affect bacterial evenness. There was also a season-dependent warming trend on Shannon diversity, although this effect was not significant ($p = 0.072$). Since in the organic horizon in the summer warming decreased bacterial evenness but did not affect Chao1 richness, we carried out a *post-hoc* differential abundance analysis on dominant ASVs to determine if changes in the abundance of dominant ASVs could explain the decrease in evenness. None of the dominant ASVs were differentially abundant between the warmed and control plots.

Chao1 estimated richness significantly differed between the two different sites, Prospect Hill (28 year warmed) and SWaN (13 year warmed) in the organic horizon. At the younger experimental site, Chao1 estimated richness was increased by 12% compared to the older site (Table 1). Unheated control plots from the 13-year warmed and 28-year warmed experiments had significantly different bacterial diversity metrics and so were not combined when bacterial diversity was the response variable.

4.2 Ecosystem multifunctionality

Ecosystem multifunctionality was influenced more by season than by warming treatment or warming duration. In the organic horizon, EMF was significantly higher in fall compared to summer ($p = 0.022$, Figure 1A). Similarly, in the mineral soil, EMF was somewhat higher in fall compared to summer ($p = 0.090$, Figure 1B). Due to known differences between the organic horizon and mineral soil (Pold et al. 2017), ecosystem multifunctionality was calculated for the soils separately.

For both the organic horizon and mineral soils, neither 13 years nor 28 years of warming significantly altered EMF. In mineral soils, EMF tended to increase as warming duration increased, but these differences were not significant. In the organic horizon soils, we did not observe any trend in EMF with longer duration of heating.

4.3 Ecosystem multifunctionality-bacterial diversity relationship

In the organic horizon, season had a significant effect on the EMF-diversity (bacterial Chao1 estimated richness) relationship, where fall had significantly higher intercept for EMF than summer (Figure 2A). We observed a similar, seasonal trend in the relationship between EMF and diversity based on Shannon's diversity ($p = 0.052$, Figure S2A) and Pielou's evenness ($p = 0.055$, Figure S2C). In the organic horizon soils, ecosystem multifunctionality did not correlate with bacterial Chao1 estimated richness, Shannon diversity, or Pielou evenness. The relationship between EMF and Chao1 estimated richness had a progressively lower intercept as warming duration increased. However, neither the 13-year warmed nor the 28-year warmed plots showed a relationship between EMF and Chao1 estimated richness that was significantly different from the control plots.

In the mineral horizon, Shannon diversity, Chao1 estimated richness, or Pielou evenness were not correlated with EMF, and the intercept for the relationship between EMF and Chao1 estimated richness did not change as warming duration increased. There also was no effect of season on the intercept for EMF for all three of the diversity metrics in the mineral soil.

4.4 Individual ecosystem functions-bacterial diversity relationship

In the organic horizon, no individual ecosystem function or nutrient pool had a significant relationship with any diversity metric. MBC and oxidative enzyme activity both varied significantly by season, with MBC

higher in fall compared to summer and oxidative enzyme activity higher in fall compared to summer for both the Shannon diversity and Chao1 estimated richness models (Table 2, Table S1).

In the mineral soil, β-glucosidase activity was the only ecosystem function that had a relationship with any bacterial diversity metric, where Shannon's H had a negative relationship with β-glucosidase activity (Table 3, Table S2). Similar to the organic horizon, significant seasonal differences were observed for MBC, oxidative enzyme activity, and β-glucosidase activity. Both MBC and oxidative enzyme activity were higher in fall compared to summer, whereas the opposite was observed for β-glucosidase activity, irrespective of the diversity metric.

5 Discussion

Season is a critical driver of ecosystem function, especially in deciduous forests. We observed that ecosystem multifunctionality was significantly higher in fall compared to summer. Indeed, season had a larger effect on EMF than warming treatment, which had no effect on EMF. The seasonal input of fresh litter could explain the difference in EMF between fall and summer. With leaf-fall, a priming effect could be initiated, resulting in higher microbial activity (Lyu et al. 2018; Zhang et al. 2020; Domeignoz-Horta et al. 2023). This priming could be substantial enough to mitigate the negative impact of warming on EMF. Further supporting a priming effect, we observed a significant increase in microbial biomass in fall, and an increased microbial biomass has also been noted following the addition of fresh carbon in priming experiments (Jilling et al. 2021; Lyu et al. 2019; Zhou et al. 2021). In the heated plots at Harvard Forest, soil organic matter is more depleted in simple sugars and is lower in quantity compared to soil organic matter in the control plots (VandenEnden et al. 2021), and the influx of litter in the fall might help alleviate this resource limitation (Domeignoz-Horta et al. 2023). Accordingly, seasonal influx of nutrients cancels out the warming-induced substrate limitation which can be observed by the response of the microbial biomass carbon (Domeignoz-Horta et al. 2023). This could subsequently increase enzyme production and activity and microbial biomass, both of which were included in our EMF index. While we only examined two seasons, summer and fall, our results highlight the importance of accounting for seasonal differences when measuring ecosystem function.

An additional contributor to the higher EMF observed in fall could be a two-day rainfall event that occurred two days prior to the fall sampling (Boose et al. 2022). This rainfall could have initiated a Birch effect, which is an increase in respiration that occurs after rewetting a dried soil (Birch 1958). Furthermore, the rainfall could have resulted in a flush of dissolved organic matter, which was found in Domeignoz-Horta et al. (2023). Drying-rewetting cycles can increase microbial biomass (Evans et al. 2012; Fierer et al. 2003), which is in line with the significantly higher microbial biomass that we observed in fall (Table 2, Table 3). Warmed soils have significantly lower soil moisture in the spring and fall compared to the control plots in the (Contosta et al. 2011), and the combination of warming and lower moisture can further increase CO_2 efflux after rewetting (Schindlbacher et al. 2012). Yet, we did not observe a significant increase in respiration in fall (Domeignoz-Horta et al. 2023), as would be expected with a Birch effect. This could be due to the fact that respiration was measured after the soil was taken back to the lab, up to six days after the rainfall. This falls outside of the 1.5 days when the respiration spike that is characteristic of the Birch effect is typically observed (Canarini et al. 2017). Enzyme activity can also increase after rewetting (Ouyang et al. 2020). While we did observe increased phenol oxidase activity in the fall, we did not observe similar increases in activity for β-glucosidase and N-acetyl glucosaminidase (Domeignoz-Horta et al. 2023). Even though we noted higher EMF in the fall, the ecosystem function responses we observed are not entirely consistent with

a rewetting response.

Bacterial community evenness tends to be more sensitive to warming compared to bacterial community richness. We observed that bacterial community evenness was negatively affected by soil warming and that this effect was specific to the organic horizon in the summer (Table 1). This contrasts with previous work at our site, where warming increased bacterial community evenness in the organic horizon as a result of decreases in dominant taxa abundance (DeAngelis et al. 2015). Warming driven shifts in community evenness, whether through increased dominance of a single taxon, decreases in dominant taxa, or losses of rare taxa (Anthony et al. 2021; Deslippe et al. 2012; Nottingham et al. 2022). We did not observe any significant effects of warming on bacterial community richness (Table 1), which is in alignment with other experimental warming studies or meta-analyses (Wang et al. 2019; Yu et al. 2019; Zhou et al. 2020; Zi et al. 2018). Changes in abundance of dominant taxa were not the drivers of this decrease in evenness either.

Changes in substrate quality and quantity due to warming might also explain the observed changes in evenness. At the Harvard Forest, warming has negatively affected both substrate quality and quantity (Domeignoz-Horta et al. 2023), and lower substrate availability likewise has negative effects on microbial community evenness (Dilly et al. 2004). Given that ecosystem function tends to be more resilient to stress in more even communities (Wittebolle et al. 2009), the negative effects of warming on evenness ultimately could make ecosystem function less stable. Yet these negative effects might be masked or compensated for by changes to microbial community function as a result of heating, such as increased abundance of carbohydrate activated enzymes (i.e. CAZymes) involved in degrading complex carbohydrates (Roy Chowdhury et al. 2021) or an expanded number of bacteria able to degrade lignin (Pold et al. 2015).

In this study, we observed no significant relationship between bacterial diversity and EMF, irrespective of season, soil horizon, warming treatment, or warming duration. Typically, ecosystem multifunctionality positively correlates with bacterial diversity (Chen et al. 2020; Mori et al. 2016; Wagg et al. 2019), but neutral, or marginally positive relationships are not uncommon (Jiang et al. 2008; van der Plas 2019). In fact, abiotic or environmental factors, such as soil moisture or pH, are consistently identified as stronger drivers of EMF than diversity itself (Delgado-Baquerizo et al. 2016; Delgado-Baquerizo et al. 2020; Jing et al. 2015; van der Plas 2019). Even the spatial scale of sampling can affect the relationship between EMF and diversity, where at larger scales, like continental or global scales, diversity and ecosystem function tend to be positively correlated, but at smaller scales, like plot level or regional scales, diversity and ecosystem function tend to be neutrally or negatively correlated (Hagan et al. 2021). Additionally, active microbial diversity (Bastida et al. 2016) or functional trait diversity (Hillebrand et al. 2009; Steudel et al. 2016; Trivedi et al. 2019) may be a better predictor of ecosystem multifunctionality than total or taxonomic microbial diversity. This may explain why we did not see a relationship between bacterial diversity and EMF. Our DNA-based sequencing approach captured total taxonomic diversity, which comprises both the active and inactive community, and did not provide any functional trait information. Moreover, in soils many bacteria are dormant, with minimal metabolic activity (Papp et al. 2018), and these dormant cells are not likely contributing to EMF. Altogether, this suggests that when microbial diversity positively correlates with ecosystem function, this correlation tends to be weak, mainly driven by active members of the community or specific functional groups, and ultimately can be better explained by abiotic variables.

Functional redundancy within the bacterial community can also account for the lack of relationship between EMF and bacterial diversity. In theory, if an ecosystem function is functionally redundant within a community, the addition of more taxa that carry out that specific function will have negligible effects on the performance of that function at any given time. Forest soils are known to contain high levels of

functional redundancy (Zhang et al. 2016), therefore changes in diversity might not result in changes in function. Additionally, the functions included in the ecosystem multifunctionality index we calculated are known to be widespread within soil microbial communities. For instance, aerobic respiration is widespread throughout the phylogenetic tree, and the genes for β-glucosidase or N-acetyl glucosaminidase production are found or predicted in Proteobacteria, Chloroflexi, and Actinobacteria (Isobe et al. 2020; Moreno et al. 2013), all of which were abundant in our samples. Conversely, ecosystem functions that are performed by a narrower group of taxa and therefore exhibit less functional redundancy, such as xenobiotic degradation or nitrogen fixation, are more sensitive to changes in diversity (Delgado-Baquerizo et al. 2016; Li et al. 2021). Although, even within functions that are considered to be less widespread throughout the phylogenetic tree, such as nitrite oxidation, there does seem to be some level of functional redundancy or resilience in response to changes in diversity (Wertz et al. 2007). The lack of a relationship between bacterial diversity and EMF could partially be attributed to the functional redundancy of the ecosystem functions in this study.

While we did not observe a relationship between EMF and bacterial diversity, we did observe that β-glucosidase activity had a significant relationship with bacterial Shannon diversity in the mineral soils. Unexpectedly, this relationship was negative (Table 3), meaning that soils with higher diversity tended to have lower β-glucosidase activity potential. We only observed this negative relationship for bacterial Shannon diversity and not bacterial Chao1 richness, suggesting that more even bacterial communities have lower BG activity. This could be explained by negative selection effects, which arise when the most abundant or dominant taxa do not perform or add to a specific ecosystem function, making the diversity-ecosystem function relationship negative or neutral (Jiang 2007). In fact, β-glucosidase activity can be driven by individual taxa that are not the most abundant within a community (Bailey et al. 2013), and the energetic costs of extracellular enzyme production might negatively impact growth of extracellular enzyme producers. As a result, the presence or absence of individual taxa that are associated with high BG activity might not be reflected in diversity metrics that assess total community diversity. Further, metabolic overlap, which occurs when different taxa within a community can utilize the same substrate or compound, might also account for the negative relationship between diversity and function, since more diverse communities tend to have less metabolic overlap and more diverse metabolic pathways (Hester et al. 2019). As it relates to β-glucosidase activity, higher diversity communities might have more diverse metabolisms that can utilize other substrates besides glucosyl, the substrate of BG, and therefore might have lower overall BG activity. Negative selection effects and metabolic overlap can both account for the negative relationship between bacterial diversity and β-glucosidase activity, which has also been observed in other studies (Chen et al. 2023; Han et al. 2022).

Controlled manipulative laboratory incubations and observational field studies are complementary methods to understand the drivers of the BEF relationship, and both of these methods have validated the relationship between ecosystem function and microbial diversity. Mycorrhizal and saprotrophic fungal diversity and bacterial diversity were all noted as having positive correlations with ecosystem multifunctionality in a study that sampled from 80 different sites across the globe (Delgado-Baquerizo et al. 2020). Manipulative laboratory experiments, where diversity is altered via serial dilutions, have also observed a similar positive trend (Osburn et al. 2023; Wagg et al. 2014). However, the strength of the positive correlation tends to decrease when comparing manipulative studies to observational studies, due to factors such as lack of immigration and dispersal, reduced environmental complexity, or short experimental duration (Hillebrand et al. 2009). Additional variability in the strength of the relationship between microbial diversity and ecosystem function arises from abiotic factors, like season or soil type (van der Plas 2019). Indeed, we observed no relationship between bacterial diversity and ecosystem function in this observational field study. However,

the removal of abiotic variables can affect how diversity confers benefits to ecosystem function. The benefits of diversity arise from mechanisms such as niche partitioning (Hong et al. 2021) or maintaining a pool of taxa that can thrive under different environmental conditions (De Mazancourt et al. 2013; Loreau et al. 2013; Wittebolle et al. 2009; Yachi et al. 1999). Niche partitioning, in particular, is dependent on the presence of environmental heterogeneity in order for organisms to colonize the niche they are best adapted to (Cardinale 2011). Further emphasizing the importance of abiotic variables, we found that season had the largest impact on ecosystem function. Thus, while controlled laboratory experiments have helped to elucidate the mechanisms driving the BEF relationship, observational field studies are still necessary to fully understand the role that abiotic factors, such as season, have on this relationship under more natural conditions.

This study investigated if long-term soil warming influenced the relationship between ecosystem multifunctionality and bacterial diversity. We observed a strong seasonal effect on ecosystem function, where ecosystem multifunctionality was significantly higher in the fall than in the summer. The effects of soil warming on bacterial evenness were also dependent on season in the organic horizon, where in the summer warming negatively affected bacterial evenness, but in the fall warming did not affect bacterial evenness. However, we found that warming treatment or warming duration ultimately had no effect on the relationship between EMF and bacterial diversity. Indeed, we found that EMF had no relationship with bacterial diversity, which is not uncommon in natural or non-laboratory systems. The younger experimental site also had increased bacterial richness in the organic horizon. Overall, we found that season exerts a strong influence on ecosystem functionality, and season modulates the effects of warming in temperate deciduous forests. We emphasize that cross-season sampling is needed to best assess an ecosystem's total function and that diversity metrics other than taxonomic diversity may be better suited to capturing the role of diversity on ecosystem function.

6 Data availability

The primer-trimmed bacterial sequencing data are available at the NCBI Sequence Read Archive under the BioProject accession number PRJNA957454. Ecosystem function data is available in the Harvard Forest Data Archive under the Dataset ID HF431.

7 Acknowledgments

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MSS and KMD conceptualized the study. MSS, LADH, MJC, and RR conducted experiments and

collected the data. SDF maintains the long-term soil warming experiments at Harvard Forest, facilitated sample collection, and provided input on data interpretation. MSS and MFM analyzed the data. MSS wrote the first draft of the manuscript. All authors contributed to editing and revising the manuscript.

Table 1. The effects of warming treatment, season, or site on diversity metrics in the organic horizon and mineral soil. Shannon diversity models and organic horizon Pielou evenness models used a Gaussian distribution, Chao1 estimated richness models used a negative binomial distribution, and mineral soil Pielou evenness models used a gamma distribution. Chao1 estimated richness and mineral soil Pielou evenness estimates are reported without back-transformation. Estimates are reported where the reference for warming treatment is control non-heated plots, the reference for season is summer, and the reference for site is the 13 year experiment. Number of samples for the contrasts are reported in parentheses. Errors are reported for a 95% confidence interval. Any significant interactions were further investigated using a Tukey HSD test. Benjamini-Hochberg adjusted p-values are reported, and any adjusted p-values that were greater than 0.05 are reported as not significant (n.s.).

	diversity metric	predictor	estimate	error	p value
Organic horizon	Shannon diversity	season (15)	-0.076	0.157	n.s.
		warming (16)	-0.134	0.151	n.s.
		warming:season (9)	0.247	0.220	0.072
	Chao1 estimated richness	site (16)	-0.110	0.086	0.019 *
		warming (16)	0.038	0.0.86	n.s.
	Pielou evenness	season (15)	-0.011	0.016	n.s.
		warming (16)	-0.024	0.016	0.011 *
		warming:season (9)	0.031	0.024	0.015 *
Mineral soil	Shannon diversity	season (19)	0.011	0.151	n.s.
		warming (19)	0.001	0.0151	n.s.
	Chao1 estimated richness	site (19)	-0.047	0.122	n.s.
		warming (19)	0.017	0.122	n.s.
	Pielou evenness	site (19)	-0.006	0.022	n.s.
		warming (19)	0.002	0.022	n.s.

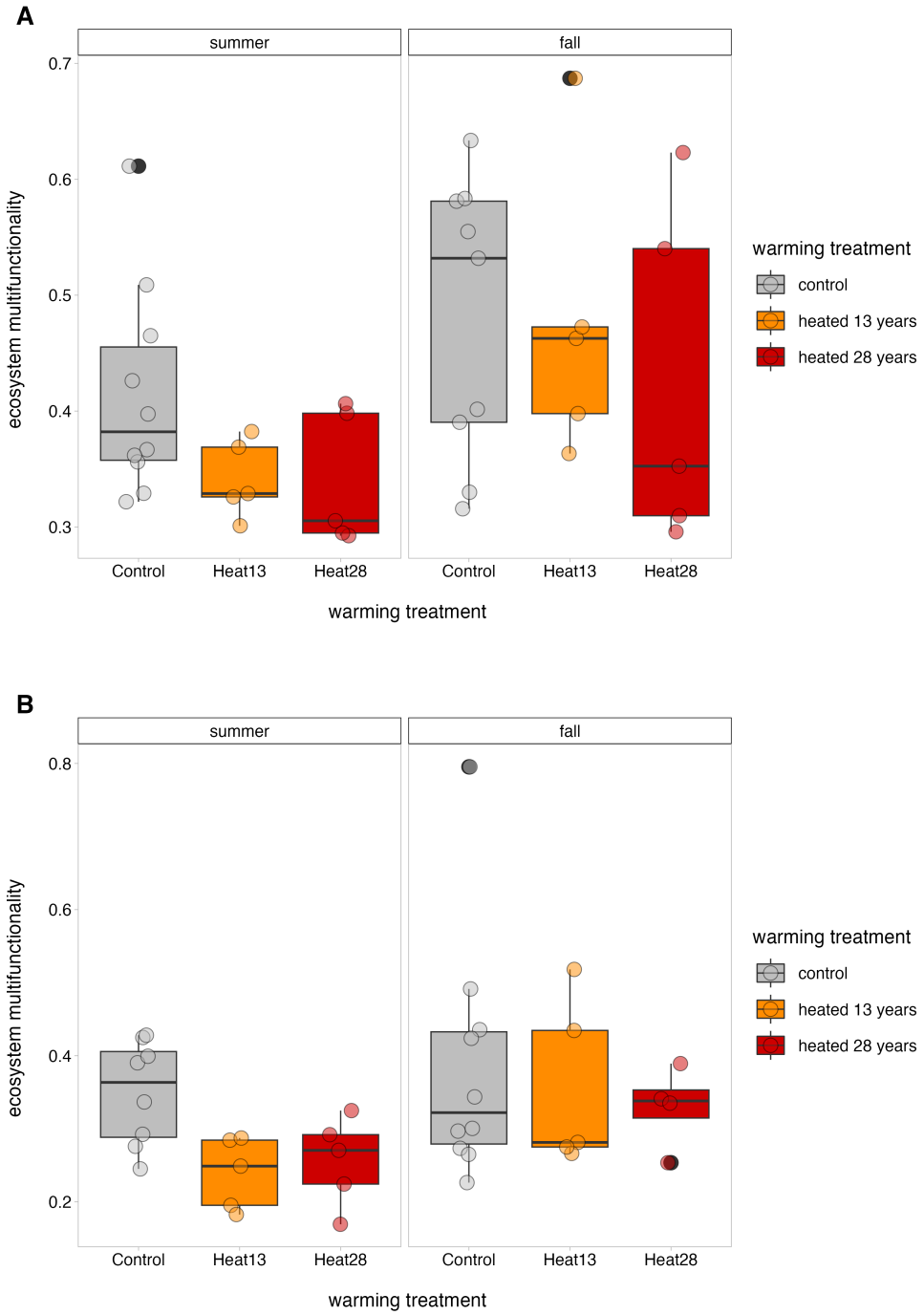

Figure 1. Ecosystem multifunctionality (EMF) in the organic horizon (A) and the mineral soils (B). EMF was calculated by averaging z-score standardized ecosystem function measurements for each sample. In the mineral soils, EMF was log-transformed and is displayed without back-transformation. The reported p-values are Benjamini-Hochberg corrected to account for multiple comparisons. In both horizons, there was a seasonal trend where EMF was higher in the fall, but this was only significant in the organic horizon (A) (0.089 ± 0.065, $p = 0.022$).

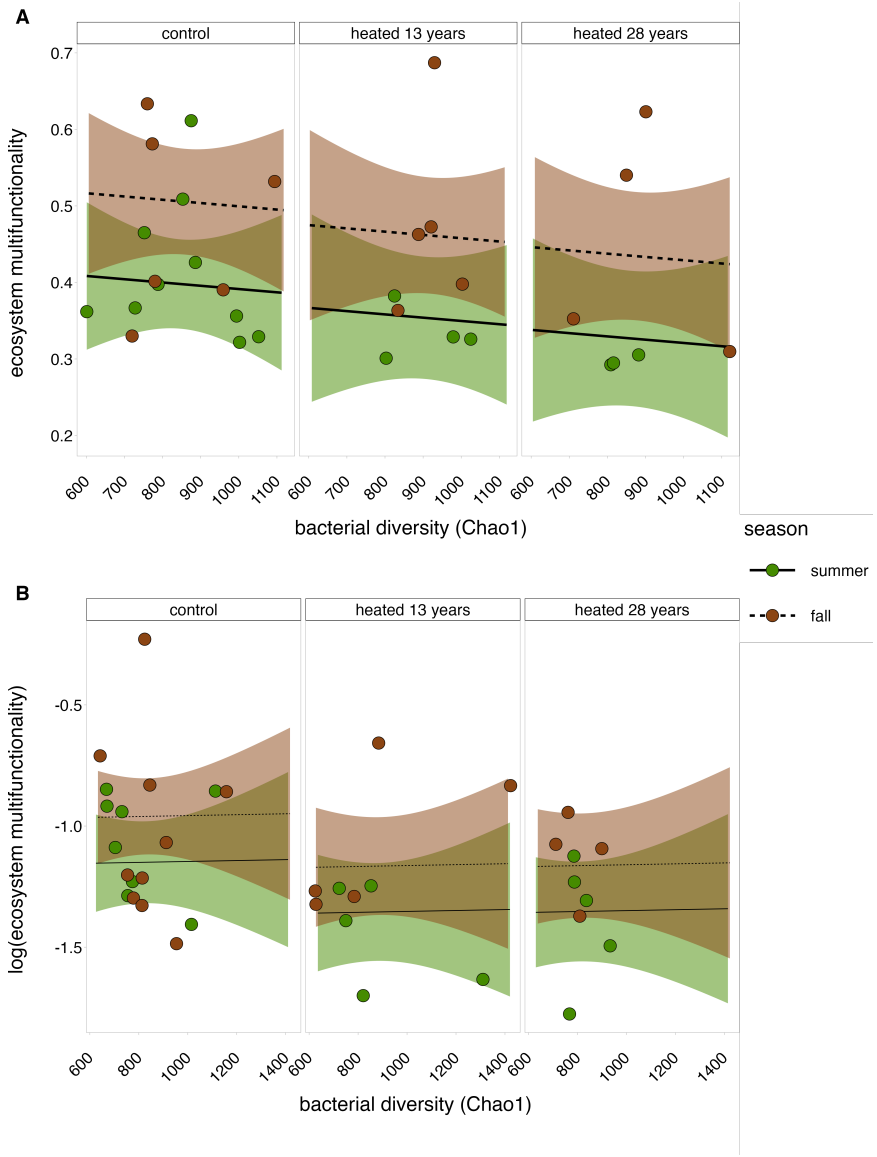

Figure 2. The relationship between ecosystem multifunctionality (EMF) and bacterial Chao1 estimated richness in the organic horizon (A) and the mineral soil (B). EMF was calculated by averaging z-score standardized function measurements for each sample. Shaded regions represent a 95% confidence interval. Mineral soil EMF was log-transformed and is displayed without back-transformation. The reported p-values are Benjamini-Hochberg corrected to account for multiple comparisons. There was no significant difference between the heated 13 years, heated 28 years, and control plots for either of the relationships. For EMF and bacterial Chao1 richness in the organic horizon (A), there was a significant effect of season, where fall had an overall higher baseline EMF than summer (0.108 ± 0.074, $p = 0.020$). In the mineral soil, there was no significant effect of season (B).

Table 2. Single ecosystem function-diversity relationships in the organic horizon. Microbial biomass (MBC) models used a Gaussian distribution and oxidative enzyme (OX) models used an inverse Gaussian distribution. Oxidative enzyme model estimates are reported without back-transformation. All model estimates are reported where the reference for warming treatment is control non-heated plots and the reference for season is summer. Number of samples are reported in parentheses. Errors are reported for a 95% confidence interval. Benjamini-Hochberg adjusted p-values are reported. Any adjusted p-values greater than 0.05 are reported as not significant (n.s.).

ecosystem function	diversity metric	predictor	estimate	error	p value
MBC	Shannon diversity	season (15)	2149.600	537.432	9.942e-08 ***
		13 years heating (9)	-230.400	622.104	n.s.
		28 years heating (7)	-470.800	675.612	n.s.
		Shannon (32)	341.100	1654.436	n.s.
	Chao1 estimated richness	season	2165.129	533.296	7.456e-08 ***
		13 years heating (9)	-246.062	636.418	n.s.
		28 years heating (7)	-477.112	678.174	n.s.
		Chao1 (32)	0.1276	2.248	n.s.
OX activity	Shannon diversity	season	-11573.7	1724.212	1.468e-12 ***
		13 years heating (9)	122.400	231.476	n.s.
		28 years heating (7)	101.300	233.828	n.s.
		Shannon (32)	245.900	545.664	n.s.
	Chao1 estimated richness	season	-1.157e+04	1765.960	2.579e-12 ***
		13 years heating (9)	1.419e+02	229.712	n.s.
		28 years heating (7)	1.225e+02	229.908	n.s.
		Chao1 (32)	0.186	0.706	n.s.

Table 3. Single ecosystem function-diversity relationships in the mineral soil. A log-transformation was applied to microbial biomass carbon (MBC) and oxidative enzyme (OX) activity. MBC models, OX models, and β-glucosidase (BG) models all used a Gaussian distribution. MBC model estimates and OX model estimates are reported without back-transformation. All model estimates are reported where the reference for warming treatment is control non-heated plots and the reference for season is summer. Number of samples for contrasts/predictor are reported in parentheses. Errors are reported for a 95% confidence interval. Benjamini-Hochberg adjusted p-values are reported. Any adjusted p-values greater than 0.05 are reported as not significant (n.s.).

ecosystem function	diversity metric	predictor	estimate	error	p-value
MBC	Shannon diversity	season (19)	0.944	0.278	8.355e-07 ***
		13 years heating (10)	-0.294	0.333	n.s.
		28 years heating (9)	-0.318	0.345	n.s.
		Shannon (37)	0.004	0.623	n.s.
	Chao1 estimated richness	season (19)	0.942	0.276	3.833e-07 ***
		13 years heating (10)	-0.309	0.333	n.s.
		28 years heating (9)	-0.313	0.343	n.s.
		Chao1 (37)	0.0002	0.0008	n.s.
OX activity	Shannon diversity	season (19)	1.927	0.247	1.479e-15 ***
		13 years heating (10)	-0.329	0.296	n.s.
		28 years heating (9)	-0.320	0.308	n.s.
		Shannon (37)	-0.024	0.555	n.s.
	Chao1 estimated richness	season (19)	1.926	0.247	7.401e-16 ***
		13 years heating (10)	-0.334	0.298	0.058
		28 years heating (9)	-0.318	0..298	0.063
		Chao1 (37)	8.007e-05	7.207e-04	n.s.
BG activity	Shannon diversity	season (19)	-744.41	216.306	6.389e-07 ***
		13 years heating (10)	-96.970	258.720	n.s.
		28 years heating (9)	-157.880	268.598	n.s.
		Shannon (37)	-623.500	482.866	0.028 *
	Chao1 estimated richness	season (19)	-747.101	232.613	2.316e-06 ***
		13 years heating (10)	-91.320	280.160	n.s.
		28 years heating (9)	-150.038	288.798	n.s.
		Chao1 (37)	-0.375	0.678	n.s.

8 Supplementary material

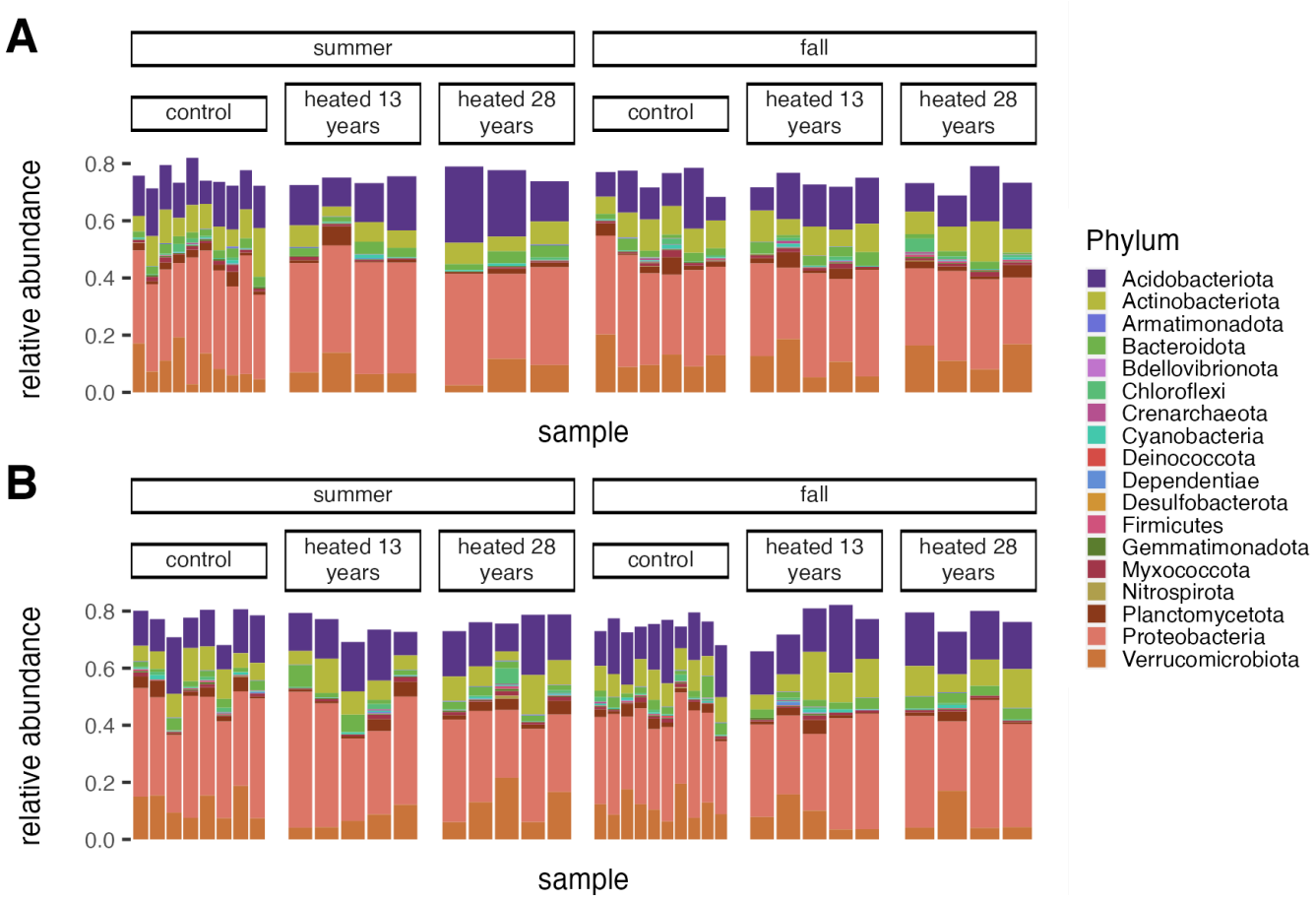

Figure S1. Relative abundances of the phyla of dominant bacteria based on 16S rRNA sequencing in the organic horizon (A) and the mineral soils (B) between the heated +5°C and control treatments in summer and fall. Taxonomy was assigned using SILVA (v 138.1). Bacterial amplicon sequence variants (ASV) were normalized using sample-specific DESeq2 library size estimation correction factor. Dominant ASVs were determined as ASVs with a relative abundance of 0.001 or higher within a sample.

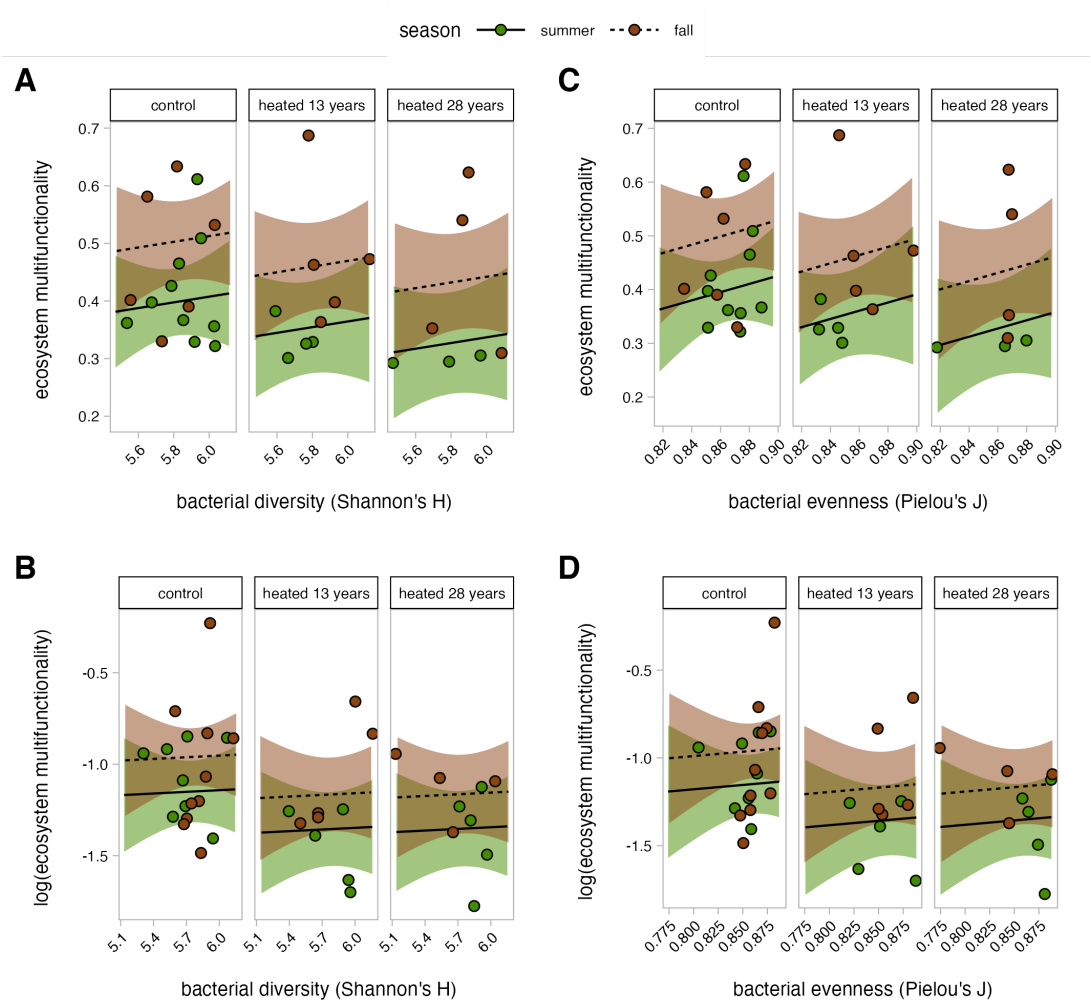

Figure S2. The relationship between ecosystem multifunctionality (EMF) and bacterial Shannon diversity and bacterial Pielou evenness in the organic horizon (A, C) and the mineral soil (B, D). EMF was calculated by averaging z-score standardized function measurements for each sample. Shaded regions represent a 95% confidence interval. Mineral soil EMF was log-transformed and is displayed without back-transformation. The reported p-values are Benjamini-Hochberg corrected to account for multiple comparisons. There was no significant difference between the heated 13 years, heated 28 years, and control plots for any of the relationships. In the organic horizon, there was a slight seasonal trend for both Shannon diversity (A, $p = 0.052$) and Pielou evenness (C, $p = 0.055$), where fall had a higher EMF intercept compared to summer. There were no seasonal trends observed in the mineral soils.

Table S1. Single ecosystem function-diversity relationships in the organic horizon. All models using a Gaussian distribution and model residuals were checked for normality. To meet normality of model residuals, β-glucosidase (BG) activity, respiration, and total nitrogen in the Chao1 model were log-transformed. The model estimates and errors for the ecosystem functions that were log-transformed are reported without back-transformation. All model estimates are reported where the reference for warming treatment is control non-heated plots and the reference for season is summer. Number of samples are reported in parentheses. Errors are reported for a 95% confidence interval. Benjamini-Hochberg adjusted p-values are reported. Any adjusted p-values greater than 0.05 are reported as not significant (n.s.).

ecosystem function	diversity metric	predictor	estimate	errors	p value
NAG activity	Shannon diversity	season (15)	-155.666	1958.802	n.s.
		13 years heating (9)	-1846.554	2267.306	n.s.
		28 years heating (7)	-1238.011	2461.991	n.s.
		Shannon (32)	4.701	6029.377	n.s.
	Chao1 estimated richness	season	-121.155	1923.250	n.s.
		13 years heating (9)	-1690.075	2295.138	n.s.
		28 years heating (7)	-1194.214	2445.721	n.s.
		Chao1 (32)	-2.671	8.107	n.s.
BG activity	Shannon diversity	season	-0.520	0.433	n.s.
		13 years heating (9)	0.200	0.502	n.s.
		28 years heating (7)	-0.269	0.545	n.s.
		Shannon (32)	-0.169	1.334	n.s.
	Chao1 estimated richness	season	-0.514	0.417	n.s.
		13 years heating (9)	0.270	0.531	n.s.
		28 years heating (7)	-0.248	229.908	n.s.
		Chao1 (32)	-0.001	0.002	n.s.

Table S1 continued:

ecosystem function	diversity metric	predictor	estimate	errors	p value
Respiration	Shannon diversity	season	0.164	0.276	n.s.
		13 years heating (9)	-0.317	0.320	n.s.
		28 years heating (7)	-0.323	0.347	n.s.
		Shannon (32)	-0.065	0.851	n.s.
	Chao1 estimated richness	season	0.162	0.273	n.s.
		13 years heating (9)	-0.310	0.326	n.s.
		28 years heating (7)	-0.320	0.347	n.s.
		Chao1 (32)	-8.578e-05	0.001	n.s.
Total carbon	Shannon diversity	season	-0.020	0.041	n.s.
		13 years heating (9)	0.063	0.047	n.s.
		28 years heating (7)	0.051	0.051	n.s.
		Shannon (32)	0.140	0.126	n.s.
	Chao1 estimated richness	season	-0.001	0.043	n.s.
		13 years heating (9)	0.005	0.051	0.073
		28 years heating (7)	0.005	0.054	n.s.
		Chao1 (32)	1.118e-04	1.800e-04	n.s.
Total nitrogen	Shannon diversity	season	0.006	0.002	n.s.
		13 years heating (9)	-0.002	0.002	n.s.
		28 years heating (7)	-0.002	0.002	n.s.
		Shannon (32)	0.001	0.006	n.s.
	Chao1 estimated richness	season	0.088	0.188	n.s.
		13 years heating (9)	-0.252	0.224	n.s.
		28 years heating (7)	-0.277	0.122	n.s.
		Chao1 (32)	5.970e-05	0.001	n.s.

Table S2. Single ecosystem function-diversity relationships in the organic horizon. All models used a Gaussian distribution, except total carbon and total nitrogen models, which used an inverse Gaussian distribution with an inverse link function and an inverse Gaussian distribution with an $1/\mu^2$ link function, respectively. Model residuals were examined, and if normality of residuals for the Gaussian models was not met, a log-transformation was applied. Subsequently, N-acetyl glucosaminidase (NAG) was log-transformed to satisfy normality of model residuals. The model estimates and errors for the ecosystem functions that were log-transformed or that used an inverse Gaussian distribution are reported without back-transformation. All model estimates are reported where the reference for warming treatment is control non-heated plots and the reference for season is summer. Number of samples are reported in parentheses. Errors are reported for a 95% confidence interval. Benjamini-Hochberg adjusted p-values are reported. Any adjusted p-values greater than 0.05 are reported as not significant (n.s.).

ecosystem function	diversity metric	predictor	estimate	errors	p value
NAG activity	Shannon diversity	season (15)	-0.578	0.555	n.s.
		13 years heating (9)	0.004	0.664	n.s.
		28 years heating (7)	0.163	0.689	n.s.
		Shannon (32)	1.078	1.244	n.s.
	Chao1 estimated richness	season	-0.577	0.565	n.s.
		13 years heating (9)	-0.029	0.680	n.s.
		28 years heating (7)	0.157	0.358	n.s.
		Chao1 (32)	0.001	0.001	n.s.
Respiration	Shannon diversity	season	0.086	0.072	n.s.
		13 years heating (9)	-0.069	0.087	n.s.
		28 years heating (7)	-0.098	0.046	n.s.
		Shannon (32)	0.023	0.083	n.s.
	Chao1 estimated richness	season	0.08635	0.073	n.s.
		13 years heating (9)	-0.070	0.087	n.s.
		28 years heating (7)	-0.098	0.090	n.s.
		Chao1 (32)	2.143e-05	2.115e-04	n.s.

Table S2 continued:

ecosystem function	diversity metric	predictor	estimate	errors	p value
Total carbon	Shannon diversity	season	-5.954	5.749	n.s.
		13 years heating (9)	5.701	6.933	n.s.
		28 years heating (7)	5.451	7.348	n.s.
		Shannon (32)	-4.091	12.893	n.s.
	Chao1 estimated richness	season	-172.024	175.828	n.s.
		13 years heating (9)	156.102	215.646	n.s.
		28 years heating (7)	160.703	232.259	n.s.
		Chao1 (32)	0.085	0.445	n.s.
Total nitrogen	Shannon diversity	season	-44235	55174	n.s.
		13 years heating (9)	48151	67798.36	n.s.
		28 years heating (7)	53284	74613.28	n.s.
		Shannon (32)	-6720	122076.64	n.s.
	Chao1 estimated richness	season	-45373.09	54775.179	n.s.
		13 years heating (9)	46648.91	67727.741	n.s.
		28 years heating (7)	54701.17	74130.512	n.s.
		Chao1 (32)	48.42	165.659	n.s.

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