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

# ABSTRACT

**Aim:** 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-65DAP **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 to December 2022.

**Methodology:** Two sweet potato cultivars, Beauregard and Whatley/Loretan, were planted and exposed to high ( $32/26^{\circ}$ C) and optimal ( $28/22^{\circ}$ 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), was analyzed using a standard DNA-Based Quantitative Technique The extracellular enzymes, Acid Phosphatases (P-acid),  $\beta$ -Glucosidase (GLU), N-acetylglucosaminidase ( $\beta$ -NAG) and phosphodiesterase (DIEST) were analyzed using fluorometric microplate enzyme assays.

**Results:** Bacterial communities were dominated by phyla *Acidobacteria* and *Proteobacteria*, whereas fungi were dominated by the *Ascomycota* across the samples. Temperature significantly affected the abundance of some bacterial phyla (*Proteobacteria, Firmicutes, Verrucomicrobia* and *Chloroflexi*) and all identified fungal phyla (*Ascomycota, Mucoromycota, and Entomophthoromycota*). Cultivars affected bacterial Phylum *Gemmatimonadetes* and fungi phyla *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 phyla *Acidobacteria, Verrucomicrobia, Firmicutes* and Fungi *Entomophthoromycota*. Additionally, high temperatures influenced the activities of key soil enzymes—acid phosphatase,  $\beta$ -glucosidase, and  $\beta$ -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 cultivars were significantly impacted by high temperatures.

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

# **1.0 INTRODUCTION**

Sweet potato [*Ipomea batatas* (*L*.) Lam.] is an important root crop known for its nutritional, medicinal, industrial and economic values (El Sheikha & Ray, 2017). 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(Tedesco et al., 2023). 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 (Sapakhova et al., 2023).

The rhizosphere is an interface of dynamic interactions among plants, soil and microorganisms (Mommer et al., 2016). It is essential for nutrient cycling and uptake, plant growth regulation and soil health (Berg & Smalla, 2009). This zone also provides a habitat for microorganisms to multiply, grow and function (Raaijmakers et al., 2008). Rhizosphere soil microorganisms, such as bacteria and fungi, are essential for the decomposition of organic matter, nutrient recycling and the regulation of plant growth (Dlamini et al., 2022; Höfte, 2021; Wilhelm et al., 2022). Soils with high microbial diversity are considered to have high ecological functions and resistance to environmental stresses, including climate change (Jansson & Hofmockel, 2019). Most biochemical processes in the rhizosphere are regulated by extracellular soil enzymes secreted by soil microbes and plants (Błońska et al., 2017). For example,  $\beta$ -Glucosidase is a group of carbon-cycling enzymes crucial for breaking down cellulose residues into glucose, an important energy source for soil microorganisms (Martinez & Tabatabai, 1997). Another key enzyme, β-1,4-Nacetylglucosaminidase, facilitates the breakdown of chitin into amino sugars, contributing to carbon and nitrogen cycling in the soil (Ekenler & Tabatabai, 2004). Phosphatase enzymes play a vital role in phosphorous cycling by releasing inorganic phosphate complexes, making them available for plant and microbial uptake (Nannipieri et al., 2011). Soil enzyme activity is linked to microbial functions and hence, is also used as an early indicator of microbial abundance in ecosystems (Deng et al., 2015; Ekenler & Tabatabai, 2004).

Temperature and plant species influence microbial communities and enzymatic activity in the rhizosphere either directly or indirectly (T Classen et al., 2015). Temperature directly affects the metabolism, growth and function of soil microorganisms (Carey et al., 2016; Cheng et al., 2017; Wang et al., 2021a) 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 (Vishwakarma et al., 2017; Wang et al., 2021b). 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 (Fekete et al., 2016). Furthermore, plant attributes such as growth stage, fine root number and metabolic activity influence soil enzyme activity (Hou et al., 2020; Zhao et al., 2014; Zhou et al., 2023).

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 (Borowik & Wyszkowska, 2016; Romero-Olivares et al., 2017). 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 swee tpotato. 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.

# 2.0 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.

# 2.1 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,  $28/22^{\circ}$ C (optimum) and  $32/26^{\circ}$ 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<sup>-1</sup> NPK. The plants were watered based on field capacity according to the oven-dry weight method (Gardner, 1986). Additionally, plant growth chamber conditions included relative humidity of 75±5% and photosynthetic photon flux of 123 µmol m<sup>2</sup>s<sup>-1</sup> were maintained.

# 2.2 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.

# 2.3 Soil enzyme analysis

The activities of  $\beta$ -glucosidase (GLU), acid phosphomonoesterase (acid-P), N-acetyl- $\beta$ -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  $\mu$ mol (4-methylumbelliferone) released per gram of dry soil per hour ( $\mu$ mol g<sup>-1</sup> h<sup>-1</sup>).

### 2.4 Microbial analysis

### 2.4.1 DNA extraction

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.

### 2.4.2 DNA amplification and sequencing

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.

### 2.4.3 Sequence analysis

The Q25 sequence data derived from the sequencing process was processed using the MR DNA ribosomal and functional gene analysis pipeline (<u>www.mrdnalab.com</u>, 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 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 (<u>www.ncbi.nlm.nih.gov</u>). 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.

# 2.5 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).

# 3.0 RESULTS AND DISCUSSION

### 3.1 Effects of temperature and cultivars on enzyme activities

The main effect of temperature significantly affected the activities of  $\beta$ -glucosidase (GLU), acid phosphomonoesterase (acid-P) and N-acetyl- $\beta$ -D-glucosamidase (NAD). Conversely, the cultivar effects were significant only for  $\beta$ -glucosidase ( $\beta$ -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 <sup>ii</sup> | βNAG <sup>iii</sup> | βGLU <sup>i</sup> ν | DIEST <sup>v</sup> |
|------------------------|----|-------------------|---------------------|---------------------|--------------------|
| 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               |

<sup>i</sup> Significance at Tukey Kramer's test, \*(P =0.05) or \*\* (P=0.01) levels

<sup>ii</sup> Acid-Phosphatase

<sup>iii</sup> β-1,4-N-acetylglucosaminidase

<sup>iv</sup> βeta-Glucosidase

<sup>v</sup> Phosphodiesterase

3.1.1 Main effect of temperature on soil enzyme activities. Most rhizosphere soil enzyme activities were significantly affected by temperature. At 32/26°C, the activities of P-Acid,  $\beta$ -GLU and  $\beta$ -NAG were significantly higher than at 28/22°C, equivalent to a twofold increase in P-Acid, threefold in  $\beta$ -GLU and fivefold in  $\beta$ -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 (Steinweg et al., 2008) and may have been within the optimal range, which could potentially influence the kinetic energy of the enzyme and its substrate, leading to an increase in enzyme activity (Davidson & Janssens, 2006).

P-acid is secreted by fungi, bacteria and plant roots and is responsible for catalyzing the transformation of complex organic P into assimilable phosphate (Caldwell, 2005). 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  $32/26^{\circ}$ 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 (Marklein & Houlton, 2012). 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 Acid-phosphatase enzyme activity from 9.2 to 297.5 µg of 4-nitrophenol g<sup>-1</sup> soil h<sup>-1</sup> (Kumari et al., 2018).

 $\beta$ -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 (Mariscal-Sancho et al., 2018). Studies have demonstrated the sensitivity of  $\beta$ -GLU enzymes to temperature change (Song et al., 2019; Steinweg et al., 2012). Similarly, our observations showed an increase in  $\beta$ -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  $\beta$ -GLU activity under high-temperature conditions. For example, an increase in temperature from 15 °C to 35°C significantly increased  $\beta$ -GLU activity in soils (Steinweg et al., 2012). Similarly [41] reported that  $\beta$ -GLU activity doubled at high temperatures (30°C) compared with the optimal conditions of 20°C.

 $\beta$ -NAG is a key glycosidase enzyme produced mainly by fungi. It is responsible for the hydrolysis of N-acetylglucosamine residues from chitin and other  $\beta$ -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 ((Schimel & Bennett, 2004). In our study, we observed a significant increase in  $\beta$ -NAG activity in rhizosphere soils at 32/26°C compared to that at 28/22°C. This increase activity could be due to enhanced optimal temperature conditions for microbial activity, substrate availability, or an increase in enzyme efficiency. This 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 (Luo et al., 2017). Our findings were consistent with those of (Machuca et al., 2018a), who demonstrated a similar temperature-dependent increase in N-acetylglucosaminidase activity in forest soils.

| Temperature (°C) | ACP <sup>ii</sup> | βNAĠ <sup>iii</sup> | βGLU <sup>iv</sup> | DIEST <sup>v</sup> |
|------------------|-------------------|---------------------|--------------------|--------------------|
| 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**             | 0.01**             | 0.94               |

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

<sup>i</sup> Note: Significant at Tukey Kramer's test 0.05 level

<sup>ii</sup> Acid-Phosphatase

<sup>iii</sup>β-1,4-N-acetylglucosaminidase

<sup>i</sup>v βeta-Glucosidase

<sup>v</sup> Phosphodiesterase

3.1.2 Main Effect of Cultivars on soil enzyme activity. Cultivar significantly influenced the activity of the  $\beta$ NAG (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  $\beta$ NAG activity in the rhizosphere of sweet potato was cultivar dependent.

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

| Cultivar        | ACF  | "βNAG"      | βGLU <sup>iv</sup>      | DIEST <sup>v</sup> |
|-----------------|------|-------------|-------------------------|--------------------|
|                 | C    | (µmol g⁻¹ s | soil hr <sup>-1</sup> ) |                    |
| Whatley Loreran | 0.19 | 8 0.035     | 0.033                   | 0.091              |
| Beauregard      | 0.28 | 5 0.068     | 0.049                   | 0.077              |
| Significance    | 0.14 | 0.001**     | 0.28                    | 0.79               |

Note: Significant at Tukey Kramer's test 0.05 level

<sup>ii</sup> Acid-Phosphatase

<sup>iii</sup>β-1,4-N-acetylglucosaminidas

<sup>iv</sup> βeta-Glucosidase

v Phosphodiesterase

# 3.2 Microbial community composition across soil rhizosphere and temperature levels

3.2.1 Bacteria composition. The bacterial composition in response to temperature and cultivars across the samples is shown in Figure 1. Acidobacteria and Proteobacteria were dominant phyla across all samples, comprising more than 45% of the total population. Other notable bacteria present at >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 phyla as being prevalent in soil bacterial communities across diverse environments. For instance, (Machuca et al., 2018b) reported that phyla such as Chloroflexi, Actinobacteria. Proteobacteria and Acidobacteria are commonly dominant in various tropical soils. Similarly, (Mateos-Rivera et al., 2016) found that Proteobacteria was the most predominant phylum at elevated temperatures (5°C to 10°C), followed by Chloroflexi, Acidobacteria and Verrucomicrobia. Another study, (Fang et al., 2021) reported that Proteobacteria and Actinobacteria were the predominant phyla in soils incubated at 0-40°C. Additionally, (Zhang et al., 2022) 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 = Watley/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 (Chauhan et al., 2023).



**Figure 2:** Relative abundance of soil bacterial 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 = Watley/Loreton at high temperature, B63-Opt = Bourguard at optimal temperature and B63-Opt = Bourguard at high temperature. Only abundance for > 1% of either soil.)

**3.2.2 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).

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 sweet potato rhizosphere (Ding et al., 2020; Gao et al., 2019). 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 (Manici et al., 2024).

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 = Watley/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 = Watley/Loreton at high temperature, B63-Opt = Bourguard at optimal temperature and B63-Opt = Bourguard at high temperature.)

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

### 3.3.1 Response of bacterial community to temperature and cultivars.

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 main bacterial phyla and classes

| Phylum           | Class              |          | Source of variat | on             |
|------------------|--------------------|----------|------------------|----------------|
|                  |                    | Temp.    | Cultivar Te      | mp. 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           |

The table summarizes the results of a two-factor Analysis of Variance (ANOVA) evaluating the effects of cultivar (sweet potato cultivars), temperature (growth conditions) and their interaction (cultivar  $\times$  temperature) on the relative abundance of bacteria taxa at the phylum and class levels. The superscript indicates that the mean difference is significant at Tukey Kramer's test, \*(P=0.05) or \*\*(P=0.01) respectively.

**3.3.1.1 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 ((Allison et al., 2010)

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 (Zhang et al., 2022).

Previous studies have also highlighted the temperature sensitivity of various bacterial phyla, including *Acidobacteria, Planctomycetes, Bacteroidetes, Actinobacteria, Chloroflexi, Firmicutes* and *Verrucomicrobia* (Oliverio et al., 2017). 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 the main bacteria

| Phylum         | Те      | mp. (°C) |
|----------------|---------|----------|
|                | 28/22   | 32/26    |
| Proteobacteria | 30.8    | 24.0     |
| Chloroflexi    | 4.2     | 7.7      |
| Significance   | 0.001** | 0.001**  |

phyla

The table indicates the means of relative abundance (%) of major Bacteria taxa at the phylum

level in soil samples under two diurnal temperatures 28/22°C (optimal) and 32.28°C (high). The

significance values indicate the statistically significant differences between groups determined

by Tukey Kramer's test at 0.05 level of probability.

**3.3.1.2 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 plantmicrobe 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 (Zhou et al., 2023). 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.

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

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

The table indicates the means of relative abundance (%) of major bacteria taxa at phylum and

class levels in soil samples from two sweet potato cultivars (WaltleyLoreton and Beaurguard). The

significance values indicate the statistically significant differences between groups determined by

Tukey Kramer's test at 0.05 level of probability.

classes

**3.3.1.3** 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.

| Phylum          |         | Significance            |         |         |         |
|-----------------|---------|-------------------------|---------|---------|---------|
|                 | Watley  | atleyLoreton Beaurguard |         |         |         |
|                 | 28/22ºC | 32/26ºC                 | 28/22°C | 32/26ºC | _       |
| Proteobacteria  | 34.0    | 23.9                    | 27.7    | 24.1    | 0.04*   |
| Acidobacteria   | 23.9    | 32.1                    | 27.9    | 25.6    | 0.04*   |
| Verrucomicrobia | 13.5    | 10.0                    | 17.8    | 08.5    | 0.03*   |
| Firmicutes      | 08.6    | 13.1                    | 09.0    | 16.8    | 0.03*   |
| Actinobacteria  | 08.1    | 06.7                    | 05.5    | 09.1    | 0.002** |

The table indicates the means of relative abundance (%) of major bacterial phyla in soil samples from two sweet potato cultivars (WaltleyLoreton and Beaurguard) grown under two diurnal temperatures of 28/22°C (optimal) and 32.28°C (high). The significance values indicate the statistically significant differences between groups determined by Tukey Kramer's test at 0.05 level.

### 3.3.2 Response of fungal communities to temperatures and cultivars.

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 *Mucoromycetes* 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.

| Phylum              | Class           | So          | n        |         |
|---------------------|-----------------|-------------|----------|---------|
|                     |                 | Temperature | Cultivar | тхс     |
| 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*   |

The table summarizes the results of a two-factor Analysis of Variance (ANOVA) evaluating the effects of cultivar (sweet potato cultivars), temperature (growth conditions) and their interaction (cultivar  $\times$  temperature) on the relative abundance of fungal taxa at the phylum and class levels. The superscript indicates significant differences based on Tukey-Kramer's test, \*(P=0.05) or \*\*(P=0.01) respectively.

**3.3.2.1 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 (Asemaninejad et al., 2018). For instance, (Nottingham et al., 2018) 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*        |

and classes

The table indicates the means of relative abundance (%) of major fungal taxa at phylum and class

levels in soil samples under two diurnal temperatures, 28/22°C (optimal) and 32.28°C (high). The

significance values indicate the statistically significant differences between groups determined by

Tukey Kramer's test at 0.05 level.

**3.3.2.2** *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 the main fungal phyla and classes

| 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*        |

The table indicates the means of relative abundance (%) of major fungal taxa at phylum and class levels

in soil samples from two sweet potato cultivars (WaltleyLoreton and Beaurguard). The significance values

indicate the statistically significant differences between groups determined by Tukey Kramer's test at

0.05 level.

**3.3.2.3** 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 sweet potato 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 the main soil fungal phyla and

classes in response to temperature and cultivar interaction.

| Phylum              | Class           | Cultivar       |          |                  | Significance |         |
|---------------------|-----------------|----------------|----------|------------------|--------------|---------|
|                     |                 | Watley Loreton |          | oreton 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*   |

The table indicates the means of relative abundance (%) of major fungal taxa at phylum and class levels in soil samples

from two sweet potato cultivars (WaltleyLoreton and Beaurguard) grown under two diurnal temperatures 28/22°C

(optimal) and 32.28°C (high). The significance values indicate the statistically significant differences between groups

determined by Tukey Kramer's test at 0.05 level.

# **4.0 CONCLUSION**

High temperatures significantly affected key soil enzymes, such as P-acid-phosphatase,  $\beta$ -glucosidase, N-acetyl- $\beta$ -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 influenced 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 the understanding of soil ecological dynamics and develop sustainable strategic farming, particularly in the context of global climate change.

# **Disclaimer (Artificial intelligence)**

Author(s) hereby declare that generative AI technology Mendeley Reference Manager has been used during the writing or editing of manuscripts to generate the references. Details of the AI usage are given below:

# 1. Mendeley Reference Manager Version 2.128.0

### REFERENCES

- Allison, S. D., Wallenstein, M. D., & Bradford, M. A. (2010). Soil-carbon response to warming dependent on microbial physiology. *Nature Geoscience*, *3*(5), 336–340. https://doi.org/10.1038/ngeo846
- Asemaninejad, A., Thorn, R. G., Branfireun, B. A., & Lindo, Z. (2018). Climate change favours specific fungal communities in boreal peatlands. *Soil Biology and Biochemistry*, 120, 28–36. https://doi.org/10.1016/J.SOILBIO.2018.01.029
- Berg, G., & Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology*, *68*(1), 1–13. https://doi.org/10.1111/J.1574-6941.2009.00654.X
- Błońska, E., Lasota, J., & Zwydak, M. (2017). The relationship between soil properties, enzyme activity and land use. *Forest Research Papers*, *78*(1), 39–44. https://doi.org/10.1515/FRP-2017-0004
- Borowik, A., & Wyszkowska, J. (2016). Soil moisture as a factor affecting the microbiological and biochemical activity of soil. *Plant, Soil and Environment*, 62(6), 250–255. https://doi.org/10.17221/158/2016-PSE
- Caldwell, B. A. (2005). Enzyme activities as a component of soil biodiversity: A review. *Pedobiologia*, 49(6), 637–644. https://doi.org/10.1016/J.PEDOBI.2005.06.003
- Carey, J. C., Tang, J., Templer, P. H., Kroeger, K. D., Crowther, T. W., Burton, A. J., Dukes, J. S., Emmett, B., Frey, S. D., Heskel, M. A., Jiang, L., Machmuller, M. B., Mohan, J., Panetta, A. M., Reich, P. B., Reinschj, S., Wang, X., Allison, S. D., Bamminger, C., ... Tietema, A. (2016). Temperature response of soil respiration largely unaltered with experimental warming. *Proceedings* of the National Academy of Sciences of the United States of America, 113(48), 13797–13802. https://doi.org/10.1073/PNAS.1605365113/SUPPL\_FILE/PNAS.1605365113.SAPP.PDF
- Chauhan, P., Sharma, N., Tapwal, A., Kumar, A., Verma, G. S., Meena, M., Seth, C. S., & Swapnil, P. (2023). Soil Microbiome: Diversity, Benefits and Interactions with Plants. *Sustainability 2023, Vol. 15, Page 14643, 15*(19), 14643. https://doi.org/10.3390/SU151914643
- Cheng, L., Zhang, N., Yuan, M., Xiao, J., Qin, Y., Deng, Y., Tu, Q., Xue, K., Van Nostrand, J. D., Wu, L., He, Z., Zhou, X., Leigh, M. B., Konstantinidis, K. T., Schuur, E. A. G., Luo, Y., Tiedje, J. M., & Zhou, J. (2017). Warming enhances old organic carbon decomposition through altering functional microbial communities. *The ISME Journal*, *11*(8), 1825–1835. https://doi.org/10.1038/ISMEJ.2017.48
- CIP. Sweetpotato Facts and Figures International Potato Center. Retrieved October 17, 2022, from https://cipotato.org/sweetpotato/sweetpotato-facts-and-figures/
- Davidson, E. A., & Janssens, I. A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature 2006 440:7081*, *440*(7081), 165–173. https://doi.org/10.1038/nature04514
- Deng, S., Kang, H., & Freeman, C. (2015). Microplate Fluorimetric Assay of Soil Enzymes. *Methods of Soil Enzymology*, 311–318. https://doi.org/10.2136/SSSABOOKSER9.C14
- Ding, Y., Jin, Y., He, K., Yi, Z., Tan, L., Liu, L., Tang, M., Du, A., Fang, Y., & Zhao, H. (2020). Low Nitrogen Fertilization Alter Rhizosphere Microorganism Community and Improve Sweetpotato Yield in a Nitrogen-Deficient Rocky Soil. *Frontiers in Microbiology*, *11*, 515507. https://doi.org/10.3389/FMICB.2020.00678/BIBTEX
- Dlamini, S. P., Akanmu, A. O., & Babalola, O. O. (2022). Rhizospheric microorganisms: The gateway to a sustainable plant health. *Frontiers in Sustainable Food Systems*, 6. https://doi.org/10.3389/FSUFS.2022.925802
- Ekenler, M., & Tabatabai, M. A. (2004). β-Glucosaminidase Activity as an Index of Nitrogen Mineralization in Soils. *Communications in Soil Science and Plant Analysis*, *35*(7–8), 1081–1094. https://doi.org/10.1081/CSS-120030588
- El Sheikha, A. F., & Ray, R. C. (2017). Potential impacts of bioprocessing of sweet potato: Review. *Critical Reviews in Food Science and Nutrition*, *57*(3), 455–471. https://doi.org/10.1080/10408398.2014.960909
- Fang, J., Wei, S., Shi, G., Cheng, Y., Zhang, X., Zhang, F., Lu, Z., & Zhao, X. (2021). Potential effects of temperature levels on soil bacterial community structure. *E3S Web of Conferences*, 292. https://doi.org/10.1051/E3SCONF/202129201008
- FAO. (2016). FAOSTAT. https://www.fao.org/faostat/en/#data/QCL

- Fekete, I., Varga, C., Biró, B., Tóth, J. A., Várbíró, G., Lajtha, K., Szabó, G., & Kotroczó, Z. (2016). The effects of litter production and litter depth on soil microclimate in a central european deciduous forest. *Plant and Soil*, 398(1–2), 291–300. https://doi.org/10.1007/S11104-015-2664-5/METRICS
- Gao, Z., Han, M., Hu, Y., Li, Z., Liu, C., Wang, X., Tian, Q., Jiao, W., Hu, J., Liu, L., Guan, Z., & Ma, Z. (2019). Effects of Continuous Cropping of Sweet Potato on the Fungal Community Structure in Rhizospheric Soil. *Frontiers in Microbiology*, *10*, 477420. https://doi.org/10.3389/FMICB.2019.02269/BIBTEX
- Gardner, W. H. (1986). 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*. https://www.scirp.org/(S(i43dyn45teexjx455qlt3d2q))/reference/ReferencesPapers.aspx?ReferenceI D=1189528
- Höfte, M. (2021). The use of Pseudomonas spp. as bacterial biocontrol agents to control plant diseases. 301–374. https://doi.org/10.19103/AS.2021.0093.11
- Hou, Q., Wang, W., Yang, Y., Hu, J., Bian, C., Jin, L., Li, G., & Xiong, X. (2020). Rhizosphere microbial diversity and community dynamics during potato cultivation. *European Journal of Soil Biology*, 98, 103176. https://doi.org/10.1016/J.EJSOBI.2020.103176
- Jansson, J. K., & Hofmockel, K. S. (2019). Soil microbiomes and climate change. *Nature Reviews Microbiology 2019 18:1, 18*(1), 35–46. https://doi.org/10.1038/s41579-019-0265-7
- Kumari, J. A., Rao, P. C., Padmaja, G., & Madhavi, M. (2018). Effect of Temperature on Soil Enzyme Acid Phosphatase. International Journal of Current Microbiology and Applied Sciences, 7(09), 2830– 2845. https://doi.org/10.20546/IJCMAS.2018.709.352
- Luo, L., Meng, H., & Gu, J. D. (2017). Microbial extracellular enzymes in biogeochemical cycling of ecosystems. *Journal of Environmental Management*, 197, 539–549. https://doi.org/10.1016/J.JENVMAN.2017.04.023
- Machuca, Á., Córdova, C., Stolpe, N. B., Barrera, J. A., Chávez, D., Almendras, K., & Bonilla, A. M. (2018a). In vitro sensitivity of forest soil enzymes to temperature increase in Western Patagonia. *Journal of Soil Science and Plant Nutrition*, 18(1), 202–219. https://doi.org/10.4067/S0718-95162018005000801
- Machuca, Á., Córdova, C., Stolpe, N. B., Barrera, J. A., Chávez, D., Almendras, K., & Bonilla, A. M. (2018b). In vitro sensitivity of forest soil enzymes to temperature increase in Western Patagonia. *Journal of Soil Science and Plant Nutrition*, 18(1), 202–219. https://doi.org/10.4067/S0718-95162018005000801
- Manici, L. M., Caputo, F., De Sabata, D., & Fornasier, F. (2024). The enzyme patterns of Ascomycota and Basidiomycota fungi reveal their different functions in soil. *Applied Soil Ecology*, *196*, 105323. https://doi.org/10.1016/J.APSOIL.2024.105323
- Margalef, O., Sardans, J., Fernández-Martínez, M., Molowny-Horas, R., Janssens, I. A., Ciais, P., Goll, D., Richter, A., Obersteiner, M., Asensio, D., & Peñuelas, J. (2017). Global patterns of phosphatase activity in natural soils. *Scientific Reports 2017 7:1*, 7(1), 1–13. https://doi.org/10.1038/s41598-017-01418-8
- Mariscal-Sancho, I., Ball, B., & McKenzie, B. (2018). 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, 2(2), 21. https://doi.org/10.3390/SOILSYSTEMS2020021
- Marklein, A. R., & Houlton, B. Z. (2012). Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytologist*, *193*(3), 696–704. https://doi.org/10.1111/J.1469-8137.2011.03967.X
- Martinez, C. E., & Tabatabai, M. A. (1997). Decomposition of Biotechnology By-Products in Soils. *Journal of Environmental Quality*, *26*(3), 625–632.
  - https://doi.org/10.2134/JEQ1997.00472425002600030006X
- Mateos-Rivera, A., Yde, J. C., Wilson, B., Finster, K. W., Reigstad, L. J., & Øvreås, L. (2016). 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 Microbiology Ecology*, 92(4). https://doi.org/10.1093/FEMSEC/FIW038
- Mommer, L., Hinsinger, P., Prigent-Combaret, C., & Visser, E. J. W. (2016). Advances in the rhizosphere: stretching the interface of life. *Plant and Soil*, 407(1–2), 1–8. https://doi.org/10.1007/S11104-016-3040-9/METRICS

- Nannipieri, P., Giagnoni, L., Landi, L., & Renella, G. (2011). *Role of Phosphatase Enzymes in Soil*. 215–243. https://doi.org/10.1007/978-3-642-15271-9\_9
- Nottingham, A. T., Fierer, N., Turner, B. L., Whitaker, J., Ostle, N. J., McNamara, N. P., Bardgett, R. D., Leff, J. W., Salinas, N., Silman, M. R., Kruuk, L. E. B., & Meir, P. (2018). Microbes follow Humboldt: temperature drives plant and soil microbial diversity patterns from the Amazon to the Andes. *Ecology*, 99(11), 2455–2466. https://doi.org/10.1002/ECY.2482
- Oliverio, A. M., Bradford, M. A., & Fierer, N. (2017). Identifying the microbial taxa that consistently respond to soil warming across time and space. *Global Change Biology*, *23*(5), 2117–2129. https://doi.org/10.1111/GCB.13557
- Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C., & Moënne-Loccoz, Y. (2008). The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil 2008 321:1, 321*(1), 341–361. https://doi.org/10.1007/S11104-008-9568-6
- Romero-Olivares, A. L., Allison, S. D., & Treseder, K. K. (2017). Soil microbes and their response to experimental warming over time: A meta-analysis of field studies. *Soil Biology and Biochemistry*, 107, 32–40. https://doi.org/10.1016/J.SOILBIO.2016.12.026
- Sapakhova, Z., Raissova, N., Daurov, D., Zhapar, K., Daurova, A., Zhigailov, A., Zhambakin, K., & Shamekova, M. (2023). Sweet Potato as a Key Crop for Food Security under the Conditions of Global Climate Change: A Review. *Plants 2023, Vol. 12, Page 2516, 12*(13), 2516. https://doi.org/10.3390/PLANTS12132516
- Schimel, J. P., & Bennett, J. (2004). NITROGEN MINERALIZATION: CHALLENGES OF A CHANGING PARADIGM. *Ecology*, 85(3), 591–602. https://doi.org/10.1890/03-8002
- Song, Y., Song, C., Ren, J., Ma, X., Tan, W., Wang, X., Gao, J., & Hou, A. (2019). 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, 11(3), 590. https://doi.org/10.3390/SU11030590
- Steinweg, J. M., Dukes, J. S., & Wallenstein, M. D. (2012). Modeling the effects of temperature and moisture on soil enzyme activity: Linking laboratory assays to continuous field data. Soil Biology and Biochemistry, 55, 85–92. https://doi.org/10.1016/J.SOILBIO.2012.06.015
- Steinweg, J. M., Plante, A. F., Conant, R. T., Paul, E. A., & Tanaka, D. L. (2008). Patterns of substrate utilization during long-term incubations at different temperatures. *Soil Biology and Biochemistry*, 40(11), 2722–2728. https://doi.org/10.1016/J.SOILBIO.2008.07.002
- T Classen, A. E., Sundqvist, M. K., Henning, J. A., Newman, G. S., M Moore, J. A., Cregger, M. A., Moorhead, L. C., Patterson, C. M., Sundqvist, M. K., Henning, J. A., Newman, G. S., M Moore, J. A., Cregger, M. A., Moorhead, L. C., & Patterson, C. M. (2015). Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? *Ecosphere*, 6(8), 1– 21. https://doi.org/10.1890/ES15-00217.1
- Tedesco, D., Moreira, B. R. de A., Barbosa Júnior, M. R., Maeda, M., & Silva, R. P. da. (2023). Sustainable management of sweet potatoes: A review on practices, strategies, and opportunities in nutrition-sensitive agriculture, energy security, and quality of life. *Agricultural Systems*, *210*, 103693. https://doi.org/10.1016/J.AGSY.2023.103693
- Vishwakarma, K., Mishra, M., Jain, S., Singh, J., Upadhyay, N., Verma, R. K., Verma, P., Tripathi, D. K., Kumar, V., Mishra, R., & Sharma, S. (2017). 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*, 1, 489–499. https://doi.org/10.1007/978-981-10-5813-4\_23/COVER
- Wang, C., Morrissey, E. M., Mau, R. L., Hayer, M., Piñeiro, J., Mack, M. C., Marks, J. C., Bell, S. L., Miller, S. N., Schwartz, E., Dijkstra, P., Koch, B. J., Stone, B. W., Purcell, A. M., Blazewicz, S. J., Hofmockel, K. S., Pett-Ridge, J., & Hungate, B. A. (2021a). The temperature sensitivity of soil: microbial biodiversity, growth, and carbon mineralization. *The ISME Journal*, *15*(9), 2738–2747. https://doi.org/10.1038/S41396-021-00959-1
- Wang, C., Morrissey, E. M., Mau, R. L., Hayer, M., Piñeiro, J., Mack, M. C., Marks, J. C., Bell, S. L.,
  Miller, S. N., Schwartz, E., Dijkstra, P., Koch, B. J., Stone, B. W., Purcell, A. M., Blazewicz, S. J.,
  Hofmockel, K. S., Pett-Ridge, J., & Hungate, B. A. (2021b). The temperature sensitivity of soil:
  microbial biodiversity, growth, and carbon mineralization. *The ISME Journal 2021 15:9*, *15*(9), 2738–2747. https://doi.org/10.1038/s41396-021-00959-1

- Wilhelm, R. C., van Es, H. M., & Buckley, D. H. (2022). Predicting measures of soil health using the microbiome and supervised machine learning. Soil Biology and Biochemistry, 164, 108472. https://doi.org/10.1016/J.SOILBIO.2021.108472
- Zhang, H., Phillip, F. O., Wu, L., Zhao, F., Yu, S., & Yu, K. (2022). Effects of Temperature and Nitrogen Application on Carbon and Nitrogen Accumulation and Bacterial Community Composition in Apple Rhizosphere Soil. *Frontiers in Plant Science*, *13*, 859395. https://doi.org/10.3389/FPLS.2022.859395/BIBTEX
- Zhang, X., Cui, K., Wen, X., Li, L., Yu, X., Li, B., Lin, H., He, H., & Wang, F. (2022). The Association between Gut Microbiome Diversity and Composition and Heat Tolerance in Cattle. *Microorganisms*, 10(8), 1672. https://doi.org/10.3390/MICROORGANISMS10081672/S1
- Zhao, M., Xue, K., Wang, F., Liu, S., Bai, S., Sun, B., Zhou, J., & Yang, Y. (2014). Microbial mediation of biogeochemical cycles revealed by simulation of global changes with soil transplant and cropping. *The ISME Journal 2014 8:10, 8*(10), 2045–2055. https://doi.org/10.1038/ismej.2014.46
- Zhou, J., Liu, C., Shi, L., & Zamanian, K. (2023). Rhizosphere influence on microbial functions: consequence for temperature sensitivity of soil organic matter decomposition at early stage of plant growth. *Plant and Soil*, 1–15. https://doi.org/10.1007/S11104-023-06258-2/METRICS