Temperature and Cultivar Influence on Enzyme Activity and Composition of the Microbial Community in the Rhizosphere of Sweet Potato During Early Growth Stage

---

ABSTRACT

Aims: To determine the influence of temperature on sweet potato yield, soil enzyme activity, and composition of the microbial community in the rhizosphere during early growth stage-65 DAP

Study design: The study was conducted as a Completely Randomized Design with a 2x2 factorial treatment arrangement of two temperatures and two cultivars —and four replications in time.

Place and duration of study: George Washington Carver Agricultural Experiment Station, Tuskegee University, Tuskegee Alabama, between February and December 2022.

Methodology: We planted cuttings of two sweet potato cultivars, Beauregard and Whatley/Loretan, and exposed them to high (32/26°C) and optimal (28/22°C) diurnal temperatures in a plant growth chamber. Standard cultural practices for irrigation and fertilizer application were followed. The plants were harvested 65 days after planting (65 DAP) and rhizosphere samples from both cultivars were collected. The microbial composition, including bacterial 16S rDNA and fungal ITS (Internal Transcribed Spacer), then analyzed using a standard DNA-Based quantitative Technique The extracellular enzymes, Acid Phosphatases (P-acid), β-Glucosidase (GLU), N-acetylglucosaminidase (β-NAG) and phosphodiesterase (DIEST) were analyzed using fluorometric microplate enzyme assays.

Results: Bacterial communities were dominated by *Acidobacteria* and *Proteobacteria*, whereas fungi were dominated by the *Ascomycota* across the samples. Temperature significantly affected the abundance of some bacterial (*Proteobacteria*, *Firmicutes*, *Verrucomicrobia,* and *Chloroflexi*) and all identified fungal (*Ascomycota, Mucoromycota*, and *Entomophthoromycota*). Cultivars affected bacterial *Gemmatimonadetes* and fungi *Mucoromycetes and Ascomycota,* with their higher relative abundances found in Whatley/Loretan than in Beauregard. There were significant interactions between temperature and cultivars for bacteria *Acidobacteria, Verrucomicrobia*, *Firmicutes*, and fungi *Entomophthoromycota.* Additionally, high temperatures influenced the activities of key soil enzymes—acid phosphatase, β-glucosidase, and β-1,4-N-acetylglucosaminidase—in the rhizosphere of sweet potato, irrespective of cultivars.

Conclusion: The bacterial and fungal populations and activities of key soil enzymes in the rhizosphere of Whatley/Loretan and Beauregard sweet potato were significantly impacted by high temperatures.

Keywords: Sweet potato, high-temperature, extracellular enzymes, rhizosphere microbial community, soil health

Commented [SN1]: The research was held between February and December 2022 meaning only 2 months. If you wrote February until December, the research was held in a few month. Please describe clearly.

INTRODUCTION **The yellow highlight is the correct vocabulary**

Sweet potato [*Ipomea batatas* (*L.*) Lam.] is an important root crop known for its nutritional, medicinal, industrial, and economic values [1]. The crop is characterized by its resilience to adverse environmental

conditions and high adaptability to a wide range of agroclimatic conditions, particularly in tropical regions [2]. Sweet potato is the fourth most important root crop in terms of the quantity of production and ranks seventh in global food production (FAO, 2024). It is cultivated in over 100 countries, ranging from tropical to subtropical and warm temperate regions (CIP, 2019.). Because of these attributes, it has been recognized as a potential climate-resilient crop that can ensure food security under changing climatic conditions [5].

The rhizosphere is an interface of dynamic interactions among plants, soil, and microorganisms [6]. It is essential for nutrient cycling and uptake, plant growth regulation, and soil health [7]. This zone also provides a habitat for microorganisms to multiply, grow, and function [8]. Rhizosphere soil microorganisms, such as bacteria and fungi, are essential for the decomposition of organic matter, nutrient recycling, and the regulation of plant growth [9], [10], [11]. Soils with high microbial diversity are considered to have high ecological functions and resistance to environmental stresses, including climate change [12]. Most biochemical processes in the rhizosphere are regulated by extracellular soil enzymes secreted by soil microbes and plants [13]. For example, β-Glucosidase is a group of carbon-cycling enzymes crucial for breaking down cellulose residues into glucose, an important energy source for soil microorganisms [14]. Another key enzyme, β-1,4-N-acetylglucosaminidase, facilitates the breakdown of chitin into amino sugars, contributing to carbon and nitrogen cycling in the soil [15]. Phosphatase enzymes play a vital role in phosphorous cycling by releasing inorganic phosphate complexes, making them available for plant and microbial uptake [16]. Soil enzyme activity is linked to microbial functions, and hence, is also used as an early indicator of microbial abundance in ecosystems [15], [17].

Temperature and plant species influence microbial communities and enzymatic activity in the rhizosphere either directly or indirectly [18]. Temperature directly affects the metabolism, growth, and function of soil microorganisms [19], [20], [21], and indirectly alter soil properties, which subsequently affect microbial compositions and enzyme activity. Plant species also shape the structure and function of the rhizosphere microbiome through mechanisms such as root development, root exudates, nutrient uptake, and litter quality [22], [23]. Additionally, crop growth characteristics, such as the root system, plant canopy, and nutrient requirements, alter soil characteristics, which may, in turn, affect soil microbial functions [24]. Furthermore, plant attributes such as growth stage, fine root number, and metabolic activity influence soil enzyme activity [25], [26], [27].

Studies have shown that the responses of microbial communities and enzymes' activities to temperature change are dependent on soil characteristics, environmental conditions, and plant genotypes [28], [29]. Therefore, understanding how high temperatures and specific plant cultivars influence enzyme activities and microbial diversity is essential for the development of advanced sustainable farming and ecosystem management, especially in the era of an increase in global warming due to climate change. However, there is a limited understanding of temperature's influence on soil ecology, especially in root crops, including sweet potatoes. Therefore, this study aimed to evaluate the relationship between high temperature and sweet potato cultivars on soil enzyme activities and microbial diversity within the rhizosphere.

MATERIAL AND METHODS

The study was conducted in a plant growth chamber (Model No: E-36VL, Percival Scientific, Boone Iowa). between February and December 2022 at Tuskegee University, Alabama, USA. Bulk soil for the experiment was collected from a certified organic farm at the George Washington Carver Agricultural Experiment Station. Composite soil samples were analyzed by the Plant and Soil Testing Laboratory at Auburn University to determine fertilizer recommendations.

Experimental Design, Planting, and Fertilizer Application.

The study was conducted as a Completely Randomized Design with a 2x2 factorial treatment arrangement and four replications in time. Three-node cuttings of Beauregard and Whatley/Loretan were planted in 3 kg greenhouse pots. The plants were exposed to two diurnal temperatures of, 28/22°C (optimum) and 32/26°C (high), and a 16h light/8h dark/ light period in a growth chamber. Fertilizers were applied one week after planting based on the recommended rate of 80-120-150kg ha^{-1} NPK. The plants were watered based on field capacity according to the

Commented [SN2]: Give the number if including in references

Commented [SN3]: Give the number of the references

UNDER PEER REVIEW

oven-dry weight method [30]. Additionally, plant growth chamber conditions included relative humidity of 75±5% and photosynthetic photon flux of 123 µmol m^2s^{-1} were maintained.

Soil sampling

At 65 DAP, the plants were harvested, and the roots were gently shaken in a container to separate the soil aggregates from the roots (rhizosphere). The rhizosphere samples were sieved to pass a 2 mm screen to remove roots and other debris. The sieved soils were homogenized and stored at -80°C for subsequent enzyme and microbial (metagenomic) analyses.

Soil enzyme analysis

The activities of β-glucosidase (GLU), acid phosphomonoesterase (acid-P), N-acetyl-β-D-glucosamidase (NAD), and phosphodiesterase (DIEST), which are involved in C, N, and P cycling, respectively, were measured using the microplate assay fluorometric method (Deng et al. 2015). Enzyme activity was expressed as µmol (4-methylumbelliferone) released per gram of dry soil per hour (µmol g^{-1} h^{-1}).

Commented [SN4]: Give the number of references

Microbial analysis

DNA extraction

Commented [SN5]: Please follow the template write a manuscript

Total microbial DNA was extracted from approximately 0.25 g of moist field soil from each soil rhizosphere (in triplicate) using a ZymoBIOMICS DNA kit (Zymo Research), following the manufacturer's instructions. The concentration of extracted DNA was determined using a Nanodrop 2000C spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and the eluted DNA was stored at -80°C for subsequent analysis. The extracted microbial DNA was then sent to MRDNA Laboratories (www.mrdnalab.com Shallowater, TX, USA) for purification, PCR optimization, amplification, and sequencing.

DNA amplification and sequencing

Commented [SN6]: Check the template how to wrote a sub-title

Microbial and fungal DNA were amplified using the Illumina NovaSeq via the bTEFAP® DNA analysis service methods. Each sample underwent a single-step 35-cycle PCR using HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) under the following conditions: 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 53°C or 52°C (microbial or fungal respectively) for 40 s, and 72°C for 1 min, after which a final elongation step at 72°C for 10 min was performed. Microbial DNA was amplified using the 16s rRNA primer pair (515F GTGYCAGCMGCCGCGGTAA and 806R GGACTACNVGGGTWTCTAAT). Fungal DNA was amplified using the fungal ITS1-2 primer pair (ITS1 CTTGGTCATTTAGAGGAAGTAA and ITS2R GCTGCGTTCTTCATCGATGC). Following PCR amplification, amplicon products were mixed in equal concentrations and purified using calibrated SPRI beads. Samples were then sequenced using Illumina NovaSeq chemistry following the manufacturer's protocol.

Sequence analysis

Commented [SN7]: Check template of sub-title

The Q25 sequence data derived from the sequencing process was processed using the MR DNA ribosomal and functional gene analysis pipeline (www.mrdnalab.com, MR DNA, Shallowater, TX). Sequences were depleted of primers, and short sequences (<150bp) and sequences with ambiguous base calls were removed. Sequences were quality-filtered using a maximum expected error threshold of 1.0 and then dereplicated. The dereplicated or unique sequences were

UNDER PEER REVIEW

denoised; unique sequences identified with sequencing or PCR point errors were removed, followed by chimera removal, resulting in denoised sequences or zOTU (zero-radius Operational Taxonomic Unit). Taxonomic classification of the final zOTUs was performed using BLASTn against a curated database derived from NCBI (www.ncbi.nlm.nih.gov). The results were compiled into taxonomic count files containing the absolute number of sequences and percentage files, which represented the relative proportions of sequences assigned to each taxonomic classification within each sample.

Statistical analysis

All data were analyzed using analysis of variance (ANOVA StataCorp, 2017). The significant effects of temperature and cultivar on soil enzyme activity and microbial populations were evaluated using a two-way analysis of variance (ANOVA). Means were separated using the Tukey-Kramer test (0.05).

RESULTS AND DISCUSSION

Effects of temperature and cultivars on enzyme activities

The main effect of temperature significantly affected the activities of β-glucosidase (GLU), acid phosphomonoesterase (acid-P), and N-acetyl-β-D-glucosamidase (NAD). Conversely, the cultivar effects were significant only for β-glucosidase (β-GLU) activity. Additionally, phosphodiesterase (DIEST) activity was not significantly influenced by temperature or cultivar (Table 1).

Table 1: Statistical significance from Analysis of Variance (ANOVA) of temperature, cultivars, and temperature x cultivars effect on soil enzyme activity

Source of Variation	df	ACP[ii]	βNAG[iii]	βGLU[iv]	DIEST[v]
Temperature	1	0.02**	<0.001***	0.01***	0.94
Cultivar	2	0.14	<0.001***	0.28	0.79
Temperature x Cultivar	2	0.40	0.27	0.87	0.76

[i] Significance, *** P (.001), **P (.05)

[ii] Acid-Phosphatase

[iii] β-1,4-N-acetylglucosaminidase

[iv] βeta-Glucosidase

[v] Phosphodiesterase

Temperature effect on soil enzyme activities. Most rhizosphere soil enzyme activities were significantly affected by temperature. At 32/26°C, the activities of P-Acid, β-GLU, and β-NAG were significantly higher than at 28/22°C, equivalent to a twofold increase in P-Acid, threefold in β-GLU, and fivefold in β-NAG while there was no effect on DIEST (Table 2). Thus, a higher temperature enhanced microbial activities and functions, potentially leading to accelerated synthesis of enzymes [31] and may have been within the optimal range, which could potentially

Commented [SN8]: Table title in single space

Commented [SN9]: Single space

Commented [SN10]: Single space

Commented [SN11]: All of them in single space

Commented [SN12]: No need the underline

UNDER PEER REVIEW

influence the kinetic energy of the enzyme and its substrate, leading to an increase in enzyme activity [32].

P-acid is secreted by fungi, bacteria, and plant roots and is responsible for catalyzing the transformation of complex organic P into assimilable phosphate [33]. Variations in P-acid activity are linked to soil organic carbon and inorganic nitrogen availability (Luo et al., 2017). An increase in P-acid at 32/26°C may indicate enhanced P mobilization in the soil, which can potentially boost plant growth. Additionally, an increase in P-acid can improve soil health by stimulating the breakdown of organic P compounds and nutrient cycling [35]. Our results are consistent with those of previous studies, reporting an increase in P-acid activity with increasing temperature in different ecosystems (Margalef et al., 2017). For example, in an incubation study, an increase in temperatures ranging from 20°C to 90°C in Alfisols and Vertisols soils resulted in an increase in Acid-phosphatase enzyme activity from 9.2 to 297.5 µg of 4-nitrophenol g^{-1} soil h^{-1} [37].

Commented [SN13]: Number in the references

Commented [SN14]: Reference number?

β-GLU is an important enzyme in the C cycle involved in the terminal process of cellulose degradation, essential for the release of nutrients from organic compounds, and is used as an indicator of organic matter status and turnover in soils [38]. Studies have demonstrated the sensitivity of β-GLU enzymes to temperature change [39], [40]. Similarly, our observations showed an increase in β-GLU activity at 32/26°C compared to that at 28/22°C and are consistent with those of previous studies that have reported an increase in β-GLU activity under high-temperature conditions. For example, an increase in temperature from 15 °C to 35°C significantly increased β-GLU activity in soils [40]. Similarly [41] reported that β-GLU activity doubled at high temperatures (30°C) compared with the optimal conditions of 20°C.

β-NAG is a key glycosidase enzyme produced mainly by fungi. It is responsible for the hydrolysis of N-acetylglucosamine residues from chitin and other β-1,4-linked glucosamine polymers in organic materials present in soils. Through the breakdown of chitin, NAG enzymes contribute to the release of simple amino sugars, which are important sources of readily mineralizable C and N in the soil ([41]. In our study, we observed a significant increase in β-NAG activity in rhizosphere soils at 32/26°C compared to that at 28/22°C. This increased activity could be due to enhanced optimal temperature conditions for microbial activity, substrate availability, or an increase in enzyme efficiency. The increase in NAG activity indicates a potential increase in chitin degradation, which can contribute to the availability of soil-mineralizable C and N for plant uptake during plant growth [34]. Our findings were consistent with those of [42], who demonstrated a similar temperature-dependent increase in N-acetylglucosaminidase activity in forest soils.

UNDER PEER REVIEW

Table 2. The main effect of temperature on soil enzyme activity

Temperature (°C)	ACP[ii]	βNAG[iii]	βGLU[iv]	DIEST[v]
28/22	0.166	0.017	0.019	0.082

32/28	0.317	0.084	0.062	0.086
Significance	0.02	0.001	0.010	0.94

[i] Note: Significant at Tukey Kramer's test at 0.05 level of probability

[ii] Acid-Phosphatase

[iii] β-1,4-N-acetylglucosaminidase

[iv] βeta-Glucosidase

[v] Phosphodiesterase

Main Effect of Cultivars on soil enzyme activity. Cultivar significantly influenced the activity of the βNAG (Table 3) which was higher in Beauregard than in Whatley/Loretan. This suggests that this soil may have a greater capacity to enhance the mineralization of chitin. Higher enzyme activity in Beauregard indicates the potential for improved nutrient cycling and soil fertility in its rhizosphere. Our results suggest that βNAG activity in the rhizosphere of sweet potato is cultivar-dependent.

Table 3. The main effect of cultivar on soil enzyme activity

Cultivar	**ACP[ii]**	**βNAG[iii]**	**βGLU[iv]**	**DIEST[v]**
		(μmol g^{-1} soil hr^{-1})		
Whatley Loreran	0.198	0.035	0.033	0.091
Beauregard	0.285	0.068	0.049	0.077
Significance	0.14	0.001	0.28	0.79

[i]Note: Significant at Tukey Kramer's test at 0.05 level of probability

[ii] Acid-Phosphatase

[iii] β-1,4-N-acetylglucosaminidas

[iv] βeta-Glucosidase

[v] Phosphodiesterase

Microbial community composition across soil rhizosphere and temperature levels

Bacteria composition. The bacterial composition in response to temperature and cultivars across the samples is shown in Figure 1. *Acidobacteria* and *Proteobacteria* were dominant genera across all samples, comprising more than 45% of the total population. Other notable bacteria present at

Commented [SN15]: Single space

Commented [SN16]: Single space

Commented [SN17]: Not underline

Commented [SN18]: Single space

Commented [SN19]: Single space

Commented [SN20]: Not underline

UNDER PEER REVIEW

>1% included *Verrucomicrobia*, *Firmicutes*, *Actinobacteria, Chloroflexi, Planctomycetes, Gemmatimonadetes*, and *Bacteroidetes*. The dominance of *Acidobacteria* and *Proteobacteria* is consistent with previous research in agricultural ecosystems, which frequently identified these as bacterial phyla being prevalent in soil bacterial communities across diverse environments. For instance, [42] reported that *Chloroflexi, Actinobacteria, Proteobacteria*, and *Acidobacteria* are commonly dominant in various tropical soils. Similarly, [43] found that *Proteobacteria* was the most predominant at elevated temperatures (5°C to 10°C), followed by *Chloroflexi*, *Acidobacteria*, and *Verrucomicrobia*. Another study, [44] reported that *Proteobacteria* and *Actinobacteria* were predominant in soils incubated at 0-40°C. Additionally, [45] highlighted that under different temperature conditions in apple rhizosphere soil, *Proteobacteria, Bacteroidetes, Acidobacteria*, and *Verrucomicrobia* were the most dominant phyla, followed by *Firmicutes* and *Gemmatimonadetes*.

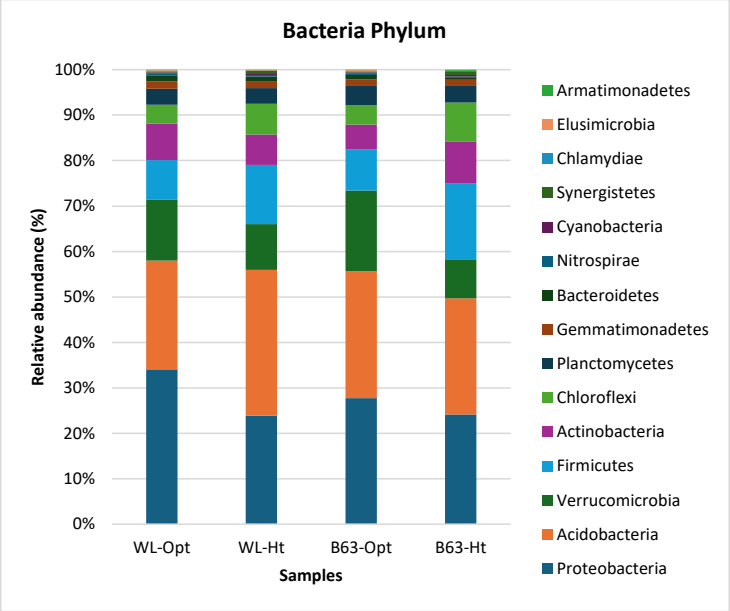

Figure 1: Relative abundance of soil bacterial phyla on sweet potato rhizosphere of Watley/Loreton and Boaurguard under high and optimal temperature conditions. WL-Opt = Watley/Loreton at optimal temperature, WL-Ht = Waltely/Loreton at high temperature, B63-Opt = Bourguard at optimal temperature, and B63-Opt = Bourguard at high temperature. Only abundance for > 1% of either soil.

At the class level, among the forty-six identified bacterial classes, ten with greater than 1% abundance were detected in all samples (Figure 2). *Acidobacteria* and *Alphaproteobacteria* of the phyla *Acidobacteria* and *Proteobacteria*, respectively, were the most dominant across all the samples. Other classes with more than 1% abundance included *Betaproteobacteria, Gammaproteobacteria,* and *Deltaproteobacteria* of the phylum *Proteobacteria*; *Spartobacteria* of the phylum *Acidobacteria; Rubrobacteria*; and *Thermoleophilia* of the phylum *Actinobacteria*; *Bacilli* and *Clostridia* of the phylum *Firmicutes*; and classes *Ktedonobacteria, Verrucomicrobiae*,

and *Planctomycetia* from the phyla *Chloroflexi, Verrucomicrobia*, and *Planctomycetes*, respectively.

The distribution of bacterial classes across the samples provided interesting insights into the microbial community structure in the soil. For example, *Acidobacteriia* and *Alphaproteobacteria* emerged as the dominant classes, aligning with the broader dominance of their respective phyla, *Acidobacteria* and *Proteobacteria*. The presence of diverse classes is indicative of diverse soil microbes, which are essential for nutrient cycling and soil health maintenance [46].

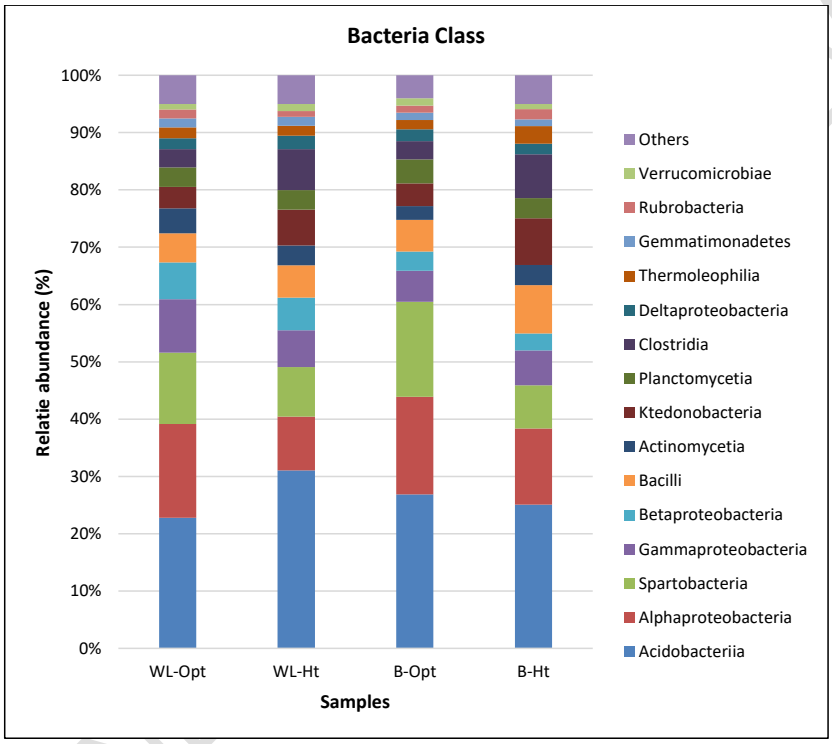

Figure 2: Relative abundance of soil bacterial classes on sweetpotato rhizosphere of Watley/Loreton and Boaurguard under high and optimal temperature conditions. WL-Opt = Watley/Loreton at optimal temperature, WL-Ht = Waltely/Loreton at high temperature, B63-Opt = Bourguard at optimal temperature, and B63-Opt = Bourguard at high temperature. Only abundance for > 1% of either soil.

Fungi composition. There were three prominent phyla in the fungal community namely *Mucoromycota, Ascomycota*, and *Entomophthoromycota*, with *Ascomycota* being the dominant phylum across all the samples, ranging from 58% to 98% of the total fungal sequences observed (Figure 3).

Commented [SN21]: Not underline

At the class level, eight classes represented > 1% of the total sequences (Figure 4). Six of these belonged to the phylum *Ascomycota: Dothideomycetes, Eurotiomycetes, Pezizomycetes, Lecanoromycetes, Sordariomycetes,* and *Leotiomycetes*. The other two classes included *Mucoromycetes* from the phylum *Mucoromycota* and *Entomophthoromycetes* from the phylum *Entomophthoromycota*. The dominant fungal groups varied depending on temperature. Specifically, *Lecanoromycetes* and *Eurotiomycetes* were the most dominant classes at high-temperature conditions, whereas *Dothideomycetes* and Mucoromycetes were the dominant groups at optimal temperatures (Figure 4).

The dominance of *Ascomycota* was consistent with previous studies that highlighted the abundance of this phylum in the sweetpotato rhizosphere [47], [48]. Generally, the presence of *Ascomycota* is an indicator of soil health because it is primarily responsible for the degradation of polysaccharides in plant cell walls and is known for its ecological versatility and role in decomposing organic material, making it a critical component of soil health and nutrient cycling [49].

The observed variation in dominance across temperature conditions reflected the adaptability of fungal communities to environmental factors. Under high temperatures, *Lecanoromycetes* and *Eurotiomycetes* were the most prevalent, suggesting that these classes may have thermotolerant traits that allow them to thrive under warmer conditions. In contrast, the dominance of *Dothideomycetes* and *Mucoromycetes* under optimal temperature conditions may indicate their potential preference for more moderate environments.

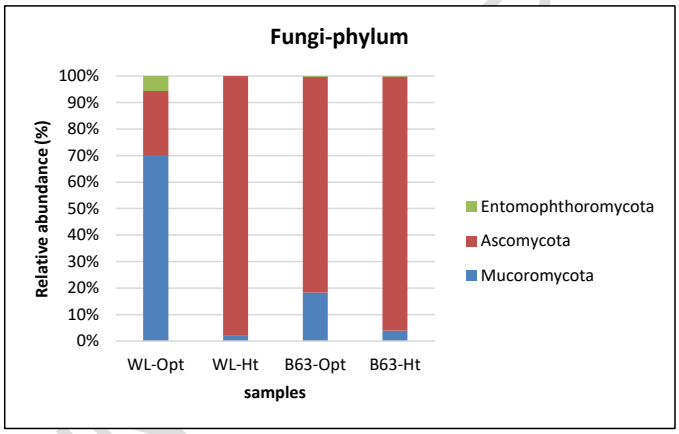

Figure 3: Relative abundance of soil fungi phyla on sweet potato rhizosphere of Watley/Loreton and Boaurguard under high and optimal temperature conditions. WL-Opt = Watley/Loreton at optimal temperature, WL-Ht = Waltely/Loreton at high temperature, B63-Opt = Bourguard at optimal temperature, and B63-Opt = Bourguard at high temperature.

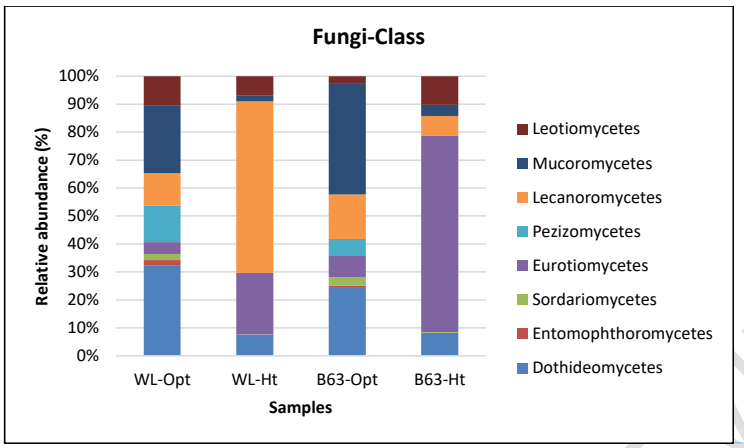

Figure 4: Relative abundance of soil fungal phyla classes on sweet potato rhizosphere of Watley/Loreton and Boaurguard under high and optimal temperature conditions. WL-Opt = Watley/Loreton at optimal temperature, WL-Ht = Waltely/Loreton at high temperature, B63-Opt = Bourguard at optimal temperature, and B63-Opt = Bourguard at high temperature.

The microbial community responses to temperatures, cultivars, and their interaction

Response of bacterial community.

Analysis of variance showed that temperature significantly affected the composition of several bacterial phyla, including *Proteobacteria, Firmicutes, Verrucomicrobia*, and *Chloroflexi.* Cultivar significantly affected the phyla *Firmicutes* and *Gemmatimonadetes*. The interaction between temperature and cultivar influenced the relative abundances of phyla *Acidobacteria, Verrucomicrobia, Firmicutes,* and *Actinobacteria* (Table 4).

At the class level, temperature had a significant effect on *Ktedonobacteria* within the phylum *Chloroflexi*, whereas cultivars significantly affected the class *Betaproteobacteria* within the phylum *Proteobacteria*. There was also a significant interaction between temperature and cultivar influencing the relative abundance of the classes *Rubrobacteria and Thermoleophilia* from the phylum Actinobacteria, as well as *Acidobacteriia* and *Spartobacteria* from the phylum *Acidobacteria*.

Table 4: Analysis of Variance (ANOVA) of temperature, cultivars, and temperature x cultivars on bacterial phyla

Commented [SN22]: Not using line in the border of pictures

Commented [SN23]: Not underline

UNDER PEER REVIEW

Phylum	Class	Source of variation		
		Temp.	Cultivar	Temp. x Cultivar
Proteobacteria		<0.001***	0.05**	0.04**
Acidobacteria		NS	NS	0.04
Verrucomicrobia		<0.001***	0.24	0.03**
Firmicutes		<0.001 ***	0.01**	0.03**
Actinobacteria		0.05	0.99	0.002**
Chloroflexi		<0.001***	0.15	0.23
Planctomycetes		NS	0.50	0.34
Gemmatimonadetes		0.80	0.02**	0.81
Acidobacteria	Acidobacteriia	0.04**	0.47	0.01**
	Spartobacteria	<0.001***	0.21	0.05**
Actinobacteria	Rubrobacteria	0.88	0.15	0.006**
	Thermoleophilia	0.01**	0.03**	0.003**
Proteobacteria	Betaproteobacteria	0.41	0.001**	0.73
Chloroflexi	Ktedonobacteria	<0.001***	0.15	0.24

Note: **P<0.05, ***P<0.01

Effect of temperature on bacterial community. Temperature influenced the phyla *Chloroflexi* and *Proteobacteria*, the higher temperatures had an 83% increase in the relative abundance of *Chloroflexi* and a 28% decrease in *Proteobacteria* (Table 5). Temperature is a crucial factor in the growth and function of soil microorganisms, and temperature changes can significantly affect the diversity and population of microbial communities. The differential response of bacterial phyla to increased temperatures observed in this study highlights the varying sensitivities and adaptabilities of these groups to thermal changes.

The significant increase in *Chloroflexi* under high temperatures suggests that this phylum may have a higher heat tolerance, allowing it to thrive under elevated temperature conditions. Conversely, the decrease in *Proteobacteria* indicates that this phylum may be less adaptable to high temperatures, potentially because it prefers moderate conditions or interactions with other microbes. These observations are consistent with previous studies, which have shown that higher temperatures can reduce the prevalence of bacteria adapted to cooler environments while favoring those that are heat-resistant and resilient ([50]

UNDER PEER REVIEW

Variability in bacterial responses to temperature changes can be attributed to differences in thermal tolerance, optimal growth temperatures, and microbial interactions ([51] For instance, high temperatures may suppress the growth of bacteria adapted to cooler conditions while promoting the proliferation of heat-tolerant species. For example, Firmicutes are known for their ability to withstand high temperatures, which may explain their increased abundance under such conditions [51].

Previous studies have also highlighted the temperature sensitivity of various bacterial phyla, including *Acidobacteria, Planctomycetes, Bacteroidetes, Actinobacteria, Chloroflexi*, *Firmicutes*, and *Verrucomicrobia* [52]. Our findings align with these studies, particularly in the case of Proteobacteria, which are predominantly mesophiles and microorganisms that prefer moderate temperatures. In the present study, the significant reduction in the relative abundance of *Proteobacteria* under high-temperature conditions across both cultivars further supports the idea that this phylum is less capable of thriving in warmer environments.

Table 5. The main effect of temperature is on the percentage of relative abundance of bacteria

Phylum	**Temperature (°C)**	
	28/22	**32/26**
Proteobacteria	30.8	24.0
Chloroflexi	4.2	7.7
Significance	<0.001	<0.001

Note: Significant at Tukey Kramer's test at 0.05 level of probability

Effect of cultivars on bacterial communities. Cultivar significantly affected the composition of the bacterial communities (Table 6). *Gemmatimonadetes* and class *Betaproteobacteria* (of the phylum *Proteobacteria*) had a higher relative abundance in the rhizosphere of the Whatley/Loretan than in that of Beauregard. The significantly higher relative abundance of *Gemmatimonadetes* and *Betaproteobacteria* in Whatley/Loretan suggests that specific plant-microbe interactions may be driving these variations. Such differences could be linked to the distinct root exudates or soil environments associated with each cultivar, which in turn select different microbial populations. *Gemmatimonadetes* and *Betaproteobacteria* are known for their roles in soil nutrient cycling and adaptability to various environmental conditions [27]. The increased presence of these groups in Whatley/Loretan may indicate that this cultivar fosters a more favorable environment for these bacteria, potentially enhancing soil health and nutrient availability.

UNDER PEER REVIEW

Table 6. The main effect of cultivar is on the percentage of relative abundance of bacteria

Phylum	Class	Cultivars		Significance
		Watley Loreton	Bourguard	
Gemmatimonadetes		1.55 a	1.23	0.02**
Proteobacteria	Betaproteobacteria	3.13 b	6.05	0.05**

Note: Significant at Tukey Kramer's test at 0.05 level of probability

Interaction of temperature and cultivar on the bacterial community. At elevated temperatures, Whatley/Loretan exhibited a higher relative abundance of phylum *Acidobacteria* but a lower relative abundance of *Actinobacteria* and *Verrucomicrobia* compared to those in Beauregard (Table 7). In contrast, Beauregard showed a significant increase in the relative abundance of phylum *Firmicutes* relative to those of Whatley/Loretan. At the class level, there was a decrease in the abundance of *Rubrobacteria* and *Thermoleophilia* in Whatley/Loretan but an increase in Beauregard at the higher temperature, highlighting distinct cultivars' differences in response to temperature perturbations.

Table 7: Analysis Percentages indicate the relative abundance of soil bacterial phyla in response to temperature and cultivar interaction.

Commented [SN24]: Title in single space

	Cultivar				Significance
	WatleyLoreton		Beaurguard		
	28/22^{0}C	32/26^{0}C	28/22^{0}C	32/26^{0}C	
Proteobacteria	34.0 a	23.9 b	27.7 b	24.1 b	0.04
Acidobacteria	23.9 b	32.1 a	27.9 b	25.6 b	0.04
Verrucomicrobia	13.5 b	10.0 bc	17.8 a	08.5 c	0.03
Firmicutes	08.6 c	13.1 b	09.0 c	16.8 a	0.03
Actinobacteria	08.1 ab	06.7 bc	05.5 c	09.1 a	0.002

Note: Significant at Tukey Kramer's test at 0.05 level of probability

UNDER PEER REVIEW

Response of fungal community.

Commented [SN25]: Not underline

Analysis of variance showed that temperature and cultivar significantly influenced the composition of all identified fungal phyla. The interaction between temperature and cultivar specifically affected the phylum *Entomophthoromycota* (Table 8). At the class level, temperature significantly impacted the relative abundances of *Dothideomycetes, Eurotiomycetes, Pezizomycetes, Lecanoromycetes,* and *Leotiomycetes* within the phylum *Ascomycota,* as well as *Mucoromycete*s within the phylum *Mucoromycota*. Cultivars significantly affected the classes *Lecanoromycetes* and *Mucoromycetes*, with *Lecanoromycetes* being the only class influenced by the interaction between temperature and cultivars (Table 8)

Temperature and cultivar played significant roles in shaping fungal community composition, with clear impacts observed across various phyla and classes. The response of *Ascomycota* classes to temperature underscores their sensitivity to temperature changes, while the significant effect of cultivars on *Lecanoromycetes* and *Mucoromycetes* suggests that plant-specific factors also drive the fungal community structure.

Table 8: Statistical significance from two-factor Analysis of Variance (ANOVA) showing F-value and significance of cultivar, temperature, and cultivar × temperature effects on fungal taxa at the phylum and class levels.

Commented [SN26]: Table title in single space

Phylum	Class	Source of variation		
		Temperature	Cultivar	T X C
Mucoromycota		<0.001***	0.03**	0.24
Ascomycota		<0.001***	0.04**	0.29
Entomophthoromycota		<0.001***	<0.001***	<0.001***
Ascomycota	Mucoromycetes	<0.001***	0.03**	0.24
	Dothideomycetes	0.001**	0.43	0.17
	Eurotiomycetes	0.01**	0.10	0.20
	Pezizomycetes	0.03**	0.34	0.34
	Lecanoromycetes	0.01**	0.02**	0.01**
	Leotiomycetes	0.44	0.50	0.03**

Note. **P<0.05, ***P<0.01

UNDER PEER REVIEW

***Effect of temperature on fungal community*.** Temperature significantly influenced the relative abundance of all identified phyla, with the phylum *Ascomycota* increaseing by 80%, while that of *Mucoromycota* and *Entomophthoromycota* decreased by 40 and 77%, respectively, compared to the optimal temperature (Table 9).

Also impacted were important fungal classes, including *Mucoromycetes* of phylum *Mucoromycota* and classes *Dothideomycetes*, *Pezizomycetes*, and *Eurotiomycetes* of phylum *Ascomycota* (Table 9). Classes within phyla differed in their responses, at the higher temperature, with the relative abundance of class *Eurotiomycetes* belonging to the *Ascomycota* phylum showing a substantial 484% increase, while the relative abundance of *Dothideomycetes* and *Pezizomycetes* of phylum *Ascomycota* decreased by 69% and 100%, respectively. These findings suggest that Ascomycota was the most diverse phylum and that the observed increase in its abundance was more pronounced in the class Eurotiomycetes.

Our findings are consistent with prior research that highlighted the substantial influence of warming on soil fungal community structures across various ecosystems [53]. For instance, [54] reported a decrease in the abundance of the *Dothideomycetes* and *Eurotiomycetes* classes of *Ascomycota* with decreasing temperature. Although the changes in the relative abundance of the microbial community align with previous findings, there are marginal differences that may be attributable to the soil type, duration of exposure, vegetation type, and degree of warming.

Table 9: The main effect of temperature on the percentage relative abundance of fungal phyla

Phylum	**Class**	**Temp. (°C)**		**Significance**
		28/22	**32/26**	
Mucoromycota		31.4	5.6	<0.001
Ascomycota		67.4	94.4	<0.001
Mucoromycota	Mucoromycetes	5.58	31.37	<0.001
Ascomycota	Dothideomycetes	28.2	8.7	<0.001
	Eurotiomycetes	6.3	36.8	0.01
	Pezizomycetes	10.1	0.0	0.03

Note: Significant at Tukey Kramer's test at 0.05 level of probability

***Effect of cultivars on fungal community*.** Cultivar had a significant effect on the composition of the fungal phyla, *Mucoromycetes* and *Ascomycota*. In the rhizosphere of Beauregard, there was a higher relative abundance of *Mucoromycetes* and a lower abundance of *Ascomycota* than in Watley/Loreton (Table 10). These findings suggest that the two cultivars may have specific interactions with these fungi, or they may produce different types or quantities of root exudates, leading to differential attraction of specific fungal groups.

Table 10: Main effect of Cultivar. on the percentage relative abundance of fungal phyla

UNDER PEER REVIEW

Phylum	Class	Cultivar		Significance
		Watley Loreton	Bourguard	
Mucoromycota		12.14	24.82	0.03
Ascomycota		86.10	74.75	0.04
Mucoromycota	Mucoromycetes	12.14	24.81	0.03

Note: Significant at Tukey Kramer's test at 0.05 level of probability

***Interaction of temperature and cultivar on fungal community*.** The phylum *Entomophthoromycota* and the classes *Lecanoromycetes* and *Leotiomycetes* within the phylum *Ascomycota* were influenced by both temperature and cultivar (Table 11). At the higher temperature, the relative abundance of *Entomophthoromycota* decreased by 88% in the rhizosphere of Beauregard or was nonexistent in that of Whatley/Loretan. The high temperature led to a significant increase in the relative abundance of the class *Lecanoromycetes*, with a more pronounced increase observed in Whatley/Loretan compared to Beauregard. The class *Leotiomycetes* showed a contrasting response between the two cultivars, significantly increasing in Whatley/Loretan and decreasing in Beauregard under a high-temperature condition.

These findings demonstrate that the effect of temperature on specific fungal taxa differed depending on the sweetpotato cultivar grown and vice versa. The decrease in *Entomophthoromycota*, especially its complete absence in the rhizosphere of Whatley/Loretan, suggests that this phylum is highly sensitive to temperature increase, with cultivar-specific factors further influencing its abundance. The increase in *Lecanoromycetes* under the higher temperature, especially in Whatley/Loretan, indicated a possible thermal advantage or adaptability of this class in this cultivar. Conversely, the opposite responses of *Leotiomycetes* between the two cultivars highlight the complex and varied effects of temperature and cultivar interactions on fungal community composition. These results emphasize the importance of considering both environmental conditions and plant cultivars when studying soil fungal dynamics, as their interplay can lead to significant shifts in the community structure.

Table 11: Analysis Percentages indicate the relative abundance of soil bacterial phyla in response to temperature and cultivar interaction.

Commented [SN27]: Single space

Phylum	Class	Cultivar				Significance
		Watley Loreton		Bourguard		
		28/22°C	32/26 °C	28/22 °C	32/26 °C	
Entomophthoromycota		1.73	0.00	0.81	0.10	<0.001
Ascomycota	Lecanoromycetes	11.46	61.42	14.96	16.21	0.01
	Leotiomycetes	11.53	6.54	2.44	11.82	0.03

Note: Significant at Tukey Kramer's test at 0.05 level of probability

UNDER PEER REVIEW

CONCLUSION

High temperatures significantly affected key soil enzymes, such as P-acid-phosphatase, β-glucosidase, N-acetyl-β-glucosaminidase, and phosphodiesterase in the sweet potato rhizosphere, all of which are crucial for the decomposition of organic matter, P, N, and carbon cycling, and nutrient availability for plant uptake. Additionally, both temperature and cultivar played vital roles in shaping the distribution and population of essential bacterial and fungal communities in the rhizosphere of sweet potato. Therefore, when studying microbial diversity in sweet potato-growing environments, it is important to consider the specific cultivar being grown. This approach will enhance our understanding of soil ecological dynamics and develop sustainable strategic farming, particularly in the context of global climate change.

REFERENCES

[1] A. F. El Sheikha and R. C. Ray, "Potential impacts of bioprocessing of sweet potato: Review," *Crit Rev Food Sci Nutr*, vol. 57, no. 3, pp. 455–471, Feb. 2017, https://www.doi.org/10.1080/10408398.2014.960909

[2] D. Tedesco, B. R. de A. Moreira, M. R. Barbosa Júnior, M. Maeda, and R. P. da Silva, "Sustainable management of sweet potatoes: A review on practices, strategies, and opportunities in nutrition-sensitive agriculture, energy security, and quality of life," *Agric Syst*, 210, p. 103693, Aug. 2023, doi: 10.1016/J.AGSY.2023.103693.

[3] FAO, "FAOSTAT." Accessed: Oct. 17, 2022. [Online]. Available: https://www.fao.org/faostat/en/#data/QCL

[4] CIP, "Sweetpotato Facts and Figures - International Potato Center." Accessed: Oct. 17, 2022. [Online]. Available: https://cipotato.org/sweetpotato/sweetpotato-facts-and-figures/

[5] Z. Sapakhova *et al.*, "Sweet Potato as a Key Crop for Food Security under the Conditions of Global Climate Change: A Review," *Plants 2023, Vol. 12, Page 2516*, vol. 12, no. 13, p. 2516, Jun. 2023, doi: 10.3390/PLANTS12132516.

[6] L. Mommer, P. Hinsinger, C. Prigent-Combaret, and E. J. W. Visser, "Advances in the rhizosphere: stretching the interface of life," *Plant Soil*, vol. 407, no. 1–2, pp. 1–8, Oct. 2016, doi: 10.1007/S11104-016-3040-9/METRICS.

[7] G. Berg and K. Smalla, "Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere," *FEMS Microbiol Ecol*, vol. 68, no. 1, pp. 1–13, Apr. 2009, doi: 10.1111/J.1574-6941.2009.00654.X.

[8] J. M. Raaijmakers, T. C. Paulitz, C. Steinberg, C. Alabouvette, and Y. Moënne-Loccoz, "The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms," *Plant and Soil 2008 321:1*, vol. 321, no. 1, pp. 341–361, Feb. 2008, doi: 10.1007/S11104-008-9568-6.

[9] M. Höfte, "The use of Pseudomonas spp. as bacterial biocontrol agents to control plant diseases," pp. 301–374, Nov. 2021, doi: 10.19103/AS.2021.0093.11.

[10] R. C. Wilhelm, H. M. van Es, and D. H. Buckley, "Predicting measures of soil health using the microbiome and supervised machine learning," *Soil Biol Biochem*, vol. 164, p. 108472, Jan. 2022, doi: 10.1016/J.SOILBIO.2021.108472.

[11] S. P. Dlamini, A. O. Akanmu, and O. O. Babalola, "Rhizospheric microorganisms: The gateway to a sustainable plant health," *Front Sustain Food Syst*, vol. 6, Aug. 2022, doi: 10.3389/FSUFS.2022.925802.

[12] J. K. Jansson and K. S. Hofmockel, "Soil microbiomes and climate change," *Nature Reviews Microbiology 2019 18:1*, vol. 18, no. 1, pp. 35–46, Oct. 2019, doi: 10.1038/s41579-019-0265-7.

[13] E. Błońska, J. Lasota, and M. Zwydak, "The relationship between soil properties, enzyme activity and land use," *Forest Research Papers*, vol. 78, no. 1, pp. 39–44, Mar. 2017, doi: 10.1515/FRP-2017-0004.

[14] C. E. Martinez and M. A. Tabatabai, "Decomposition of Biotechnology By-Products in Soils," *J Environ Qual*, vol. 26, no. 3, pp. 625–632, May 1997, doi: 10.2134/JEQ1997.00472425002600030006X.

Commented [SN28]: If author cited a reference, please use the family name and year, for example, El Sheika AF and Ray RC, 2017.

Commented [SN29]: The correct wrote of doi

Commented [SN30]: This is family name, put in the beginning of citation

Commented [SN31]: Complete first author and all of co-authors

UNDER PEER REVIEW

[15] M. Ekenler and M. A. Tabatabai, “β-Glucosaminidase Activity as an Index of Nitrogen Mineralization in Soils,” *Commun Soil Sci Plant Anal*, vol. 35, no. 7–8, pp. 1081–1094, Dec. 2004, doi: 10.1081/CSS-120030588.
[16] P. Nannipieri, L. Giagnoni, L. Landi, and G. Renella, “Role of Phosphatase Enzymes in Soil,” pp. 215–243, 2011, doi: 10.1007/978-3-642-15271-9_9.
[17] S. Deng, H. Kang, and C. Freeman, “Microplate Fluorimetric Assay of Soil Enzymes,” *Methods of Soil Enzymology*, pp. 311–318, Oct. 2015, doi: 10.2136/SSSABOOKSER9.C14.
[18] A. E. T Classen *et al.*, “Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead?,” *Ecosphere*, vol. 6, no. 8, pp. 1–21, Aug. 2015, doi: 10.1890/ES15-00217.1.
[19] L. Cheng *et al.*, “Warming enhances old organic carbon decomposition through altering functional microbial communities,” *ISME J*, vol. 11, no. 8, pp. 1825–1835, Aug. 2017, doi: 10.1038/ISMEJ.2017.48.
[20] C. Wang *et al.*, “The temperature sensitivity of soil: microbial biodiversity, growth, and carbon mineralization,” *ISME J*, vol. 15, no. 9, pp. 2738–2747, Sep. 2021, doi: 10.1038/S41396-021-00959-1.
[21] J. C. Carey *et al.*, “Temperature response of soil respiration largely unaltered with experimental warming,” *Proc Natl Acad Sci U S A*, vol. 113, no. 48, pp. 13797–13802, Nov. 2016, doi: 10.1073/PNAS.1605365113/SUPPL_FILE/PNAS.1605365113.SAPP.PDF.
[22] K. Vishwakarma *et al.*, “Exploring the role of plant-microbe interactions in improving soil structure and function through root exudation: A key to sustainable agriculture,” *Plant-Microbe Interactions in Agro-Ecological Perspectives*, vol. 1, pp. 489–499, Oct. 2017, doi: 10.1007/978-981-10-5813-4_23/COVER.
[23] C. Wang *et al.*, “The temperature sensitivity of soil: microbial biodiversity, growth, and carbon mineralization,” *The ISME Journal 2021 15:9*, vol. 15, no. 9, pp. 2738–2747, Mar. 2021, doi: 10.1038/s41396-021-00959-1.
[24] I. Fekete *et al.*, “The effects of litter production and litter depth on soil microclimate in a central european deciduous forest,” *Plant Soil*, vol. 398, no. 1–2, pp. 291–300, Jan. 2016, doi: 10.1007/S11104-015-2664-5/METRICS.
[25] M. Zhao *et al.*, “Microbial mediation of biogeochemical cycles revealed by simulation of global changes with soil transplant and cropping,” *The ISME Journal 2014 8:10*, vol. 8, no. 10, pp. 2045–2055, Apr. 2014, doi: 10.1038/ismej.2014.46.
[26] Q. Hou *et al.*, “Rhizosphere microbial diversity and community dynamics during potato cultivation,” *Eur J Soil Biol*, vol. 98, p. 103176, May 2020, doi: 10.1016/J.EJSOBI.2020.103176.
[27] J. Zhou, C. Liu, L. Shi, and K. Zamanian, “Rhizosphere influence on microbial functions: consequence for temperature sensitivity of soil organic matter decomposition at early stage of plant growth,” *Plant Soil*, pp. 1–15, Sep. 2023, doi: 10.1007/S11104-023-06258-2/METRICS.
[28] A. Borowik and J. Wyszkowska, “Soil moisture as a factor affecting the microbiological and biochemical activity of soil,” *Plant Soil Environ*, vol. 62, no. 6, pp. 250–255, 2016, doi: 10.17221/158/2016-PSE.
[29] A. L. Romero-Olivares, S. D. Allison, and K. K. Treseder, “Soil microbes and their response to experimental warming over time: A meta-analysis of field studies,” *Soil Biol Biochem*, vol. 107, pp. 32–40, Apr. 2017, doi: 10.1016/J.SOILBIO.2016.12.026.
[30] W. H. Gardner, “ In Methods of Soil Analysis, Part 1. Physical and Mineralogical Methods—Agronomy Monograph No 9 (2nd Edition) American Society of Agronomy, Soil Science Society of America, Madison. - References - Scientific Research Publishing,” *American Society of Agronomy*, 1986, Accessed: Oct. 28, 2022. [Online]. Available: https://www.scirp.org/(S(i43dyn45teexjx455qlt3d2q))/reference/ReferencesPapers.aspx?ReferenceID=1189528
[31] J. M. Steinweg, A. F. Plante, R. T. Conant, E. A. Paul, and D. L. Tanaka, “Patterns of substrate utilization during long-term incubations at different temperatures,” *Soil Biol Biochem*, vol. 40, no. 11, pp. 2722–2728, Nov. 2008, doi: 10.1016/J.SOILBIO.2008.07.002.
[32] E. A. Davidson and I. A. Janssens, “Temperature sensitivity of soil carbon decomposition and feedbacks to climate change,” *Nature 2006 440:7081*, vol. 440, no. 7081, pp. 165–173, Mar. 2006, doi: 10.1038/nature04514.

UNDER PEER REVIEW

[33] B. A. Caldwell, “Enzyme activities as a component of soil biodiversity: A review,” *Pedobiologia (Jena)*, vol. 49, no. 6, pp. 637–644, Nov. 2005, doi: 10.1016/J.PEDOBI.2005.06.003.
[34] L. Luo, H. Meng, and J. D. Gu, “Microbial extracellular enzymes in biogeochemical cycling of ecosystems,” *J Environ Manage*, vol. 197, pp. 539–549, Jul. 2017, doi: 10.1016/J.JENVMAN.2017.04.023.
[35] A. R. Marklein and B. Z. Houlton, “Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems,” *New Phytologist*, vol. 193, no. 3, pp. 696–704, Feb. 2012, doi: 10.1111/J.1469-8137.2011.03967.X.
[36] O. Margalef *et al.*, “Global patterns of phosphatase activity in natural soils,” *Scientific Reports 2017 7:1*, vol. 7, no. 1, pp. 1–13, May 2017, doi: 10.1038/s41598-017-01418-8.
[37] J. A. Kumari, P. C. Rao, G. Padmaja, and M. Madhavi, “Effect of Temperature on Soil Enzyme Acid Phosphatase,” *Int J Curr Microbiol Appl Sci*, vol. 7, no. 09, pp. 2830–2845, Sep. 2018, doi: 10.20546/IJCMAS.2018.709.352.
[38] I. Mariscal-Sancho, B. Ball, and B. McKenzie, “Influence of Tillage Practices, Organic Manures and Extrinsic Factors on β-Glucosidase Activity: The Final Step of Cellulose Hydrolysis,” *Soil Systems 2018, Vol. 2, Page 21*, vol. 2, no. 2, p. 21, Apr. 2018, doi: 10.3390/SOILSYSTEMS2020021.
[39] Y. Song *et al.*, “Short-Term Response of the Soil Microbial Abundances and Enzyme Activities to Experimental Warming in a Boreal Peatland in Northeast China,” *Sustainability 2019, Vol. 11, Page 590*, vol. 11, no. 3, p. 590, Jan. 2019, doi: 10.3390/SU11030590.
[40] J. M. Steinweg, J. S. Dukes, and M. D. Wallenstein, “Modeling the effects of temperature and moisture on soil enzyme activity: Linking laboratory assays to continuous field data,” *Soil Biol Biochem*, vol. 55, pp. 85–92, Dec. 2012, doi: 10.1016/J.SOILBIO.2012.06.015.
[41] J. P. Schimel and J. Bennett, “NITROGEN MINERALIZATION: CHALLENGES OF A CHANGING PARADIGM,” *Ecology*, vol. 85, no. 3, pp. 591–602, Mar. 2004, doi: 10.1890/03-8002.
[42] Á. Machuca *et al.*, “In vitro sensitivity of forest soil enzymes to temperature increase in Western Patagonia,” *J Soil Sci Plant Nutr*, vol. 18, no. 1, pp. 202–219, Mar. 2018, doi: 10.4067/S0718-95162018005000801.
[43] A. Mateos-Rivera, J. C. Yde, B. Wilson, K. W. Finster, L. J. Reigstad, and L. Øvreås, “The effect of temperature change on the microbial diversity and community structure along the chronosequence of the sub-arctic glacier forefield of Styggedalsbreen (Norway),” *FEMS Microbiol Ecol*, vol. 92, no. 4, Apr. 2016, doi: 10.1093/FEMSEC/FIW038.
[44] J. Fang *et al.*, “Potential effects of temperature levels on soil bacterial community structure,” *E3S Web of Conferences*, vol. 292, Sep. 2021, doi: 10.1051/E3SCONF/202129201008.
[45] H. Zhang, F. O. Phillip, L. Wu, F. Zhao, S. Yu, and K. Yu, “Effects of Temperature and Nitrogen Application on Carbon and Nitrogen Accumulation and Bacterial Community Composition in Apple Rhizosphere Soil,” *Front Plant Sci*, vol. 13, p. 859395, Apr. 2022, doi: 10.3389/FPLS.2022.859395/BIBTEX.
[46] P. Chauhan *et al.*, “Soil Microbiome: Diversity, Benefits and Interactions with Plants,” *Sustainability 2023, Vol. 15, Page 14643*, vol. 15, no. 19, p. 14643, Oct. 2023, doi: 10.3390/SU151914643.
[47] Z. Gao *et al.*, “Effects of Continuous Cropping of Sweet Potato on the Fungal Community Structure in Rhizospheric Soil,” *Front Microbiol*, vol. 10, p. 477420, Oct. 2019, doi: 10.3389/FMICB.2019.02269/BIBTEX.
[48] Y. Ding *et al.*, “Low Nitrogen Fertilization Alter Rhizosphere Microorganism Community and Improve Sweetpotato Yield in a Nitrogen-Deficient Rocky Soil,” *Front Microbiol*, vol. 11, p. 515507, Apr. 2020, doi: 10.3389/FMICB.2020.00678/BIBTEX.
[49] L. M. Manici, F. Caputo, D. De Sabata, and F. Fornasier, “The enzyme patterns of Ascomycota and Basidiomycota fungi reveal their different functions in soil,” *Applied Soil Ecology*, vol. 196, p. 105323, Apr. 2024, doi: 10.1016/J.APSOIL.2024.105323.
[50] S. D. Allison, M. D. Wallenstein, and M. A. Bradford, “Soil-carbon response to warming dependent on microbial physiology,” *Nat Geosci*, vol. 3, no. 5, pp. 336–340, May 2010, doi: 10.1038/ngeo846.
[51] X. Zhang *et al.*, “The Association between Gut Microbiome Diversity and Composition and Heat Tolerance in Cattle,” *Microorganisms*, vol. 10, no. 8, p. 1672, Aug. 2022, doi: 10.3390/MICROORGANISMS10081672/S1.

UNDER PEER REVIEW

[52] A. M. Oliverio, M. A. Bradford, and N. Fierer, “Identifying the microbial taxa that consistently respond to soil warming across time and space,” *Glob Chang Biol*, vol. 23, no. 5, pp. 2117–2129, May 2017, doi: 10.1111/GCB.13557.

[53] A. Asemaninejad, R. G. Thorn, B. A. Branfireun, and Z. Lindo, “Climate change favours specific fungal communities in boreal peatlands,” *Soil Biol Biochem*, vol. 120, pp. 28–36, May 2018, doi: 10.1016/J.SOILBIO.2018.01.029.

[54] A. T. Nottingham *et al.*, “Microbes follow Humboldt: temperature drives plant and soil microbial diversity patterns from the Amazon to the Andes,” *Ecology*, vol. 99, no. 11, pp. 2455–2466, Nov. 2018, doi: 10.1002/ECY.2482.

Commented [SN32]: Not in the manuscript

Commented [SN33]: Not in the manuscript

UNDER PEER REVIEW