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Revealing the Effects of Climate Change and Fungicides on Soil Microbial Communities in the Lower Rio Grande Valley, Texas

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REVEALING THE EFFECTS OF CLIMATE CHANGE AND FUNGICIDES ON SOIL MICROBIAL COMMUNITITES IN THE LOWER RIO GRANDE, VALLEY

A Thesis

by

ARMIDA RIVERA

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ABSTRACT

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Fungicide applications are effective to prevent fungal pathogens that are known to cause crop damage and decrease yields. However, these pesticides can exert toxic or inhibitory effects on non-targeted organisms such as soil microbial communities. In addition, it is unknown to what extent shifts in temperature and soil moisture resulting from global climate change, alter the activities of non-targeted soil organisms. The aim of this study was to evaluate soil biological parameters such as soil respiration and enzyme activities involved in the cycling of carbon, nitrogen, and phosphorous (β-glucosidase, Fluorescein Diacetate (FDA) Hydrolysis, urease, alkaline phosphatase, acid phosphatase, and N-acetyl-β-glucosaminidase) from the use of fungicides, azoxystrobin and tebuconazole, with anticipated temperatures and moisture levels based on the Intergovernmental Panel on Climate Change (IPCC) Representative Concentrated Pathway 8.5 scenario (RCP 8.5) from the end of the century (2070-2100) in the Lower Rio Grande Valley, Texas, U.S.A. A total of 30 random samples were collected from a loamy sand soil in Raymondville, Texas (26° 28’ 57.69” N and 97° 55’ 53.21” W) for a 45-day incubation period under a microcosm approach. The microbial communities’ responses to fungicide, increasing temperatures, and decreasing moisture alone and in combination were studied in a factorial experiment, resulting in a total of 12 treatments with 12 replicates each, comprised of

three fungicide treatments (azoxystrobin, tebuconazole, and no fungicide control) applied accordingly by the recommended manufacture rate, two temperature levels (24ºC and 28ºC), and two soil moisture levels (8% and 9%). Results indicated FDA was increased by tebuconazole at 28ºC, however other indicators were suppressed or unchanged. This may indicate other processes were enhanced that were not measured in this study. With increments of temperatures, β-glucosidase, and phosphatase activities reduced rates. Tebuconazole significantly decreased β-glucosidase and phosphatase activities, while azoxystrobin significantly lowered urease rates. Decreased microbial activities due to temperature, moisture, and fungicide, may therefore impact nutrient availability to other microbes and plants. Solely moisture reduction had no significant impact on soil microbial activities. Therefore, it is imperative to protect soil ecosystems to aid the sustainability of agriculture and support healthy ecosystems. Future research should consider whether microbial functioning can withstand the long-term exposure to these disturbances, exploring adaptation mechanisms (e.g., shifts in microbial diversity) upon ecosystem-based strategies for prevention of crop diseases.

DEDICATION

The completion of my master studies would have not been possible without the love and support of my family, my boyfriend, and God. My mother, Marisa Rivera, my sister, Larisa Rivera, my boyfriend, Aldo Tinajero and God, wholeheartedly inspired, motivated, and provided me strength in completing this degree.

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CHAPTER I

LITERATURE REVIEW

The Lower Rio Grande Valley's Climate and Its Averages

The Lower Rio Grande Valley (LRGV) is located along the borderlands of Texas and Mexico. It is composed of four counties (Starr, Hidalgo, Willacy, and Cameron) (Figure 1) and is categorized as a subtropical and a semi-arid region (Thornthwaite, 1948; Whitney, Solano, & Hubbard, 2019; Alvarez, 2016; Vaughan, et al., 2012; Baker & Dale, 1964). Due to the LRGV's proximity to the center of the east-west continental of aridity gradient, wetness or rain levels increase eastward (Leslie, 2016; Melillo, 2014). The mean rainfall in the LRGV ranges between 508 to 762mm (20-30 inches) (NOAA, n.d.; Alvarez, 2016). Annual rainfall is primarily composed by highly erratic single events; in general, wet periods usually spell in early summer and late summer/early autumn, with normally drier periods during the rest of the year (Leslie, 2016). Moreover, the LRGV typical temperatures consist of average maximum and minimum of 29.80°C (85.65 °F) and 17.51 °C (63.52°F), respectively, averaging up to 23.66°C (74.58°F) (NOAA, n.d.; Alavarez & Plocheck, 2016).

Climate change and future trends for the Lower Rio Grande Valley

Climate change is a long-term transformation in the average weather patterns that have come to define Earth's local, regional, and global climates (Shaftel, Callery, Jackson, & Bailey, 2022). These long-term alterations in mean weather trends can be both natural and human induced (Zielinski, 2002; Fahey, Doherty, Hibbard, Romanou, & Taylor, 2017; Jain, 1993; Pachauri & Meyer, 2014). It is evident that anthropogenic greenhouse gas (GHG) releases have increased since the pre-industrial era, resulting an unprecedented concentration of emissions (e.g., CH_4, NO_x, CO_2) within the atmosphere (Pachauri & Meyer, 2014; Wolff, et al., 2020). These emissions became an observable driver for warming and have altered climate variables such as mean annual precipitation and temperatures (Pachauri & Meyer, 2014; Chan & Wu, 2015; Egorova, Rozanov, Arsenovic, Peter, & Schmutz, 2018; Fischer & Knutti, 2015). Moreover, anthropogenic greenhouse gas emissions are mainly driven by population size, economic activity, lifestyle, energy use, land use patterns, technology, and climate policy (Pachauri & Meyer, 2014). The Intergovernmental Panel on Climate Change (IPCC) developed four Representative Concentrated Pathways (RCPs) scenarios that described different climate change mitigation projections based on these circumstances mentioned above (Pachauri & Meyer, 2014). Such trajectories include RCP 2.6 (rigorous mitigation), 4.5 and 6.0 (intermediate extenuation), as well as 8.5 (very high GHG discharges with no additional efforts to restrict emissions) (Pachauri & Meyer, 2014) (Figure 2). For this study, RCP 8.5 scenario was utilized for the expected temperature and rainfall towards the end of the century (2070-2100).

In the RCP 8.5 scenario, it is anticipated for areas within the dry-subtropics and arid and semi-arid regions, such as the LRGV, to experience a drier climate (Pachauri & Meyer, 2014; Seneviratne, et al., 2015). Average rainfall is estimated to decrease by 10% (Pachauri & Meyer,

2014; Seneviratne, et al., 2015) and forecasted to increase 4°C (~7°F) on average temperatures within the LRGV locale (Pachauri & Meyer, 2014; Collins, 2013).

The role of climate (precipitation and temperatures) among soil microbial communities

Soil microbes' structure and functions are known to be sensitive to changes in precipitation and temperatures (Kumar, Rawat, & Amule, 2016; Khursheed, 2016). Therefore, it is critical to describe the roles of these climatic variables on the soil (Paul, 2015) to understand its potential implications to ecosystem functioning under future climate conditions.

Precipitation (soil moisture)

Although there are various definitions of soil moisture, it is generally referred to as the amount of water stored in the unsaturated zone (Seneviratne, et al., 2015). Soil moisture levels vary with climate, soil properties, and geographic regions (Pasternak, et al., 2013; Cao, et al., 2016); thus, these interactions are complex (Sehler, Li, Reager, & Ye, 2019). Additionally, depending on these circumstances, precipitation levels can play critical roles in soil moisture levels, and be a contributor to natural soil processes. Areas where precipitation levels play these vital roles comprises of arid and semi-arid regions. Exclusively to arid and semi-arid areas, precipitation levels are reported to be correlated with moisture levels (Shreve, 1914; Zhang, Xiao, Yao, Liu, & Sun, 2020; Zhang, et al., 2010; Wang, et al., 2019; Seneviratne, et al., 2015; Sehler, Li, Reager, & Ye, 2019). Also, since water is a limiting factor within these locales, it is an important contributor to natural and soil processes such as regulating microbial properties, abundance, community composition, and activities (Borowik & Wyszkowska, 2016; Vasquez-Dean, Maza, Morel, Pulgar, & Gonzalez, 2020; Tan, Wang, Bai, Qi, & Chen, 2020; Huxman, et al., 2004; Zhao, et al., 2016) along with controlling biological processes that occur in the soil

(Tomar & Baishya, 2020). In turn, these conditions may impact nutrient cycling and decomposition. Due to these correlations, precipitation must be considered for soil moisture levels to determine microbial responses.

Temperature

Another important factor to consider when analyzing soil microbial communities is soil temperature. Soil temperature is described to be the heat flux in soil and the exchanges between the soil and the atmosphere (onwuka & Mang, 2018). However, soil temperatures are dependent upon vegetation (onwuka & Mang, 2018; Aalto, le Roux, & Luoto, 2018), as well as geologic features (e.g., slope), soil color, and amount of solar radiation of the locale (i.e., climatic zone) (onwuka & Mang, 2018). These temperatures influence the rates of biological, physical, and chemical processes (Howe & Smith, 2021; onwuka & Mang, 2018) and aids in determining microbial and biochemical soil activities (Borowik & Wyszkowska, 2016; Pietikainen, Pettersson, & Baath, 2005). As a result, temperatures may influence vital processes within the soil microbial communities, thus, must also be considered in accessing microbial reactions.

Soil moisture together with temperatures, may also impact soil microbial communities across its properties via interactive effects. Investigations have discussed with temperatures and precipitation levels, strongly control microbial structure (Vasquez-Dean, Maza, Morel, Pulgar, & Gonzalez, 2020; Cregger, Schadt, McDowell, Pockman, & Classen, 2012), responsible for microbial and biochemical soil properties (Borowik & Wyszkowska, 2016), and affects microbial growth and activities in soil (Pietikainen, Pettersson, & Baath, 2005). Hence, measuring soil moisture and temperature are vital in assessing its individual effects on microbial communities and jointly.

Specific climate change effects on soil microbial communities

The increment of greenhouse emissions result in a plethora of environmental issues among physical and biological systems (Rosenzweig, et al., 2008). These environmental issues not only effect physical and biological systems, but also one of the most under looked systems, the soil ecosystem. Such ecosystem is rarely considered for the focus of climate change and should therefore be analyzed (Cavicchioli, et al., 2019; Maestre, et al., 2015; Dutta & Dutta, 2016) to understand the significant changes precipitation and temperature can have to soil's natural processes.

Precipitation (soil moisture)

With climate change implications, microbial communities may change (Classen, et al., 2015; Zhou, Wang, & Luo, 2020; Naylor, et al., 2020) from shifts in precipitation levels. These changes may modify processes associated with soil enzymatic activities, and organic matter content/respiration. Zeglin and others discussed the changes in precipitation trends might indirectly impact through the reduction of enzyme potential by reducing soil organic pools and directly via carbon respiration (Zeglin, et al., 2013). In another study, changes in precipitation patterns significantly impacted soil nitrogen cycling dynamics (Barnard, Osborne, & Firestone, 2015), a major nutrient in the soil that aids in crop growth (Zou, et al., 2018) and is associated with organic matter (Serrasolses, Diego, & Bonilla, 1999). Moreover, less rainfall and an increase in annual variability, as expected within the LRGV, could lead to a reduction of dry-matter production, thus lowering soil organic matter contents (Brinkman & Sombroek, 1996).

Temperature increases

Soil microbes are sensitive to temperature increments in soil ecosystems, even from minuscule increases, as can be expected from climate change (Barreiro et al., 2020). This sensitivity can be expressed through soil enzymes that are involved in nutrient cycles (Liu, et al., 2021) and soil respiration analysis, which can serve as indicators of nutrient and microbial respiration changes caused by climate change. Typically, with increases in temperatures, it is expected for microbial activity, growth, and biomass to be stimulated (enhancing enzyme production); however, over time, this may cause shifts in microbial functioning (Mooshammer, et al., 2022). Such shift in microbial function includes restraining enzyme production, resulting in a neutral or decreased in enzymatic activities (Mooshammer, et al., 2022). Therefore, temperature may have a positive effect on a short-term scale, but eventually over time may be opposite due to substrate affinity (Mooshammer, et al., 2022). Furthermore, according to a review, increased temperatures enhanced the rates of microbial decomposition, and CO_2 by soil respiration, producing positive feedback to global warming (Kumar, Rawat, & Amule, 2016). Likewise, studies have indicated similar responses in semi-arid areas where microbial CO_2 e (i.e., CO_2 that have the equivalent global warming impact) exhibited an increased ability in decomposing soil organic matter as well as a sensitivity for respiration, contributing to positive feedbacks (stimulating carbon loss) (Nie, et al., 2013; Ferreira Maia, Medeiros Gonzaga, dos Santos Silva, Bastos Lyra, & de Araujo Gomes, 2019). Thus, temperature plays a predominant role in affecting the rate of soil carbon mineralization (Tang, Sun, Luo, He, & Sun, 2018).

Soil microbial communities' responses to climate change

Collectively, soil moisture coupled with temperature changes, can directly alter soil environments through changes in the physiology and growth of specific groups within the microbial communities (Zhang, et al., 2005), influence microbial composition and functions (Classen, et al., 2015), and changes in decomposition, nitrogen mineralization, organic carbon storage, and other environmental processes (Baiser, Gutknecht, & Llang, 2010). Thus, governing microbial communities is essential in understanding how global change will affect soil processes and ecosystem function (Baiser, Gutknecht, & Llang, 2010).

Fungicides and their impact on soil microbes

Fungicide applications are used to not only prevent foliar diseases, but also soil-borne fungal pathogens known to cause crop damage and decrease yields. Thus, these chemical pesticides have contributed to improving crop production, crop quality, and revenue by reducing the risk of crop losses (Adetutu, Ball, & Osborn, 2008; Fernandez-Cornejo, et al., 2014). Moreover, fungicides may be applied in various formalities. Such modalities include dust, granules, gas, and most commonly, liquid (McGrath, 2004). Through the administration of fungicides, they can be distributed through the soil either by in-furrow planting, after planting (i.e., soil drench), as well as through a foliage spray, just to name a few (McGrath, 2004).

Despite the advantages fungicides offers, they are considered to be bio-toxicants. In other words, fungicides not only interfere with the targeted pathogen, but also influence the population or activities of non-targeted microorganisms in the soils (Chen, Edwards, & Subler, 2001; Tejada, Gomez, Garcia-Martinez, Osta, & Parrado, 2011; Arora & Sahni, 2016). Adetutu and others reported 55% of the fungicide sprayed on crops can be deposited onto the soil, especially

if applied in early growth stages, impacting non-targeted organisms. Although foliar fungicide sprays are employed to crop surfaces, they can be leaked to the soil through fungicide spray drifts and rain (Kim, Beaudette, Shim, Trevors, & Suh, 2002). It is also recorded that less than 0.1% of pesticides applied for pest control reach to the targeted pest, thus more than 99% is carried to other areas of the environment that can potentially cause adverse effects such as the soil biota (Pimentel, 1995). In another investigation, Gunstone and others accounted that the absorption of pesticides into plant tissues eventually returns to the soil through senescence of crop residues (Gunstone, Cornelisse, Klein, Dubey, & Donley, 2021), lingering in the plants and or soil (Alengebawy, Abdelkhalek, Qureshi, & Wang, 2021).

The influence of pesticides such as fungicides on soil microorganisms are dependent on the physical, chemical, and biochemical conditions of the soil, as well as the nature and concentration of the pesticides (Arora & Sahni, 2016; Tejada, Gomez, Garcia-Martinez, Osta, & Parrado, 2011; Chen, Edwards, & Subler, 2001), the dosage (Roman, Voiculescu, Filip, Ostafe, & Isvoran, 2021) or the history of management on the soils (Sulowicz, Cycon, & Piotrowska-Seget, 2016). Nonetheless, within the soil ecosystem, pesticides have the capabilities of manipulating the biological physiochemical properties of soil and which can eventually disturb microbial communities and their processes (Alengebawy, Abdelkhalek, Qureshi, & Wang, 2021), greatly affect non-targeted microbial diversity and functions related to the nutrient cycles, and soil fertility (Ding, et al., 2019; Arora & Sahni, 2016) and in cases, inhibit microbial growth (Ullah & Dijkstra, 2019). The effects of pesticides on soil microbial communities have been identified in studies in repeated fungicide applications (Katsoula, Vasileiadis, Sapountzi, & Karpouzas, 2020), various concentrations (Walia, Mehta, Guleria, Chauhan, & Shirkot, 2014; Ramudu, Modhiddin, Srinivasulu, Madakka, & Rangaswamy, 2011; Astaykina, et al., 2020; Yen,

Chang, Huang, & Wang, 2009), different dosages/rates (Ding, et al., 2019; Bacmaga, Wyszkowska, & Kucharski, 2016; Sulowicz, Cycon, & Piotrowska-Seget, 2016), as well as single fungicide applications rates at recommended field amounts (Chen, Edwards, & Subler, 2001; Ma, et al., 2021). Therefore, it is vital to consider these effects under a climate change scenario, to determine the outcomes for the future of sustainability and soil health. Specifically for this study, two of the most used fungicides groups (Correira, Rodrigues, Paiga, & Delerue-Matos, 2016), strobilurin (azoxystrobin) and triazoles (tebuconazole), were considered. Although there is limited data of fungicide use in the Lower Rio Grande Valley, these two fungicides may be utilized for major crops grown in this region such as corn, sugarcane, watermelon, sorghum, citrus, onion, tomatoes, and cotton, just to name a few (AgriLife, n.d.; Texas A&M AgriLife Research, n.d.; Jahrsdoerfer & Leslie, 1988).

Azoxystrobin

Azoxystrobin (QoI- Quinone outside Inhibitor) is a strobilurin-dervived systematic fungicide that belongs to the chemical group methoxyacrylate (FRAC, 2006) and functions by inhibiting fungal pathogen's mitochondria respiration through the binding of the cytochrome b complexes (i.e., a protein found in the mitochondria of eukaryotic cells) (Adetutu, Ball, & Osborn, 2008; Wyenandt, 2020). This process specifically blocks the electron transport from the cytochrome b to c (i.e., key role in electron transport that is associated with cellular respiration), hindering energy that interferes with cell growth (i.e., favoring the death of pathogen) (Bacmaga, Kucharski, & Wyszkowska, 2015), as well as spore germination and zoospore motility (Brauer, et al., 2019). Fungicides within this group (FRAC, 2006), are at high risk of developing cross resistance (FRAC, 2006). Additionally, azoxystrobin is often used to control several pathogens within Ascomycota (e.g., *Cerospora*, *Collectotrichium*, and *Fusarium*), Basidiomycota (e.g.,

Rhizoctonia, *Gymnosporangium*, and *Phragmidium*), Deuteromycota, and Oomycota (e.g., *Pythium*, *Phytophthora*, and *Peronospora*) (Vuyyuru, Sandhu, McCray, & Raid, 2018; Brauer, et al., 2019; Prime Source, 2014). Azoxystrobin may be utilized on a range of crops including beans, peppers, cucurbits (e.g., watermelon), citrus, onion, and variety of vegetables (e.g., carrots, lettuce, spinach, and kale), just to mention a few (AgriLife, n.d.; Texas A&M AgriLife Research, n.d.; Jahrsdoerfer & Leslie, 1988).

Regardless of the benefits azoxystrobin offers in controlling fungal pathogens, azoxystrobin has demonstrated repressive behavior towards soil microbial communities in enzymatic activities, microbial biomass, soil respiration, and the structure and function. In a study composed by (Bacmaga, Kucharski, & Wyszkowska, 2015), quantified the effects of azoxystrobin through four different dosages (recommended by manufacturer, 30x, 150x, and 300x the recommended) by accessing enzymatic activities in laboratory conditions. Ramifications of their investigation detected inhibitory effects on dehydrogenases, catalase, urease, acid, and alkaline phosphatase enzyme activities. In another study, an array of concentrations (0, 0.1, 1.0 and 10.0 mg kg^{-1}) of azoxystrobin were measured to gage the impacts on cultivatable microbial biomass and soil respiration on black soil (Guo, et al., 2015). Guo and others reported that soil respiration demonstrated repressive behaviors in response to higher dosages within the 28^{th} day (Guo, et al., 2015). Enzymes, urease, protease, and dehydrogenase were significantly impacted negatively, in which depended on the dose and time after its application (Guo, et al., 2015). Wang, et al., (2020) studied the impacts of azoxystrobin on the structure and function of microbial communities using a range of dosages (0 mg kg^{-1}, 2 mg kg^{-1}, 25mg kg^{-1}, and 50 mg kg^{-1}) by accessing four enzymes (urease, invertase, catalase, and phosphatase) in a laboratory environment. Urease, invertase, and phosphatase enzyme activities

were found to be constrained with 2 mg kg^{-1} after 35 days (Wang, et al., 2020). Wang also revealed, with the use of an Biolog Ecoplate, different carbon sources were inhibited. Aleksova, et al., (2021) described the use of Quadris^R (i.e., azoxystrobin is an active ingredient) at assorted concentration rates (0.0-35.0 mg kg^{-1}) in a mesocosm investigation to measure soil bacterial functioning. Aleksova and others discovered at field recommended concentrations, altered low available carbon sources (<0,50 optimal density), whereas those applied with higher concentrations were effective on medium (0.50-1.00 optimal density) and highly (>1.00). The investigators concluded that Quadris affects bacterial catabolic through soil environmental factors such as soil nutrients and pH (Aleksova, et al., 2021).

Tebuconazole

Tebuconazole (DeMethylation Inhibitors- DMI) is a systemic fungicide from the chemical group triazoles (FRAC, 2006) and performs by inhibiting ergosterol biosynthesis in fungi through demethylation (i.e., fungicide interferes with the structure of fungal cell wall, then inhibits reproduction and growth of pathogen) (Bacmaga, Wyszkowska, & Kucharski, 2021). Resistance within this group of fungicides (FRAC, 2006) is known in various fungal spp. and is of medium concern (FRAC, 2006). Tebuconazole is recognized for its high efficacy in plant and soil protection (Bacmaga, Wyszkowska, & Kucharski, 2021), and its control use for pathogens in the Ascomycota (e.g., *Phoma*, *Fusarium*, *Cercospora*, and *Alternaria*) and Basidiomycota (e.g., *Puccinia* and *Rhizoctonia*) (Prime Source, 2013). Tebuconazole can be utilized on groups of fruits (e.g., cucurbits, eggplant, and pepper), nuts (e.g., almonds), cereal (e.g., sorghum, corn) and vegetables (e.g., bok choy, collards) (Bacmaga, Wyszkowska, & Kucharski, 2021; Munoz-Leoz, Ruiz-Romera, Antiguedad, & Garbisu, 2011; AgriLife, n.d.; Texas A&M AgriLife Research, n.d.; Jahrsdoerfer & Leslie, 1988).

Despite of tebuconazole's ability to enhance production, yields, and crop quality, studies have indicated that this fungicide may negatively impact soil microbial communities by hindering respiration, biomass, and enzyme activities. In a short term mesocosm study, tebuconazole was applied at three different rates (5, 50, and 500 mg kg^{-1} DW soil) to measure its impact on soil microbial communities (Munoz-Leoz, Ruiz-Romera, Antiguedad, & Garbisu, 2011). It was identified that it inhibited basal respiration, substrate-induced respiration, microbial biomass (C) and enzyme activities (Munoz-Leoz, Ruiz-Romera, Antiguedad, & Garbisu, 2011). Munoz-Leoz et al. (2011) also reported nitrification rates reductions within the first 30 days as well as different functional communities (depended on amount). In a field experiment, various field rates (FR, 2x FR, and 10x FR) were sprayed to evaluate soil properties and enzyme activity impacts from tebuconazole (Saha, Pipariya, & Bhaduri, 2016). Outcomes demonstrated tebuconazole applications at FR and 2FR resulted in momentary toxic effects on properties and enzymatic activity, while in higher rates (10x) was much more extensive (Saha, Pipariya, & Bhaduri, 2016). Despite of Fluorescein diacetate (FDA) hydrolysis, urease, phosphatase, and aryl sulfatase were not affected, dehydrogenase and nitrate reductase activities decreased significantly. (Saha, Pipariya, & Bhaduri, 2016). Bacmaga et al. (2021), tested different doses of tebuconazole (0.00 (C), 0.02 (O), and 10.0 (T) mg kg^{-1}) to assess its effects on enzymatic activities. Results indicated that tebuconazole was a strong inhibitor of urease and catalase, while enhanced dehydrogenases, acid, alkaline phosphatase, and aryl sulfatase (Bacmaga, Wyszkowska, & Kucharski, 2021). In another study by Bacmaga et al. (2019), determined the effects of tebuconazole on soil microorganisms and enzymes through various dosages (0.000, 0.042, 0.083, 0.125, 1.249, and 2.499 mg kg^{-1}) in a greenhouse setting (Bacmaga, Wyszkowska, & Kucharski, 2019). Ramifications of their study demonstrated strong inhibitory effects on

urease (0.042 to 2.499 mg kg^{-1}), suppressed alkaline phosphatase (0.125 mg kg^{-1} and 2.499 mg kg^{-1}), as well as inhibitory on dehydrogenases (2.499 mg kg^{-1}), arylsulfatase mg kg^{-1}), β-glucosidase (2.499 mg kg^{-1}), and catalase (2.499 mg kg^{-1}).

Thus, monitoring soil health through enzymatic activities and microbial respiration is vital to determine potential alterations from azoxystrobin and tebuconazole that can ultimately put soil microbes at risk, directly impacting soil health and production under climate change scenarios.

Soil microbial communities important links to soil health

Soil health is defined as, "the capacity of soil to function as a living ecosystem that sustains not only animals and humans, but also plants" (USDA-NRCS, n.d.) and serves as a critical source for food production (FAO, 2015; Bridges & Van Baren, 1997) and ecosystem functioning (Wagg, et al., 2021). Such functions include the facilitation of biological activities, suppression of pathogens, decomposition of organic matter, cycling of vital nutrients, and physical stability and support of soils (USDA-NRCS, Soil Health, n.d.; Tahat, Alananbeh, Othman, & Leskovar, 2020; Hermans, et al., 2020). However, stress can be applied to soil health under agricultural pressures. It is estimated that agricultural productivity will need to triple by the end of the century (2100) to meet global demands under a "business-as-usual" scenario (i.e., RCP 8.5 scenario) (Beltran-Pena, Rosa, & D'Odorico, 2020). Thus, significant efforts have been made to increase productivity such as the use of chemical commercial pesticides (Trivedi, Delgado-Baquerizo, Anderson, & Singh, 2016) such as fungicides. Together, with these two factors, soil must be closely monitored and studied to determine the effects and understand how these changes can affect soil microbial communities' natural processes.

Soil is a complex system (Yang, Siddique, & Liu, 2020; Lehmann, Bossio, Kogel-Knabner, & Rillig, 2020). Underlying soil health is soil microorganisms (Ding, et al., 2019). These microbial communities serve for the enhancement of productivity, nutrient cycling, soil structure, and decomposition (Kennedy & Stubbs, 2006) and mediators for soil biological processes such as organic matter degradation, mineralization, and cycling of nutrients (Stege, Messina, Bianchi, Olsina, & Raba, 2010). Furthermore, while there are microbial communities with a symbiotic relation with their host (i.e., crops), free-living microbiomes (i.e., lives outside the plant cells) also have a linkage with soil health (Yang, Siddique, & Liu, 2020). Free-living soil microbes offer advantageous elements such as delivering nutrients to the crops and combating diseases via competing with nutrients and niches through the production of enzymes and hormones (Yang, Siddique, & Liu, 2020).

Thus, it is important to develop the best conditions for soil microbes in sustainable agriculture to achieve healthy soil systems (Yang, Siddique, & Liu, 2020). Indicators such as soil enzymes and respiration, may be accessed to identify changes in soil characteristics.

Indicators

Soil health can be quantified through the analysis of chemical, physical, and biological properties (Rahul, Sharma, Singh, Singh, & Kumar, 2022). Specifically, biological characteristics such as soil enzymes and respiration, serve as useful tactics to measure microbial communities and functions (Meena & Rao, 2021) due to their ability to alter rapidly under changes or disturbances within the environment than physical and chemical properties (Kennedy & Stubbs, 2006; Munoz-Leoz, Ruiz-Romera, Antiguedad, & Garbisu, 2011).

Soil enzymes

Examining biological properties can provide a measurement of responses from living organisms and changes within the environment (Alkorta, Aizpurua, Riga, Albizu, & Garbisu, 2003). Nutrient cycling among the soil biome involves a plethora of reactions (e.g., chemical and physiochemical) and are catalyzed primarily by soil enzymes, making them ideal indicators for biological activities (Alkorta, Aizpurua, Riga, Albizu, & Garbisu, 2003; Kumar & Varma, 2011). Thus, soil enzymes are responsible for increasing reaction rates to release plant available nutrients and recycling organic components and nutrients in the soil (C, N, and P) (Rahul, Sharma, Singh, Singh, & Kumar, 2022; USDA, 2010; Patle, Navnage, & Barange, 2018; Alkorta, Aizpurua, Riga, Albizu, & Garbisu, 2003) and play a key role in biochemical functions in the organic matter decomposition (Kumar & Varma, 2011). Additionally, soil enzymes are found to be related to soil microbial biomass carbon and soil organic carbon (Meena & Rao, 2021), along with physical and microbial qualities (Rahul, Sharma, Singh, Singh, & Kumar, 2022; Stege, Messina, Bianchi, Olsina, & Raba, 2010). Therefore, soil enzyme activities serve as a valuable index to nutrient cycling, nitrification, oxidation, and other processes crucial to soil quality (Adetunji, Lewu, Mulidzi, & Ncube, 2017). Furthermore, soil enzymes activities have an intimate relationship with environmental variables (e.g., temperature and moisture levels), and other factors such as acid rain, heavy metals, and pesticides that ultimately affect their activities and disrupt local metabolism (Arora & Sahni, 2016). According to Rahul and others and Alkorta, soil enzymes are noted to transform rapidly compared to other soil parameters, suggesting primary alterations in soil health (Rahul, Sharma, Singh, Singh, & Kumar, 2022; Alkorta, Aizpurua, Riga, Albizu, & Garbisu, 2003). Thus, changes within the soil environment may be identified due to their sensitivity (Sudhakaran, Ramamoorthy, Savitha, & Kirubakaran, 2019;

Adetutu, Ball, & Osborn, 2008; Adetunji, Lewu, Mulidzi, & Ncube, 2017) and reflect modifications in ecological and soil biochemical quality (Rahul, Sharma, Singh, Singh, & Kumar, 2022). Also, due to their sensitivity, they can serve as early warning indicators for soil quality, therefore, they are frequently used to assess changes among the soil environment and have been used for pollution indicators (Guo, et al., 2015; Maphuhla, Lewu, & Oyedeji, 2021) and climate change (Liu, et al., 2021).

Collectively, soil enzymes unveil ecosystem distress and have been used as indicators for various factors including those that are involved in the carbon, nitrogen, and phosphorous cycles (Lee, Kim, Kim, & Kim, 2020). Such distress maybe caused by factors such as climate change or fungicide use, thus, analyzing together is vital to assess and monitor soil health (Alkorta, Aizpurua, Riga, Albizu, & Garbisu, 2003). Ultimately, the health of the soil is essential for terrestrial ecosystems to remain resilient or to recover from disturbances such as pollution, management, and climate change; soil is thus a high priority and understanding soil enzymes is a crucial factor (Kumar & Varma, 2011).

Soil Respiration

Soil respiration is the capacity of soil to sustain life such as plants and microorganisms. These measurements help indicate the level of microbial activity, organic matter, and decomposition (USDA-NRCS, Soil Respiration, 2014; Guo, et al., 2015). In addition, soil respiration plays a significant role in regulating carbon cycling on various scales (Luo & Zhou, 2006). Identifying the levels of soil respiration (CO_2) demonstrates the state of the physical and chemical environment of the soil (USDA-NRCS, Soil Respiration, 2014). Under laboratory

conditions, soil respiration may be utilized to estimate soil microbial biomass and an outlook of nutrient cycling (USDA-NRCS, Soil Respiration, 2014).

Similar to soil enzymes, soil respiration is very sensitive to environmental changes (Luo & Zhou, 2006). Not to mention, temperature is often utilized to measure soil microorganisms to quantify respiration rates (Pietikainen, Pettersson, & Baath, 2005) from global warming and also assess atmospheric CO_2 concentrations (Meyer, Meyer, Welp, & Amelung, 2018). Kumar and others have also discussed the dependence of soil respiration on soil temperatures and moisture levels (Kumar, Rawat, & Amule, 2016). Furthermore, Arora and Sahni claimed that pesticide effects on microbial biomass (C and N) (Arora & Sahni, 2016), and soil respiration analysis are extremely useful for interpreting soil quality and health.

Thus, for this study, enzyme activities, Fluorescein Diacetate (FDA) hydrolysis, β-glucosidase, N-acetyl-β-D-glucosaminidase (NAGase), urease, acid phosphatase, and alkaline phosphatase (Table 1) and soil respiration, were considered to assess the effects of climate change and fungicides on nutrient cycling and organic matter decomposition on soil microbial communities.

Goals and Objectives

The aim of this study was to reveal how six soil enzymes involved in carbon, nitrogen, and phosphorous cycling (i.e., Fluorescein Diacetate (FDA) hydrolysis, β-glucosidase, N-acetyl-β-D-glucosaminidase (NAGase), urease, acid phosphatase, and alkaline phosphatase), and soil respiration respond to impact of azoxystrobin and tebuconazole on carbon, nitrogen, and phosphorous cycling, and microbial activities under an RCP 8.5 climate change scenario (2070-2100) in the Lower Rio Grande Valley, TX. In completing this investigation, the findings will

help clarify the relationship between fungicides, soil microbial communities, and climate change (Leslie, 2016). These communities are the basis of soil health that ultimately support the sustainability within agriculture. Not only would this study aid in closing the gap of knowledge within these topics (Zhang, et al., 2016), but also help farmers within the Lower Rio Grande Valley Region (Leslie, 2016). Additionally, aid those that live in similar climate conditions with their soil to ensure crop production in the future. Thus, this study will explore the following research question, will fungicides, temperature, and moisture inhibit or enhance microbial activities involved in the carbon, nitrogen, and phosphorous cycling? With this question, two hypotheses were formulated. Firstly, it is hypothesized, since enzymes are present in bacteria and fungus, more resources would be available for bacteria due to fungicide mode of action towards fungus thereby, increasing overall activities. Secondly, it is hypothesized that with climate change, reduced moisture, and higher temperature regimes, would decrease microbial activities.

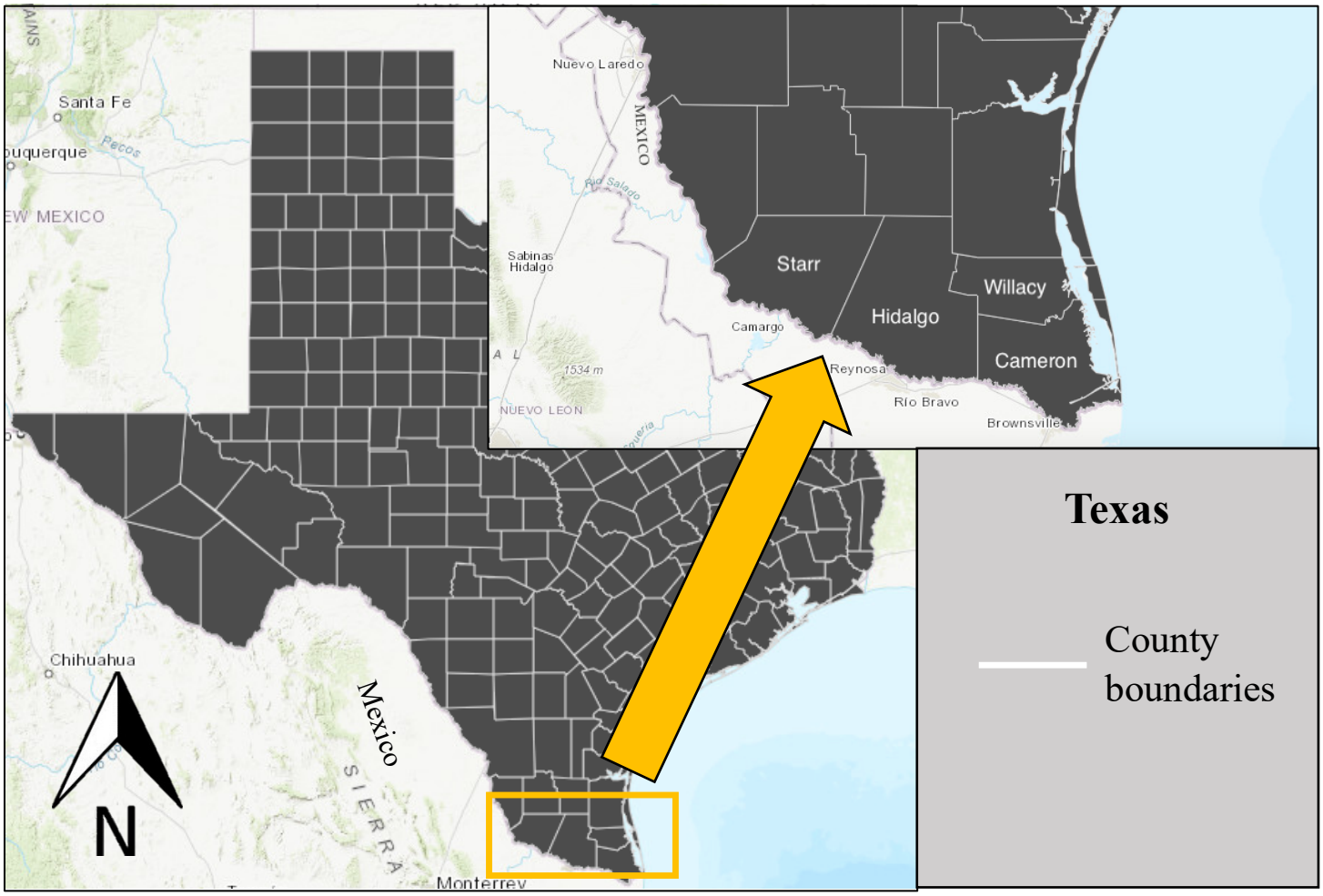

Figure 1 Map of The Lower Rio Grande Valley's counties in Texas, U.S.A. Source: Texas Department of Transportation, 2022.

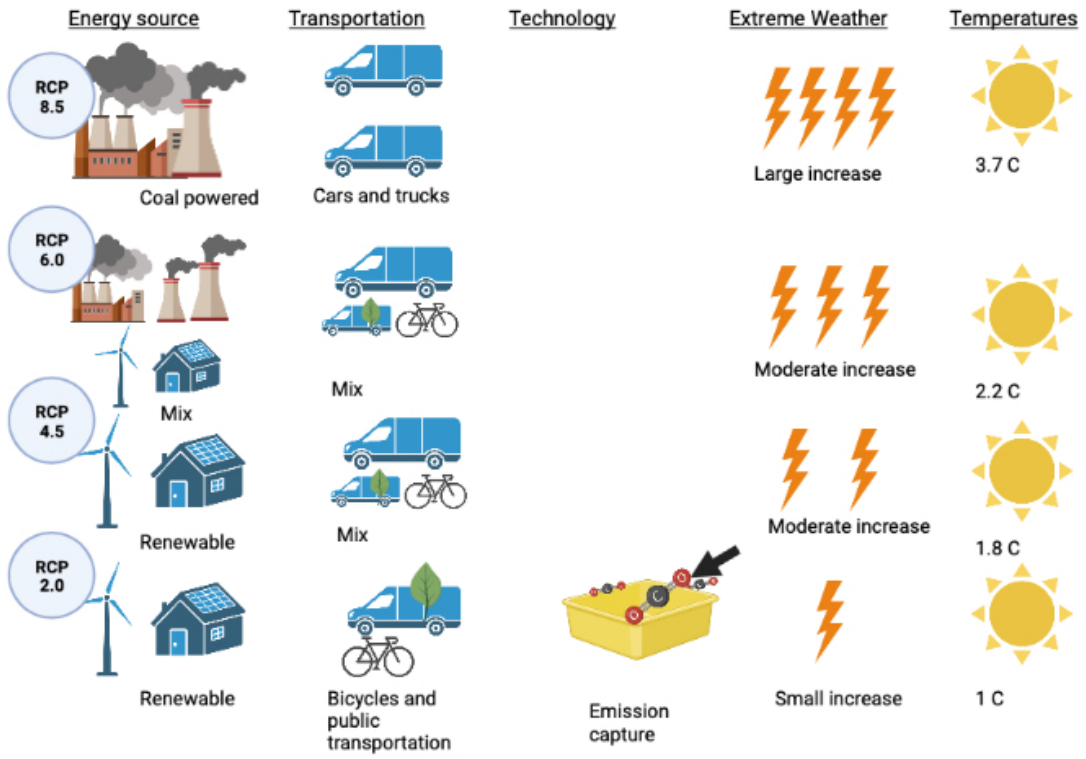

Figure 2 IPCC's estimated expected changes in climate according to mitigation efforts. Energy sources depicted the main use of energy within each scenario (Key: Renewable energy consisted of houses with solar panels (solar energy) and windmill (wind energy) icons. Fossil fuels were depicted by coal plants with smoke icons). Mode of transportation demonstrated the effort of reducing vehicle emissions (Key: Low emission transportation included vehicles with leaf (hybrid/electric), inclusion of public transportation, and bicycles. Higher emissions are shown as vehicle with no leaf (gas-driven transportation)). Emission capture depicts the development of advance technology to mitigate climate change. Changes in extreme weather and temperatures are average changes anticipated near the end of the century (2081-2100). Source of data was extracted from (CoastAdapt). Figure was recreated using BioRender.

Table 1 List of soil enzymes of interest with respective relevance to soil health.

Potential Soil Function	Enzyme	Source	Significance	References
	Fluorescein Diacetate Assay (FDA) hydrolysis	Living and dead microbes, plants, and soil organisms	Intracellular esterases hydrolyse FDA after uptake, indicating overall microbial activity in soil.	(Patle, Navnage, & Barange, 2018; USDA, 2010; Green, Stott, & Diack, 2006)
C	β-Glucosidase	Plants, animals, fungi, bacteria, and yeasts	Early indication of changes in organic matter status and its turnover; the capacity of soil to stabilize soil organic matter and can be used to detect management effects	(Adetunji, Lewu, Mulidzi, & Ncube, 2017; Stege, Messina, Bianchi, Olsina, & Raba, 2010; Tiwari, Dwivedi, Sharma, Sharma, & Dwivedi, 2019; Bakshi & Varma, 2011)
N and C	N-acetyl-β-D-glucosaminidase (NAGase)	Predominantly bacteria and fungi, but also found in plants	Availability of N, semi-quantitative indicator of soil fungal biomass, involved in biological control of plant pathogens	(Parham & Deng, 2000; Rahul, Sharma, Singh, Singh, & Kumar, 2022; Madsen, 2003; Bakshi & Varma, 2011)
N	Urease	Microorganisms, invertebrates and plants	Regulation of N supply to plants; nitrogen mineralization	(Adetunji, Lewu, Mulidzi, & Ncube, 2017; Rahul, Sharma, Singh, Singh, & Kumar, 2022; Koçak, 2020)
P	Acid Phosphatase	Bacteria, plants and fungi	Provides nutrient uptake/ inorganic phosphorus availability for crops and microbes	(USDA, 2010; Tiwari, Dwivedi, Sharma, Sharma, & Dwivedi, 2019; Janes-Bassett, et al., 2022)
P	Alkaline Phosphatase	Bacteria, plants and fungi	Provides nutrient uptake/ inorganic phosphorus availability for crops and microbes	(USDA, 2010; Tiwari, Dwivedi, Sharma, Sharma, & Dwivedi, 2019; Janes-Bassett, et al., 2022)

CHAPTER II

REVEALING THE EFFECTS OF CLIMATE CHANGE AND FUNGICIDE ON SOIL MICROBIAL COMMUNITIES IN THE LOWER RIO GRANDE VALLEY, TEXAS

Introduction

Since the pre-industrial era, anthropogenic greenhouse gases (GHG) have helped drive global warming and alter climate variables such as average annual precipitation and temperatures (Pachauri & Meyer, 2014; Chan & Wu, 2015; Egorova, Rozanov, Arsenovic, Peter, & Schmutz, 2018; Fischer & Knutti, 2015). This increment of greenhouse gas emissions result in a plethora of environmental issues among physical and biological systems (Rosenzweig, et al., 2008). These environmental issues not only effect physical and biological systems, but also one of the most under looked systems, the soil ecosystem. Such ecosystem is rarely considered for the focus of climate change and should therefore be analyzed (Cavicchioli, et al., 2019; Maestre, et al., 2015; Dutta & Dutta, 2016). Studies have emphasized the importance of rainfall (Borowik & Wyszkowska, 2016; Vasquez-Dean, Maza, Morel, Pulgar, & Gonzalez, 2020; Tan, Wang, Bai, Qi, & Chen, 2020; Huxman, et al., 2004; Zhao, et al., 2016; Tomar & Baishya, 2020) and temperatures (Howe & Smith, 2021; onwuka & Mang, 2018) critical roles in soil microbial natural processes and their sensitivity to these changes (Kumar, Rawat, & Amule, 2016).

According to the Intergovernmental Panel on Climate Change (IPCC), the Lower Rio Grande Valley's (LRGV) average rainfall and temperatures are anticipated to shift in the

Representative Concentrated Pathway (RCP) 8.5 scenario towards the end of the century (2070-2100) (Pachauri & Meyer, 2014). The LRGV is expected to experience an average decrease of 10% on rainfall (Pachauri & Meyer, 2014; Seneviratne, et al., 2015) and an increment of 4°C (~7°F) on average temperatures (Pachauri & Meyer, 2014; Collins, 2013).

Moreover, it is estimated that agricultural productivity must triple by the end of the century (2100) to meet global demands under the RCP 8.5 scenario (Beltran-Pena, Rosa, & D'Odorico, 2020). Hence, significant efforts have been made to increase productivity such as the use of commercial chemical pesticides (Trivedi, Delgado-Baquerizo, Anderson, & Singh, 2016). Specifically, fungicide applications are useful in preventing fungal diseases and have helped in improving crop production, quality, and income by reducing the risk of crop losses (Adetutu, Ball, & Osborn, 2008; Fernandez-Cornejo, et al., 2014). However, fungicide residuals may be released to the soil and manipulate biological physiochemical properties of soil, which can eventually disturb microbial communities and physiological processes (Alengebawy, Abdelkhalek, Qureshi, & Wang, 2021), greatly affect non-targeted microbial diversity and functions related to the nutrient cycles, and soil fertility (Ding, et al., 2019; Arora & Sahni, 2016) and in cases, inhibit microbial growth (Ullah & Dijkstra, 2019). In this study, two of the most used groups of fungicides (Correira, Rodrigues, Paiga, & Delerue-Matos, 2016), strobilurin (azoxystrobin) and triazoles (tebuconazole) were used. Although there is limited data of fungicide use in the Lower Rio Grande Valley, these two fungicides may be utilized for major crops grown in this region such as corn, sugarcane, watermelon, sorghum, citrus, onion, tomatoes, and cotton, just to mention a few (AgriLife, n.d.; Texas A&M AgriLife Research, n.d.; Jahrsdoerfer & Leslie, 1988).

Azoxystrobin (QoI- Quinone outside Inhibitor) is a strobilurin-dervived systematic fungicide that belongs to the chemical group methoxyacrylate (FRAC, 2006) and functions by inhibiting fungal pathogen's mitochondria respiration through the binding of the cytochrome b complexes (Adetutu, Ball, & Osborn, 2008; Wyenandt, 2020). This process specifically blocks the electron transport from the cytochrome b to c, hindering energy that interferes with cell growth (i.e., favoring the death of pathogen) (Bacmaga, Kucharski, & Wyszkowska, 2015), and spore germination and zoospore motility (Brauer, et al., 2019). Fungicides within this group (FRAC, 2006), are at high risk of developing cross resistance (FRAC, 2006). Additionally, azoxystrobin is often used to control several pathogens within Ascomycota (e.g., *Cerospora*, *Collectotrichium*, and *Fusarium*), Basidiomycota (e.g., *Rhizoctonia*, *Gymnosporangium*, and *Phragmidium*), Deuteromycota, and Oomycota (e.g., *Pythium*, *Phytophthora*, and *Peronospora*) (Vuyyuru, Sandhu, McCray, & Raid, 2018; Brauer, et al., 2019; Prime Source, 2014). Azoxystrobin may be utilized on a range of crops including beans, peppers, cucurbits (e.g., watermelon), citrus, onion, and variety of vegetables (e.g., carrots, lettuce, spinach, and kale), just to mention a few (AgriLife, n.d.; Texas A&M AgriLife Research, n.d.; Jahrsdoerfer & Leslie, 1988). Regardless of the benefits azoxystrobin offers in controlling fungal pathogens, it has demonstrated repressive behavior towards soil microbial communities in enzymatic activities, microbial biomass, soil respiration, and in structure and function (Bacmaga, Kucharski, & Wyszkowska, 2015; Guo, et al., 2015; Wang, et al., 2020; Aleksova, et al., 2021)

Tebuconazole (DeMethylation Inhibitors- DMI) is a systemic fungicide from the chemical group triazoles (FRAC, 2006) and performs by inhibiting ergosterol biosynthesis in fungi through demethylation (i.e., fungicide interferes with the structure of fungal cell wall, then inhibits reproduction and growth of pathogen) (Bacmaga, Wyszkowska, & Kucharski, 2021).

Resistance within this group of fungicide (FRAC, 2006) is known in various fungal species and is of medium concern (FRAC, 2006). Tebuconazole is recognized for its high efficacy in plant and soil protection (Bacmaga, Wyszkowska, & Kucharski, 2021), and its control use for pathogens in the Ascomycota (e.g., *Phoma*, *Fusarium*, *Cercospora*, and *Alternaria*) and Basidiomycota (e.g., *Puccinia* and *Rhizoctonia*) (Prime Source, 2013). This fungicide may be administered on groups in fruits (e.g., cucurbits, eggplant, and pepper) , nuts, cereal (e.g., sorghum) and vegetables (e.g., bok choy, collards, and corn) (Bacmaga, Wyszkowska, & Kucharski, 2021; Munoz-Leoz, Ruiz-Romera, Antiguedad, & Garbisu, 2011; AgriLife, n.d.; Texas A&M AgriLife Research, n.d.; Jahrsdoerfer & Leslie, 1988) Despite of tebuconazoles ability to enhance production, yields, and crop quality, studies have indicated that this fungicide may negatively impact soil microbial communities by impeding respiration, biomass, enzyme activities, and bacterial diversity (Bacmaga, Wyszkowska, & Kucharski, 2021; Munoz-Leoz, Ruiz-Romera, Antiguedad, & Garbisu, 2011; Saha, Pipariya, & Bhaduri, 2016; Bacmaga, Wyszkowska, & Kucharski, 2019).

Monitoring soil microbial communities are essential to develop the best conditions for sustainable agriculture and achieve healthy soil systems (Yang, Siddique, & Liu, 2020). A healthy soil system aids in functions such as the facilitation of biological activities, suppression of pathogens, decomposition of organic matter, cycling of vital nutrients, and physical stability and support of soils (USDA-NRCS, Soil Health, n.d.; Tahat, Alananbeh, Othman, & Leskovar, 2020; Hermans, et al., 2020). Soil health may be assessed through various indicators that are sensitive to changes in the natural environment such as soil enzymes and respiration (Lee, Kim, Kim, & Kim, 2020; Luo & Zhou, 2006). Soil enzymes can be utilized to unveil ecosystem distress (e.g., climate change and pesticide use) and to detect changes that are involved in the

carbon, nitrogen, and phosphorous cycles (Lee, Kim, Kim, & Kim, 2020) and overall microbial activities. Soil enzymes are also biomarkers of the soil environment contamination such as fungicides (Bacmaga, Wyszkowska, & Kucharski, 2019). Soil respiration is a useful tool to estimate the level of microbial activity, organic matter, and decomposition (USDA-NRCS, Soil Respiration, 2014; Guo, et al., 2015). In addition, soil respiration is beneficial to measure respiration rates from global warming (Meyer, Meyer, Welp, & Amelung, 2018) and pesticide effects.

Collectively, soil microbial communities are sensitive to changes in environmental factors such as precipitation and temperature and are critical to consider due to its potential changes in decomposition, nitrogen mineralization, organic carbon storage, and other environmental processes (Baiser, Gutknecht, & Llang, 2010). Thus, governing microbial communities are essential in understanding how global change will affect soil processes and ecosystem function (Baiser, Gutknecht, & Llang, 2010) coupled with fungicide use on soil health.

The aim of this study was to assess six enzymes (Fluorescein Diacetate (FDA) hydrolysis, β-glucosidase, N-acetyl-β-D-glucosaminidase (NAGase), urease, acid phosphatase, and alkaline phosphatase) and soil respiration to monitor the impact of azoxystrobin and tebuconazole on the carbon, nitrogen, and phosphorous cycling, and microbial activities under an RCP 8.5 climate change scenario (2070-2100) in the LRGV. In completing this investigation, the findings will help clarify the relationship between fungicides, soil microbial communities, and climate change (Leslie, 2016). Not only would this study aid in closing the gap of knowledge within these topics (Zhang, et al., 2016), but also help farmers within the LRGV region. Thus, this study will explore the following research question, will fungicides, temperature, and

moisture inhibit or enhance microbial activities involved in the carbon, nitrogen, and phosphorous cycling? With this question, two hypotheses were formulated. Firstly, it is hypothesized that since enzymes are present in bacteria and fungus, more resources would be available for bacteria due to fungicide mode of action towards fungus thereby, increasing overall activities. Secondly, it is hypothesized that with climate change, reduced moisture, and higher temperature regimes, would decrease microbial activities.

Material and methods

Soils

A total of 30 random soil samples were collected from the arable layer (depth 0-20 cm) from an 18 acre conventionally managed farm at Willacy County in Raymondville in Texas (26° 29'1.96" N, 97° 55'48" W) (Figure 3). The soil was a loamy sand (82% sand, 8% clay, 10.33% silt) (Figure 4). According to the Natural Resource Conservation Services (NRCS) global soil region classification, the soil of focus represents Mollisols (USDA-NRCS, 2005). Soils properties are presented in Table 2. The proportion of sand, silt, and clay was determined using the hydrometer method mixing 50 g of soils with a 0.5 M sodium hexametaphosphate solution. The hydrometer method is the based on Stokes' Law, which states that denser particles (usually larger) sink faster than less dense particles (smaller) when suspended in liquid (of a given temperature). Soil pH was measured in deionized water using a 1:1 (wt/ wt) ratio, with an equilibration time of 1 hr. The average temperature of Willacy County is 23.1 °C (73.58 °F) and annual precipitation is 645.42 mm (25.41 inches) (NOAA, n.d.).

Experimental design

Mason jars (473 ml) were filled with 100 g of air-dried soil that was passed through an 8 mm sieve twice. Pincers were utilized for further extraction of plant debris. Prior to treatments, soils were wetted to 60% of water holding capacity to reactivate microbial activities (Blazewicz, Schwartz, & Firestone, 2014; Meisner, Rousk, & Baath, 2015; Barnard, Blazewicz, & Firestone, 2020). Each sample was treated with appropriate soil moisture, incubation temperatures, and fungicide levels. Temperature and soil moisture treatments were determined according to current and projected climate conditions. Current climate conditions were obtained at the National Oceanic and Atmospheric Administration (NOAA) Climate Data Online tool (ncei.noaa.gov/cdo-web) while anticipated climate scenarios were based on estimates of the Intergovernmental Panel on Climate Change (IPCC) RCP 8.5 pathway near the end of the century (2070-2100). To calculate the amount of moisture that would emulate current soil moisture conditions, 50% of the available water storage for this soil (Web Soil Survey, USDA NRCS) was converted to gravimetric soil moisture, which indicated the amount of water needed to reach that specific available water storage (i.e., 0.09 g of water per g soil or 9% moisture). For the projected soil moisture conditions, since the IPCC estimates 10% reduction in precipitation levels for the LRGV region, 0.08 g of water per g of soil was added to each jar (i.e., 8% moisture) (Pachauri & Meyer, 2014; Seneviratne, et al., 2015). Precipitation was used due to its high correlation to soil moisture in semi-arid regions (Shreve, 1914; Zhang et al., 2020; Zhang, et al., 2010; Wang, et al., 2019; Seneviratne, et al., 2015; Sehler et al., 2019). Current and future temperatures were simulated within incubators (24 °C for the current average; 28 °C for the projected average) (Pachauri & Meyer, 2014; Collins, 2013). Air temperatures were considered due to their strong correlation with soil temperatures (Borowik & Wyszkowska, 2016; USDA,

2022). Fungicides were amended at recommended applications rates from manufacture. Commercial grades of fungicides, Azoxystrobin (methyl (E)-2-{2-(2- cyanophenoxy) pyrimidin-4-yloxy] phenyl}-3-methoxyacrylate, 22.9%) (Azoxy 2SC Select ™, Prime Source LLC, Evansville, IN) and Tebuconazole (alpha-[2-(4-chlorophenyl)ethyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethano, 38.7%) (Tebuconazole 3.6 Select ™, Prime Source LLC, Evansville, IN) were mixed with distilled water to create solutions according to treatments. Concentrations of the fungicides was administered approximately at field application rates (147.87 mL per acre for Azoxystrobin and 147.87 mL per acre for Tebuconazole). To calculate these values, it was assumed a soil bulk density of 1.6 g cm^3 and a 10 cm soil depth to dilute the amount of fungicide per jar. The following treatment combinations includes control (no fungicide, current temperature, and ambient moisture levels), fungicides with current climate scenario (azoxystrobin or tebuconazole, current temperature, and ambient moisture levels), and future climate scenario (azoxystrobin or tebuconazole, future temperature, and reduced moisture levels), resulting in 12 treatment combinations with 12 replications (Table 3). Jars were left incubated in the dark in aerobic conditions under respective temperatures (according to scenario), and 60 mL of fungicide solution for 45 days. Soil moistures were monitored two to three times a week and kept constant throughout the experiment. On day 45, samples were analyzed for enzymatic activities and soil respiration.

Soil enzymes

Soil enzymatic activity was determined after day 45 of incubation upon 12 replications. The activities of the following enzymes analyzed included Fluorescein Diacetate (FDA) hydrolysis assay according to the method described by Schnurer and Rossawall (1982), β-glucosidase (Eivazi and Tabatabai 1988; Deng and Popova 2011), alkaline and acid phosphatase

(Parham and Tabatabai 1977; Acosta-Martinez and Tabatabai 2011), N-acetyl-β-D-glucosaminidase (NAGase) (Parham and Deng 2000; Deng and Popova 2011), and urease (Allison, Steve 2001). The subsequent substrates were utilized to test the activities of enzymes: fluorescein diacetate for FDA hydrolysis, p-Nitrophenyl-β-D glucopyranoside (0.05 M) for β-glucosidase, p-Nitrophenyl-N-acetyl-β-D-glucosaminide (10.0 mM) for acid phosphatase and NAGase, p-Nitrophenyl phosphate (0.05M) for alkaline phosphatase, and urea (40 mM) for urease. Analyzed soil enzymes were measured using the Biotek's Synergy HTX Multi-Mode spectrophotometer at a wavelength of 490 nm for FDA, 410nm for β-glucosidase, alkaline and acid phosphatase, and NAGase, and 690 nm for urease.

Soil Respiration

Soil respiration was determined after day 45 of incubation based upon 12 replicates. 20g of soil and CO_2 traps (alkaline solution of 9 mL of 1 M NaOH 0.5 M) were incubated in sealed Ball jars for 5 days at room temperature. The NaOH solutions were treated with 10% barium chloride ($BaCl_2$) solution and titrated with 0.5 M HCl using Vernier's drop count. Phenolphthalein in acid and a pH probe were used as an indicator of point of neutralization.

Data analysis

The effect of the RCP 8.5 climate change scenario (2070-2100) for air temperature and soil moisture with the use of fungicides (i.e., azoxystrobin and tebuconazole) was evaluated on data obtained from soil respiration and soil enzymatic activities assays (i.e., Fluorescein Diacetate (FDA) hydrolysis, β-glucosidase, N-acetyl-β-D-glucosaminidase (NAGase), urease, acid phosphatase, and alkaline phosphatase). All data analyses were performed on R studio software (version 4.1.1, http://www.R-project.org/).

Pre-treatment

Prior to running any analysis, the dataset underwent through a logarithmic transformation (individual) based on the Shapiro-Wilk test. The Shapiro-Wilk test aids in testing the quality of data. In addition, a Levene test was utilized to access homogeneity across the data matrix.

Analysis of Variance (ANOVA)

To evaluate the differences or similarities across fungicide, temperature, and moisture treatments through temporal scale, a three-way Analysis of Variance (ANOVA), two-way ANOVA, one-way ANOVA, and Tukey's test were administered. An ANOVA is a gathering of statistical models in which measures the variation among and between groups, to analyze the differences among their averages in a sample (Cleophas & Zwinderman, 2021). Depending on the number of variables used, a three, two, or one-way ANOVA may be applied to the data matrix. In this study, a three-way ANOVA was initially applied to measure not only the differences among the fungicide, temperature, and moisture treatments, but to also access interaction effects. Based on significant levels ($p > 0.05$) from the three-way ANOVA output, a two or one-way ANOVA was administered.

Post-Hoc Tukey's Honestly Significance Difference (HSD) test

A post-hoc Tukey's Honestly Significance Difference (HSD) test helps in outlining individual averages that are significantly different from a set of averages (Haynes, 2013). Typically, Tukey's test compares multiple averages and is applied when an investigation used more than two averages and after an ANOVA analysis (Haynes, 2013). Therefore, a Tukey's test was employed to this study to identify specific significant treatments across significant ANOVA outputs ($p > 0.05$). In addition, a 95% family-wise confidence level was utilized.

Results

Main and interactive effects of fungicide, temperature, and moisture treatments were estimated on soil enzymes and general activities (Table 4). In the following subsections, these activities were described according to their association with nutrient cycling (C, N, and P).

Carbon cycle and general enzyme activities

Fluorescein Diacetate (FDA) hydrolysis. The interaction between temperature and fungicide were found to be statistically significant in the FDA hydrolysis analysis ($p<0.001$) (Figure 5). Tebuconazole and warming (28°C) enhanced FDA activities by 25.67% compared to FDA activities in soils without fungicide under warming (28°C). Although not significant, FDA activities were generally lower under warming (28°C) compared to 24°C in soils with no fungicide and azoxystrobin. In addition, moisture reductions did not exert significant effects on enzymatic activities.

β-glucosidase. Results revealed that β-glucosidase was significantly impacted by fungicide application ($p<0.0001$) and temperature increases ($p<0.05$), however there was no interaction effect between the two factors (Figure 6). When soils were incubated with tebuconazole, enzyme activities were reduced by 10.79% in comparison to control (no fungicide) (Figure 7). There were no significant differences in β-glucosidase activities between soils treated with azoxystrobin and control (no fungicide). Warming (28°C) significantly decreased β-glucosidase activities by 5.67% compared to current temperature conditions (24°C) (Figure 8). Changes in moisture regimes did not significantly alter β-glucosidase activities.

Soil respiration. The interaction between temperature, moisture, and fungicide were found to be statistically significant for soil respiration rates ($p<0.0001$) (Figure 9). The highest respiration rate was 0.711 mg CO_2-C kg^{-1} soil $^{h-1}$ (no fungicide; 24°C; reduced soil moisture) and the lowest was 0.107 mg CO_2-C kg^{-1} soil $^{h-1}$ (azoxystrobin; warming (28°C); reduced soil moisture). Warming (28°C) significantly decreased soil respiration in control (no fungicide) and soils amended with azoxystrobin, but not in soils amended with Tebuconazole. Reduced moisture levels significantly increased soil respiration in control (no fungicide) by 69.70%, while decreased 45.96% with tebuconazole. Additionally, under current temperature (24°C) azoxystrobin significantly reduced respiration rates by 47.29% compared to control (no fungicide), regardless of moisture regime. Also, under current temperature (24°C), tebuconazole hindered soil respiration rates by 80.45% under reduced moisture conditions compared to control (no fungicides).

Nitrogen Cycle

N-acetyl-B-D-glucosaminidase (NAGase). The interaction between temperature, moisture, and fungicide were found to be statistically significant in NAGase analysis ($p<0.0001$) (Figure 10). The highest NAGase activity was 1.32 mg PNP kg^{-1} soil $^{h-1}$ (no fungicide; 24°C; drought) and the lowest was 0.948 mg PNP kg^{-1} soil $^{h-1}$ (azoxystrobin; warming (28°C); drought). Warming (28°C) decreased by 19% (on average) all fungicide treatments regardless of moisture conditions. Under reduced soil moisture and 24°C incubation temperature, NAGase activities were increased under control (no fungicide) by +2.33%, but decreased under both fungicide treatments by 8.71%, though this effect was only statistically significant for tebuconazole. Impacts of fungicide were not identified to be significant under ambient moisture.

Urease. Outcomes on urease activities showed it was significantly affected by fungicide treatment (p=0.136). Azoxystrobin had an overall decrease of 5.40% in urease enzyme activities compared to no fungicide (Figure 11). Although azoxystrobin had drastic effects on urease enzymes, tebuconazole did not considerably affect these rates. The increase of temperature and reduction of moisture were also identified insignificant in altering urease.

Phosphorous cycle

Acid phosphatase. Fungicide treatments significantly altered acid phosphatase activities (p= 0.0431). Tebuconazole hindered acid phosphatase activities by 9.50% compared to no fungicide (Figure 12). Azoxystrobin did not significantly affect acid phosphatase activities. Additionally, both temperature and moisture levels did not exhibit significant changes to enzyme rates.

Alkaline phosphatase. Alkaline phosphatase activities were significantly impacted by fungicide treatment (p=0.0183) and temperature changes (p=0.0129) (Figure 13). Tebuconazole hindered alkaline phosphatase activities by 7.66% when compared to no fungicide use (Figure 14). While tebuconazole lowered alkaline phosphatase activities, azoxystrobin did not demonstrate drastic behavior. Warming (28°C) reduced activity rates by 5.29% compared to 24°C (Figure 15). Furthermore, moisture changes did not significantly alter activities.

Discussion

In this study, soil enzymes, Fluorescein Diacetate (FDA) hydrolysis, β-glucosidase, N-acetyl-β--D-glucosaminidase (NAGase), urease, acid phosphatase, and alkaline phosphatase, and soil respiration, were accessed to quantify the effects of different temperatures (24°C and 28°C), moisture (ambient and reduced), and fungicide (azoxystrobin and tebuconazole) on soil health.

Soil enzymes are excellent indicators for unveiling ecosystem distress (i.e., pesticide use and climate changes) and detect changes in the carbon, nitrogen, and phosphorous cycles (Lee, Kim, Kim, & Kim, 2020) due to their sensitivity (Sudhakaran, Ramamoorthy, Savitha, & Kirubakaran, 2019; Adetutu, Ball, & Osborn, 2008; Adetunji, Lewu, Mulidzi, & Ncube, 2017). FDA hydrolysis is an enzyme which measures overall enzyme activities within the soil ecosystem (Patle, Navnage, & Barange, 2018; USDA, 2010; Green, Stott, & Diack, 2006). This assay aids in identifying how active microbial communities are in given treatment(s). β-glucosidase is related to the carbon cycle and serves in identifying changes in organic matter and stability (Adetunji, Lewu, Mulidzi, & Ncube, 2017; Stege, Messina, Bianchi, Olsina, & Raba, 2010; Tiwari, Dwivedi, Sharma, Sharma, & Dwivedi, 2019). Moreover, NAGase is involved with the nitrogen and carbon cycle and functions in revealing the availability of nitrogen and fungal biomass (Parham & Deng, 2000; Rahul, Sharma, Singh, Singh, & Kumar, 2022; Bakshi & Varma, 2011). Urease is another enzyme in which partakes in the nitrogen cycle. This enzyme is responsible for regulating nitrogen supply to plants and play a vital role in nitrogen mineralization (Adetunji, Lewu, Mulidzi, & Ncube, 2017; Rahul, Sharma, Singh, Singh, & Kumar, 2022; Madsen, 2003). Furthermore, alkaline and acid phosphatase are critical contributors to nutrient uptake and inorganic phosphorous availability for crops and microbial communities under high or low pH values (USDA, 2010; Tiwari, Dwivedi, Sharma, Sharma, & Dwivedi, 2019; Janes-Bassett, et al., 2022). Lastly, soil respiration is also a useful tool to measure microbial activities and organic matter under changes in climate and pesticide effects (USDA-NRCS, 2014; Meyer, Meyer, Welp, & Amelung, 2018). Soil respiration exposes the capacity of soils to sustain life in plants and microorganisms (USDA-NRCS, Soil Respiration, 2014; Guo, et al., 2015).

Fungicide impacts on soil microbial activities are dependent upon the physical, chemical, and biochemical properties of the soil, the nature and concentration of the pesticide (Arora & Sahni, 2016; Tejada, Gomez, Garcia-Martinez, Osta, & Parrado, 2011; Chen, Edwards, & Subler, 2001), the dosage (Roman, Voiculescu, Filip, Ostafe, & Isvoran, 2021), and the history of management of the field soil (Sulowicz, Cycon, & Piotrowska-Seget, 2016). Nonetheless, fungicides may be more adverse towards fungi than bacterial communities due to their susceptibility to the fungicide (e.g., fungicide's mode of action) (Lopez Santisima-Trinidad, del Mar Montiel-Rozas, Diez-Rojo, Pascual, & Ros, 2018). In turn, carbon may be released from the death of the sensitive communities, resulting bacteria to use this as a substrate, (Lopez Santisima-Trinidad, del Mar Montiel-Rozas, Diez-Rojo, Pascual, & Ros, 2018), enhancing activities. Aligned with the first hypothesis of this study, fungicide was found to be significant in stimulating FDA hydrolysis when tebuconazole was applied at 28°C. A plausible explanation is the chance of microbial community shifts. Ye et al., (2018) discussed how microorganisms may be impacted through metabolic reactions, causing them to develop more resistant communities by utilizing fungicide as a carbon source (Ye, Dong, & Lei, 2018). Similarly, in another study, Filimon and others, found an increased activities of an enzyme mediated by FDA hydrolysis (Prosser, Speir, & Stott, 2011), protease, with a triazole fungicide, difenoconazole, under increased temperatures. It was also concluded that microbes adapted by utilizing this fungicide as a source of energy (Filimon, Voia, Vladoiu, Isvoran, & Ostafe, 2015). However, β-glucosidase, phosphatase, and urease activities did not confirm this hypothesis. In general, β-glucosidase activities were significantly reduced when soil samples were incubated with tebuconazole. This outcome was consistent with other studies who investigated the impacts of this fungicide on soil enzymes (Bacmaga, Wyszkowska, & Kucharski, 2019; Munoz-Leoz, Ruiz-Romera, Antiguedad,

& Garbisu, 2011; Bacmaga, Wyszkowska, & Kucharski, 2020). The suppressed rates can be a result from β-glucosidase sensitivity to tebuconazole, causing a decline in activities. Therefore, tebuconazole may lower β-glucosidase rates, disrupting the amount and stability of organic matter. Furthermore, in this study, alkaline and acid phosphatase activities were significantly hindered with tebuconazole treatments. These results agree with other studies who evaluated the effects of this fungicide on phosphatase activities (Bacmaga, Wyszkowska, & Kucharski, 2019; Munoz-Leoz, Ruiz-Romera, Antiguedad, & Garbisu, 2011; Bacmaga, Wyszkowska, & Kucharski, 2020). However, a study composed by Bacmaga et al., (2021), reported tebuconazole to stimulate acid and alkaline phosphatase activities (Bacmaga, Wyszkowska, & Kucharski, 2021). It may be hypothesized that these results differ due to the contrast of chemical formulation and preparation used. Bacmaga and other's investigation utilized tebuconazole at 99% purity (lab-grade) and dissolved it in ethanol then diluted it with distilled water. In this investigation, commercial grade Tebuconazole 3.6 Select ™ (38.7% tebuconazole and 61.3% of "other" ingredients) was employed and diluted with distilled water. This commercial chemical formula may have had a synergetic or antagonistic effects with the fungicide and enhancers (Munoz-Leoz, Ruiz-Romera, Antiguedad, & Garbisu, 2011). Thus, tebuconazole in this study impacted phosphatase activities negatively, reflecting its potential in impeding nutrient uptake to crops and soil microbes. Moreover, in this study, urease activities were drastically lowered with azoxystrobin treated soil. Other authors, (Bacmaga, Kucharski, & Wyszkowska, 2015; Wang, et al., 2020; Guo, et al., 2015; Boteva, et al., 2020), also identified azoxystrobin to have impeding behavior on urease activities. Whereas, (Wang, et al., 2018) found urease activities to fluctuate over time with azoxystrobin. These circumstances may have occurred from an interaction between the soil type and fungicide (Wang, et al., 2018). In Wang and other's study, their

experimental soil was composed of 17.68% clay, 44.62% sand, and 37.95% silt, while this study was 8% clay, 82% sand, and 10.33% silt. With higher clay content, it can be predicted that nutrients may have held together greater (Wagner, Kuhns, & Cardon, 2015; Ye, Parajuli, & Sigua, 2019) than in sandy soils (prone to leaching) (Wyatt, Arnall, & Ochsner, 2019). Thus, azoxystrobin might disturb the regulation of nitrogen supply via urease activities.

With temperature increments, it is expected for microbial activities, growth, and biomass to be stimulated (i.e., enhance enzyme production) (Mooshammer, et al., 2022). However, this effect is deemed to be temporarily. Overtime, this may shift microbial functioning by constricting enzyme production, resulting a neutral or decreased enzyme activity (Mooshammer, et al., 2022). In addition, with lowered levels of moisture, enzyme production and activities are generally expected to decline from substrate constrains (Stark & Firestone, 1995; Geisseler, Horwath, & Scow, 2011). Supporting the second hypothesis, temperature increments were found to significantly depress β-glucosidase and alkaline phosphatase, while it also effected NAGase and soil respiration solely and interactively. In this investigation, overall β-glucosidase rates were impeded in higher temperature treatments. These results were aligned with other studies in which studied climate change (McDaniel, Kaye, & Kaye, 2013) or temperature increments (Zhang, Chen, Wu, & Sun, 2011) effects on β-glucosidase. Decreasing rates of β-glucosidase with increasing temperatures may be further explained through substrate availability. According to (McDaniel, Kaye, & Kaye, 2013) and (Zhang, Chen, Wu, & Sun, 2011), greater temperatures may allow there to be higher sources of carbon, however β-glucosidase may decrease due to their high dependency of substrate (Km increase). Besides β-glucosidase, soils incubated in higher temperatures significantly lowered overall alkaline phosphatase levels. Similarly, higher temperatures lowered the rate of enzyme to substrate formation, causing a disbalance among the

two factors (Tan, et al., 2018). This was also found true in (Ma, Razavi, Holz, Blagodatskaya, & Kuzayakov, 2017). Ma and others found that warm temperatures caused substrate breakdown to increase, resulting a shorter turnover. With lower levels in β-glucosidase and alkaline phosphates from higher temperatures, organic matter content and nutrient uptake may be slowed, effecting plant growth and fertility. Furthermore, soils incubated with azoxystrobin and tebuconazole decreased NAGase rates with increasing temperatures. These changes may arise if there were alterations in the soil biota (i.e., ratio of fungal to bacteria). As mentioned previously, NAGase can be utilized as an indicator for fungal biomass. If increases of temperatures decreased NAGase, then there could be an indication of a decrease of fungus in the soil community (McDaniel, Kaye, & Kaye, 2013). Temperatures was also found to decrease respiration rates across fungicide treatments, while tebuconazole enhanced rates in one case. According to Luo and Zhou, soil respiration interacts with various factors and can often be challenging to separate the effects such as temperature and moisture levels (Luo & Zhou, 2006). However, substrate supply may also play a role in soil respiration (Luo & Zhou, 2006). With this said, respiration rates may have decreased because of low substrate availability from accelerated activities (Guoju, Qiang, Jiangtao, Fengju, & Chengke, 2012). Moreover, with increasing temperatures and reduction of moisture levels, NAGase and soil respiration rates were significantly impacted. With reduction of moisture levels, no fungicide treatment had a consistent trend of increasing under reduced moisture regimes in NAGase and soil respiration. A plausible explanation is that climatic regimes may have caused sensitive microorganisms to decease, allowing their compounds such as nitrogen or carbon to release (Bogati & Walczak, 2022). In turn, microbial organisms that thrived, could have used it as a source of energy, enhancing activities. Another possible likelihood of these outcomes is the use of Extracellular Polymetric Substance (EPS) by

bacteria (Abdul Rahman, Adul Hamid, & Nadarajah, 2021). EPS is an adaptation strategy that is used under hostile conditions such as low moisture levels (i.e., dry soils) and substrate limitations (Abdul Rahman, Adul Hamid, & Nadarajah, 2021). This mechanism attempts to trap vital nutrients and maintain moisture levels (Costa, Raaijmakers, & Kuramae, 2018). Furthermore, fungicide was found to be more impactful under reduced moisture conditions compared to ambient in both NAGase and soil respiration. This may be the case due to the properties or structure of the soil interacting with the fungicide. According to Roy and others, low moisture in soils may cause hydrophobic surfaces, causing sorption of hydrophobic fungicides such as triazoles (Roy, Gaillardon, & Montfort, 2000) as well as azoxystrobin (Rodrigues, Lopes, & Pardal, 2013). In addition, wet soils tend to absorb less pesticides than dry soils due to their competition with water particles (Fishel, 2003). Thus, enhancing the effects of pesticides in soil. With these implications stated, soil activities and fungal biomass can be reduced with increments in temperature, reduced moisture, and the use of fungicides, interfering with carbon and nitrogen cycles.

As mentioned previously, with a decrease of soil moisture or water content in the soil, it is anticipated for enzyme production and activities to decline from substrate constrains (Stark & Firestone, 1995; Geisseler, Horwath, & Scow, 2011). This is also true for areas that experience a decrease in water availability from high temperatures (Fanin, et al., 2022). However, in this study, solely moisture levels were not identified to be significant. These results were similar to (Ladwig, Sinsabaugh, Collins, & Thomey, 2015). They discovered no significant changes in rainfall (e.g., large, infrequent, small, frequent quantities) on soil enzyme activities in Chihuahuan desert soils. A possible explanation may be the presence of a lag. A lag is described as a phase that allows microorganisms to store nutrients to adapt to new environments in order to

protect themselves from threats (Bertrand, 2019). This lag effect has also been described in drought ecosystems (Ji, et al., 2021). Another probable reason for the insignificance is perhaps the amount estimated for the reduction of moisture levels may be too minuscule to make an impact on assays.

Thus, this study suggests to not utilize azoxystrobin and tebuconazole to treat fungal diseases on crops or soil due to its suppressive effects on soil microbial activities. It is recommended to utilize holistic alternatives or approaches to not only assist in crop diseases but also support healthy soil ecosystems and sustainable agriculture. Such recommendations include:

- Using organic certified fungicides such as sulfur, and bio-fungicides such as Serenade (eCFR, 2022; OMRI, 2022). These two fungicides are low in toxicity (EPA, 2022; EPA, 1991). Sulfur can be utilized within the LRGV region based on a study with similar climate conditions (Atizaz, et al., 2020) and its ability to work under higher temperatures (Koike & Tjosvold, 2020). However, it is noted that plants may be susceptible to damage with excess heat (exceeding 32.2°C (90°F)). Although there are limited studies of using Serenade in semi-arid regions, its main biological ingredient, *Bacillus subtills*, thrives under 25 to 37 °C conditions and pH of 5 to 9 (Sidorova, Asaturova, Homyak, Zhevnova, & Shternshis, 2020). In addition, these two fungicides may be used on major crops grown in the LRGV such as citrus, sorghum, watermelon, and beans (EPA, 2021; Bayer, 2020).
- Implementing farming tactics that reduces the targeted pathogen by using various genetically different crops on the field to minimize disease risk (Rottstock, Joshi, Kummer, & Fischer, 2014) and by adding appropriate compost (EPA, 1997).

Caveats

Limitations presented within this study consists of, recognizing the complexity of the outcomes from climate change, the lack of measuring the diversity of soil communities and utilizing a microcosm whereas a field approach. In reviewing these limitations, further research can be developed to enhance solutions for the future of agriculture sustainability and healthy soil systems.

Climate Change. Climate change consists of changes within the climate system's components. The climate system includes the atmosphere (e.g., surface temperatures and precipitation levels), hydrosphere, cryosphere (e.g., glacial mass loss), land surfaces, and biosphere, that constantly interact with one another and influenced by external forces (e.g., the sun) (Ahlonsou, Ding, & Schimel, 2018). Therefore, climate change is complex. For this study, only average rainfall, and temperature changes under the RCP 8.5 scenario for the LRGV were accounted. Although it is recognized that rainfall is predicted to decrease in the LRGV, hurricane system intensities are expected to enhance as a result from the warming of oceans (Knutson, 2022; Holland & Bruyere, 2014) as well as the amount of rainfall spells (EPA, 2016). On the same note, another limitation presented within this study is the use of models. Although the IPCC utilizes robust climate models (IPCC, n.d.), it can be subjected to change. Thus, this study is meant to help in estimating climate change effects on soil health with the use of fungicides under the RCP 8.5 scenario (AR5).

Microbial Communities. Bioindicators such as soil enzymatic activities and soil respiration serve as useful analysis to determine changes within the soil biome due to their sensitivity. However, no DNA analysis were completed to entail microbial diversity shifts across

treatments. In detecting changes in microbial communities, groups of phyla that were able to thrive under different conditions are revealed, reflecting the resiliency (e.g., soil health enhancers) or vulnerability (e.g., increase of a pathogenic phylum) of the soil (Onwona-Kwakye, et al., 2020). In addition, soil microbial diversity is often utilized to assess the impacts of pesticides on soil health (Wang, et al., 2020) and climatic changes. Therefore, in future studies, microbial diversity must be accessed.

Microcosm vs. Field Trials. Microcosms are practical for eliminating high variability within the natural environment (e.g., soil and microbial heterogeneity), controlling environmental conditions, (Stres, et al., 2008), along with their efficient manner to replicate and identifying responses from microorganism from stress (Beyers & Odum, 1993). However, microcosms come with tradeoffs. Such tradeoffs include excluding the behavior of chemicals in the natural environment and the responses of soil communities in environmental conditions. Studies have expressed azoxystrobin persistence or degradation may vary depending on soil moisture (e.g., flooding soil vs. non-flooding soils) and exposure to UV rays (Ghosh & Singh, 2009; Singh & Singh, 2010). Likewise, tebuconazole performance contrasts under sunlight circumstances (Carena, et al., 2022; Del Puerto, Goncalves, Medana, Prevot, & Roslev, 2022). Furthermore, soil activities may respond differently due to microclimatic variables in outdoor environments (Ranjard, et al., 2006; Salamanca, Kaneko, & Katagiri, 1997).

Conclusion

In summary, FDA hydrolysis was increased by interactive effects from tebuconazole and 28˚C, however, other indicators were suppressed or unchanged. This may indicate other processes that were enhanced but not measured in this study (Figure 16). In addition, with

increments of temperatures, β-glucosidase and phosphatase activities were reduced. Tebuconazole significantly decreased β-glucosidase and phosphatase activities while azoxystrobin drastically reduced rates in urease. Enzyme, NAGase, and soil respiration were significantly negatively impacted through interactive effects of higher temperatures, reduced moisture, and fungicide treatments. The decrease of microbial activities due to climatic variables and fungicide may impact nutrient availability to other microbial organisms and plants. Solely moisture reduction had no significant impact on soil microbial activities. Ramifications of this study does not recommend the use of azoxystrobin and tebuconazole due to its suppressive behavior towards microbial activities. Alternatives such as the use of organic fungicides and bio-fungicides, as well as, implementing holistic farming tactics should be considered. Future research should consider whether microbial functioning can withstand the long-term exposure to these disturbances, exploring adaptation mechanisms (e.g., shifts in microbial diversity) upon ecosystem-based strategies for prevention of crop diseases.

Table 2 General characteristics of experimental soil. Three replicates were used per soil characteristic. Soil texture was measured by a hydrometer.

Parameter	Value
Sand (0.05-2mm)	82%
Silt (0.002-0.05 mm)	10.33%
Clay (<0.002 mm)	8%
pH	6.6

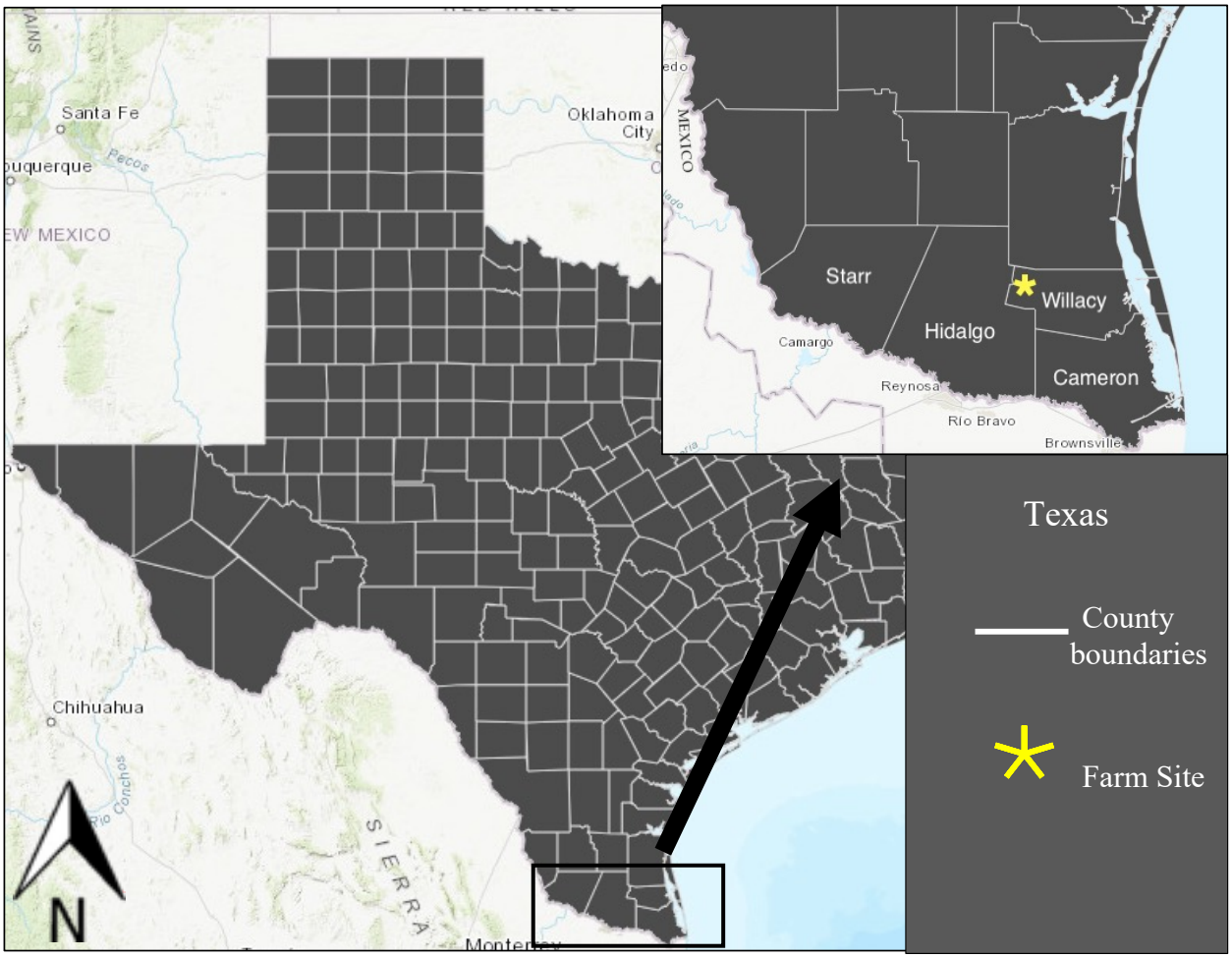

Figure 3 Map of the location of farm site (indicated with a yellow star). Farm site was located Raymondville in Willacy County, Texas, U.S.A. (26° 29'1.96" N, 97° 55'48" W). Source of map: Texas Department of Transportation, 2022.

Figure 4 Map of the 18-acre field from Raymondville in Willacy County, TX, U.S.A (26° 29'1.96" N, 97° 55'48" W), with respective soil type units: Delfina fine sandy loam (DfA), Hargill fine sandy loam (HaB), and Racombes sandy clay loam (Ra). DfA compromised 5.3 acres (29.4% of the field), HaB, 12.1 acres (67.3% of the field), and Ra, 0.6 of an acre (3.3% of the field). Source of soil data was extracted from (Soil Survey Staff, n.d.).

Table 3 List of experimental treatments according to fungicide (F), temperatures (T1 current averages; T2 future projections), and moisture (M1 ambient averages; M2 reduced averages) regimes. Fungicide and moisture levels were accommodated to microcosms.

Treatments	Fungicide	Temperature (C°)	Moisture
1	Control (F1)	24 (T1)	9% (M1)
2	Control (F1)	24 (T1)	8% (M2)
3	Control (F1)	28 (T2)	9% (M1)
4	Control (F1)	28 (T2)	8% (M2)
5	Azoxystrobin (F2)	24 (T1)	9% (M1)
6	Azoxystrobin (F2)	24 (T1)	8% (M2)
7	Azoxystrobin (F2)	28 (T2)	9% (M1)
8	Azoxystrobin (F2)	28 (T2)	8% (M2)
9	Tebuconazole (F3)	24 (T1)	9% (M1)
10	Tebuconazole (F3)	24 (T1)	8% (M2)
11	Tebuconazole (F3)	28 (T2)	9% (M1)
12	Tebuconazole (F3)	28 (T2)	8% (M2)

Table 4 Three-way analysis of variance (ANOVA) *p*-value output for the effects of fungicide (F), temperature (T), and moisture (M) on Fluorescein Diacetate (FDA) hydrolysis, β-glucosidase (BG), acid phosphatase (AciP), alkaline phosphatase (AlkP), N-acetyl-β-D-glucosaminidase (NAGase), urease (U), and soil respiration (SR).

Factors	FDA	BG	AciP	AlkP	NAGase	U	SR
F	-	<0.0001	0.0431	0.0183	<0.0001	0.0136	<0.0001
T	-	<0.05	0.0767	0.0129	<0.0001	-	<0.0001
M	-	-	-	-	<0.001	-	-
F x T	<0.001	-	-	-	0.0527	-	<0.0001
F x M	-	-	-	-	0.0201	-	<0.0001
T x M	-	-	-	-	-	-	<0.0001
F x T x M	-	-	-	-	<0.0001	-	<0.0001

(-) Not significant at p<0.1.

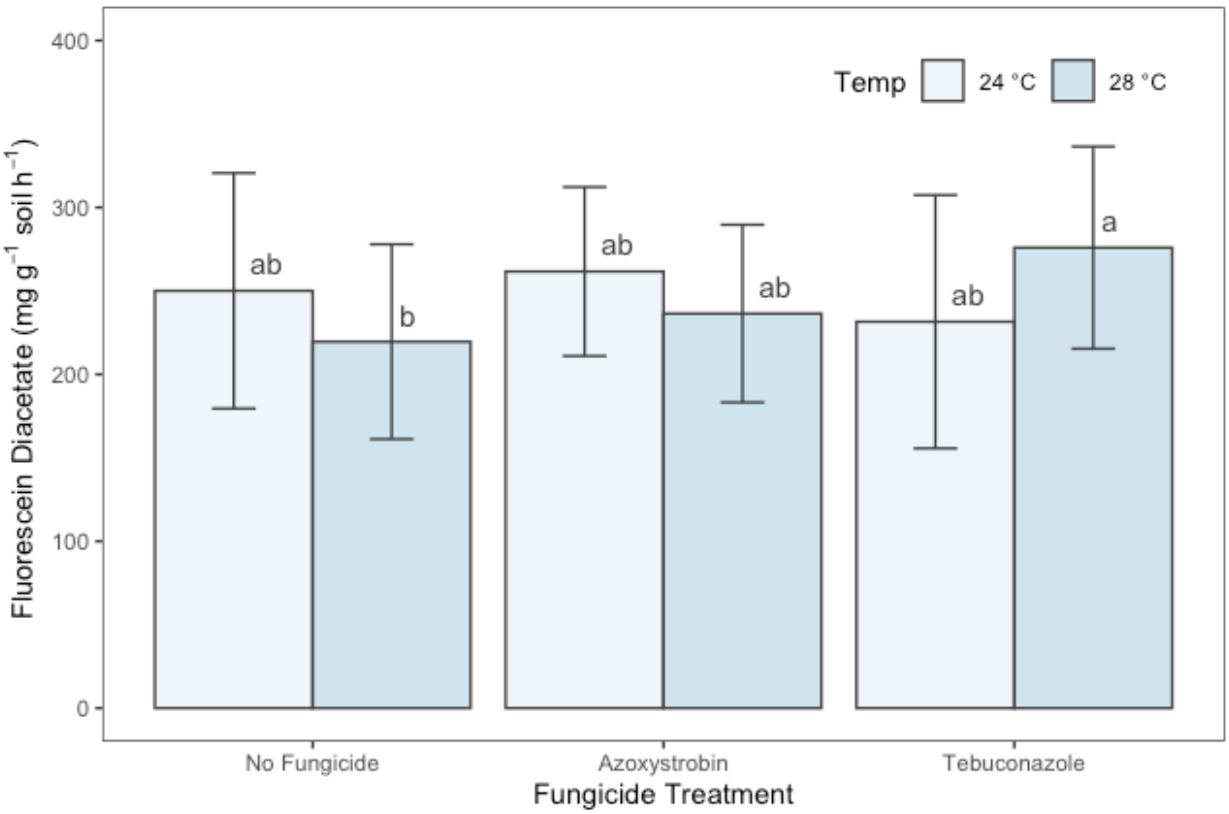

Figure 5 Effects of fungicide azoxystrobin and tebuconazole, and temperatures (24°C and 28°C) on Fluorescein Diacetate (FDA) hydrolysis. Error bars represent the standard deviation (n=24). Means followed by the same letters are not significantly different ($p < 0.05$).

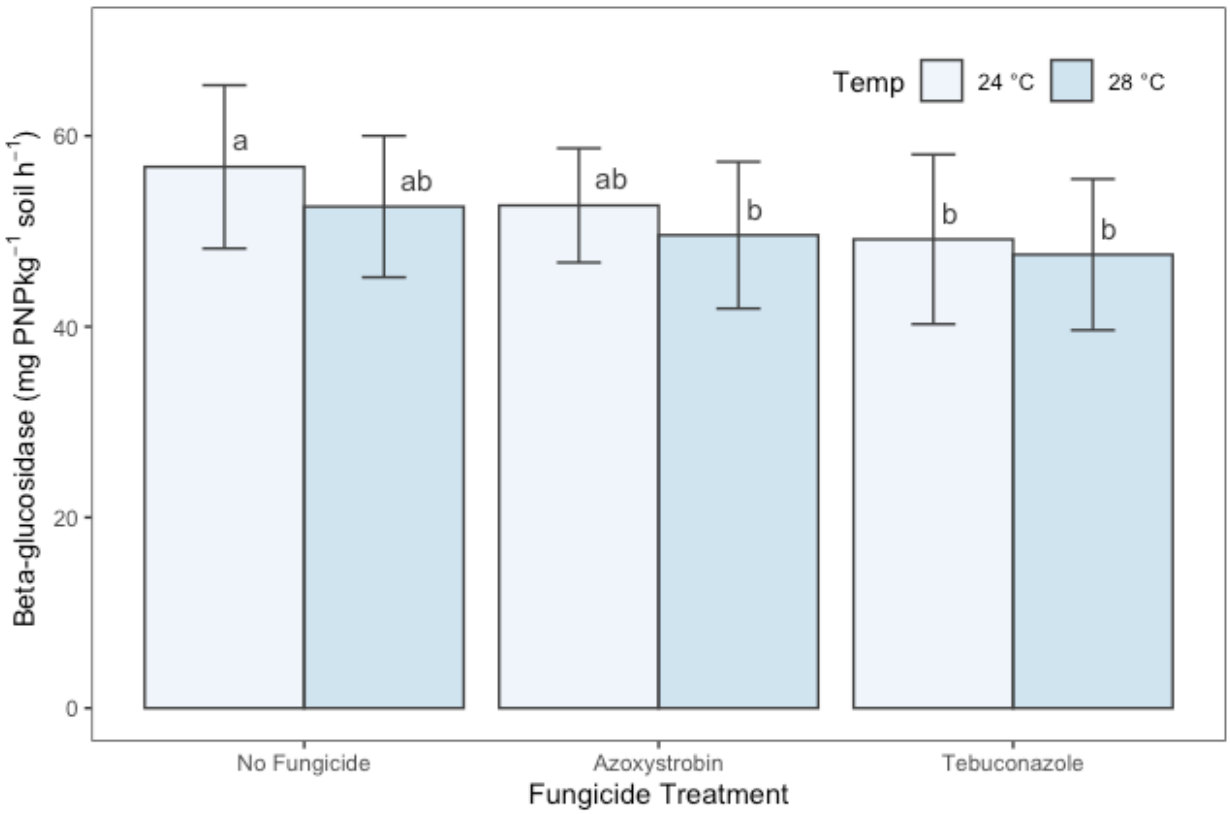

Figure 6 Effects of fungicides, azoxystrobin and tebuconazole, and temperatures (24°C and 28°C), on β-glucosidase. Error bars represent the standard deviation (n=24). Means followed by the same letters are not significantly different ($p< 0.05$).

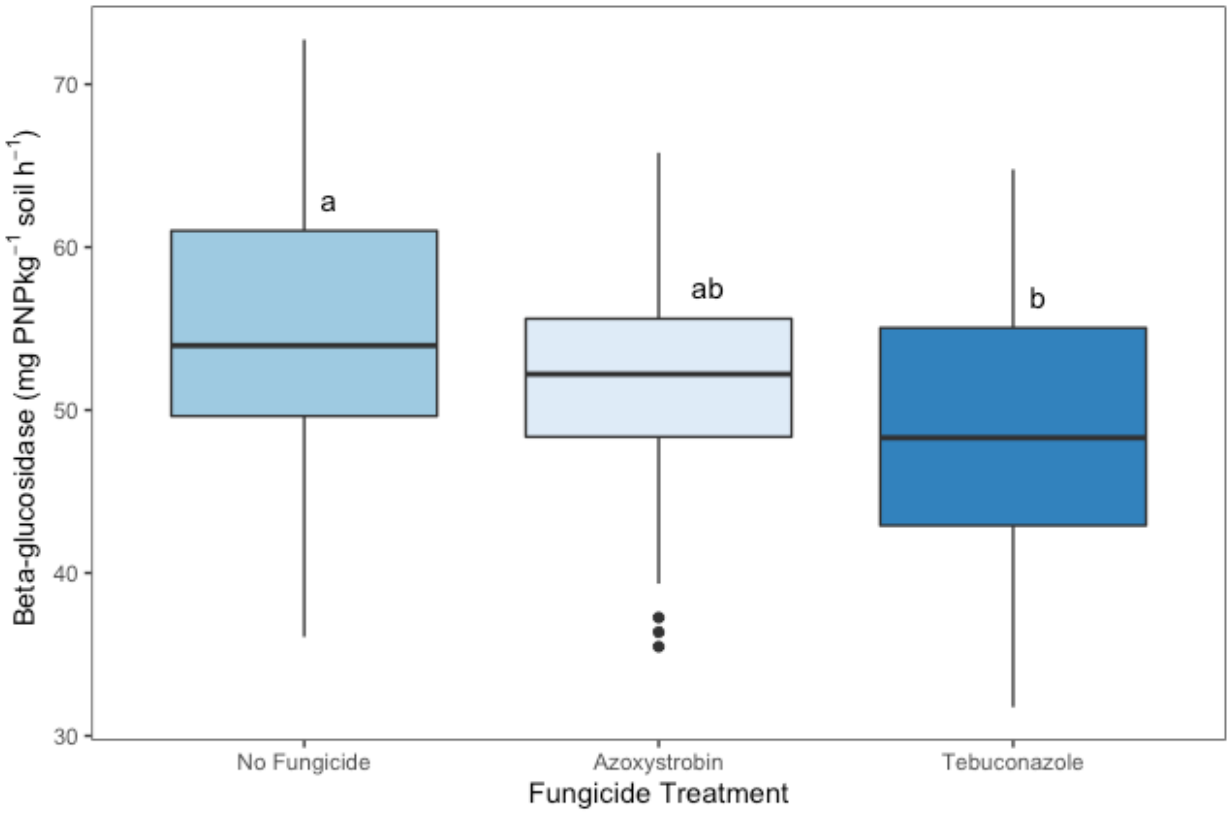

Figure 7 Fungicides, azoxystrobin and tebuconazole, effects on β-glucosidase. Error bars represent the standard deviation (n=48). Means followed by the same letters are not significantly different ($p < 0.05$).

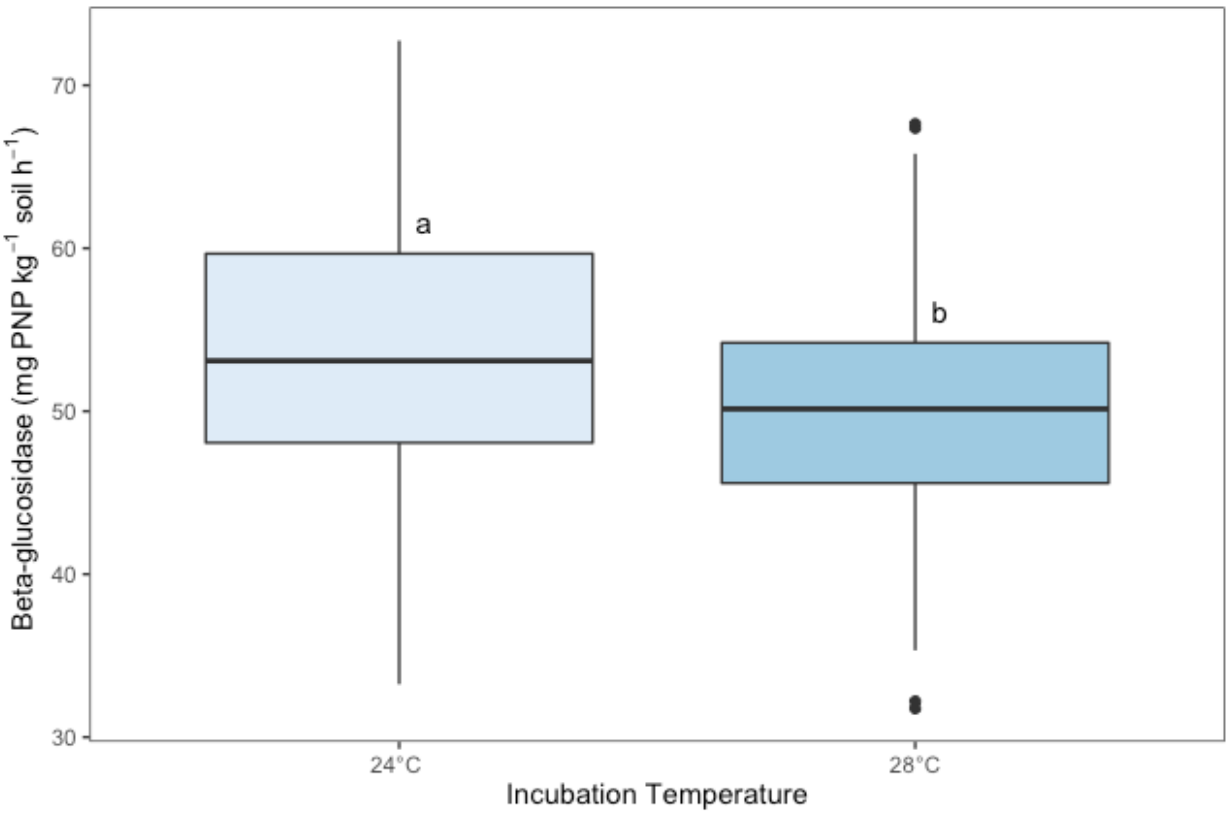

Figure 8 Incubation temperature effects, 24°C and 28°C, on β-glucosidase. Error bars represent the standard deviation (n=72).

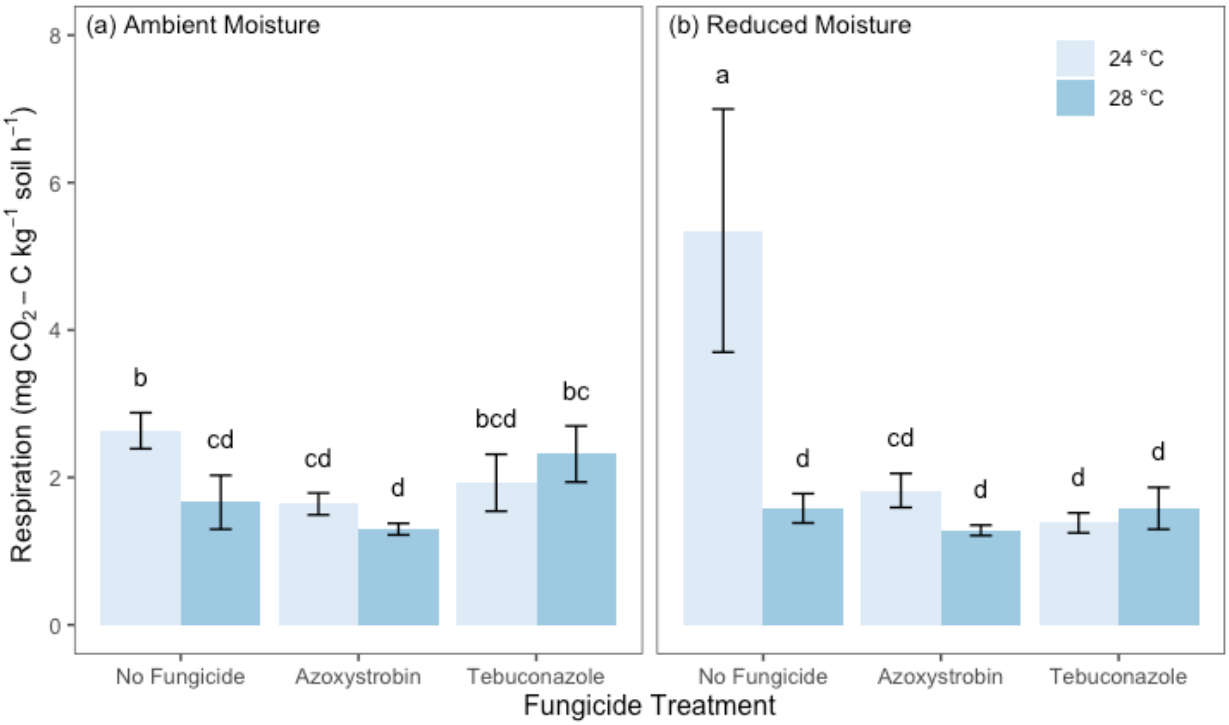

Figure 9 Effects of fungicides, azoxystrobin and tebuconazole, temperatures, 24°C and 28°C, and moisture treatments, ambient and reduced, on soil respiration. A) demonstrates temperature changes and fungicide effects under ambient moisture, while b) exhibits these effects under reduced moisture conditions. Error bars represent the standard deviation (n=12). Means followed by the same letters are not significantly different ($p < 0.05$).

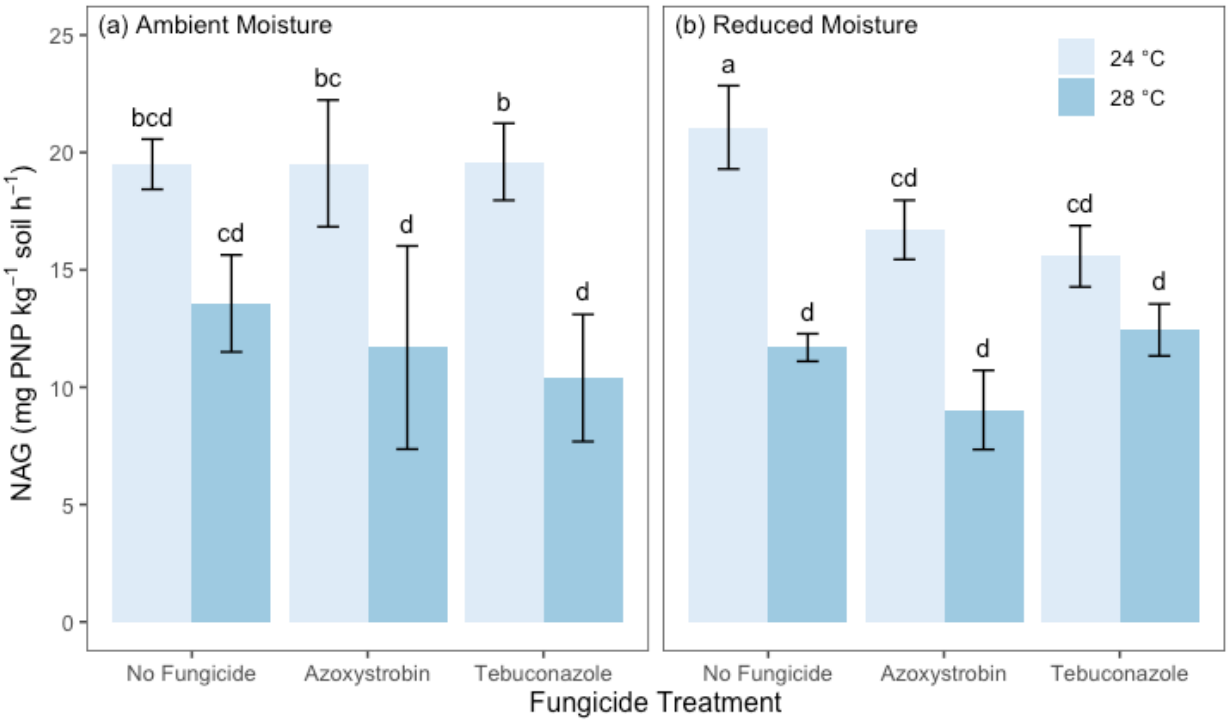

Figure 10 Effects of fungicides, azoxystrobin and tebuconazole, temperatures, 24°C and 28°C, and moisture treatments, ambient and reduced, on N-acetyl-beta-D-glucosaminidase (NAGase). A) demonstrates temperature changes and fungicide effects under ambient moisture, while b) exhibits these effects under reduced moisture conditions. Error bars represent the standard deviation (n=12). Means followed by the same letters are not significantly different ($p < 0.05$).

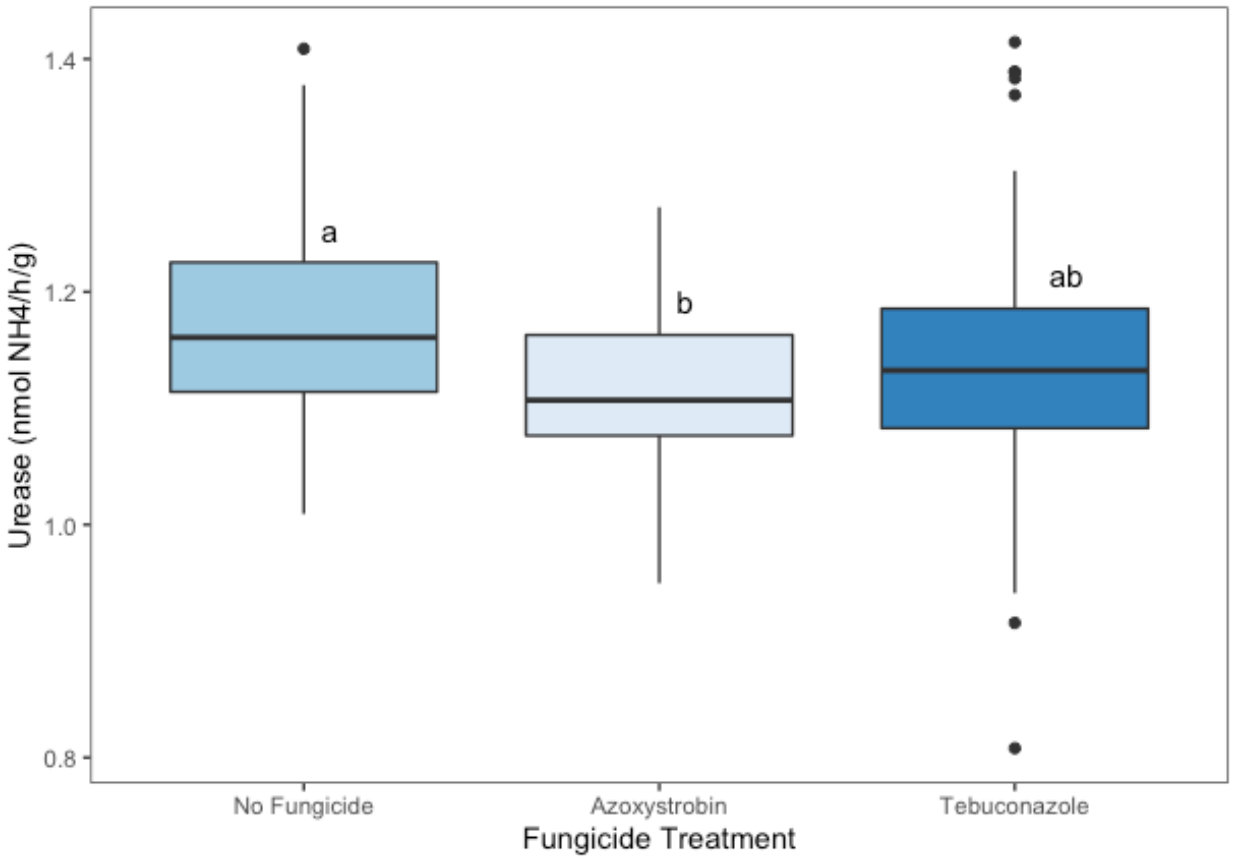

Figure 11 Fungicide treatments**,** azoxystrobin and tebuconazole, effects on urease activities. The error bars represent the standard deviation (n=48). Means followed by the same letter indicate no significant differences between treatments ($p<0.05$).

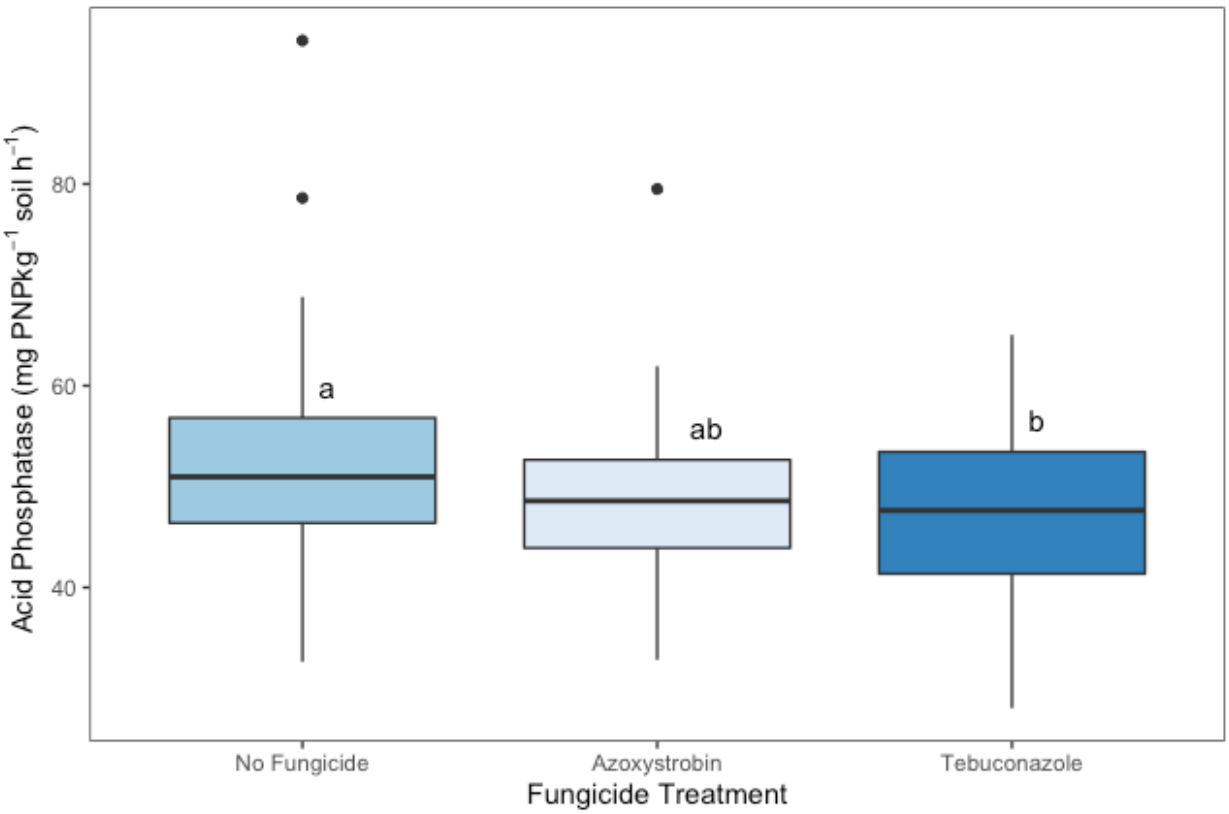

Figure 12 Fungicide treatments, azoxystrobin and tebuconazole, effects on acid phosphatase. The error bars represent the standard deviation (n=48). Means followed by the same letter indicate no significant differences between treatments ($p<0.05$).

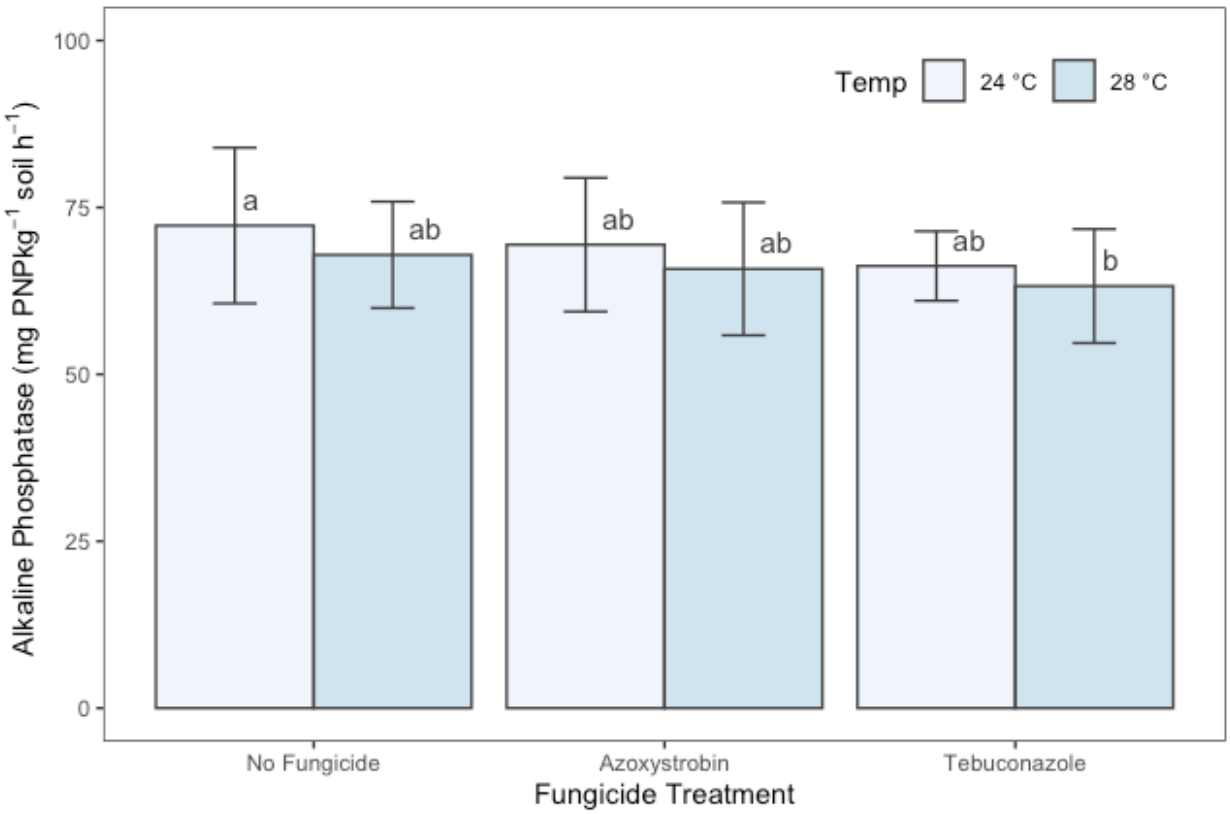

Figure 13 Effects of fungicides, azoxystrobin and tebuconazole, and temperatures (24°C and 28°C), on alkaline phosphatase. Error bars represent the standard deviation (n=24). Means followed by the same letters are not significantly different ($p < 0.05$).

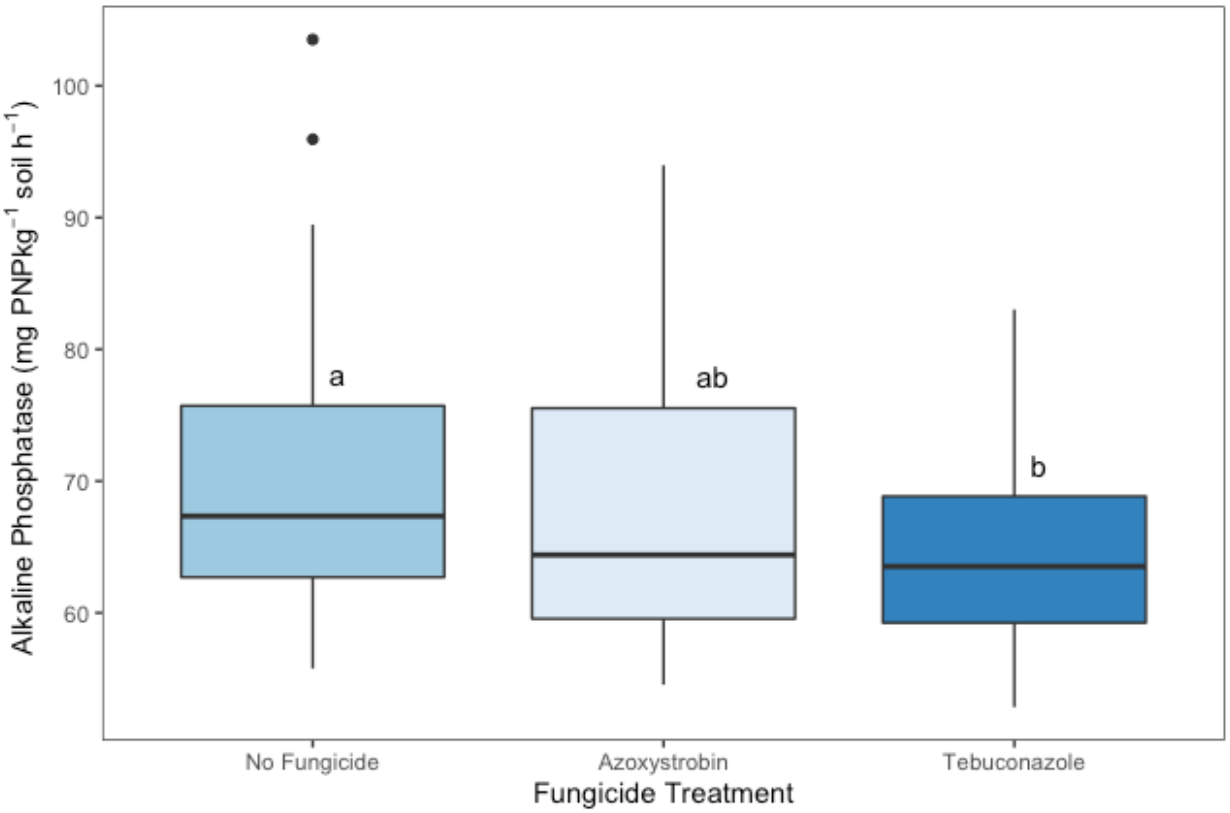

Figure 14 Fungicide treatments, azoxystrobin and tebuconazole, effects on alkaline phosphatase. The error bars represent the standard deviation (n=48). Means followed by the same letter indicate no significant differences between treatments ($p<0.05$).

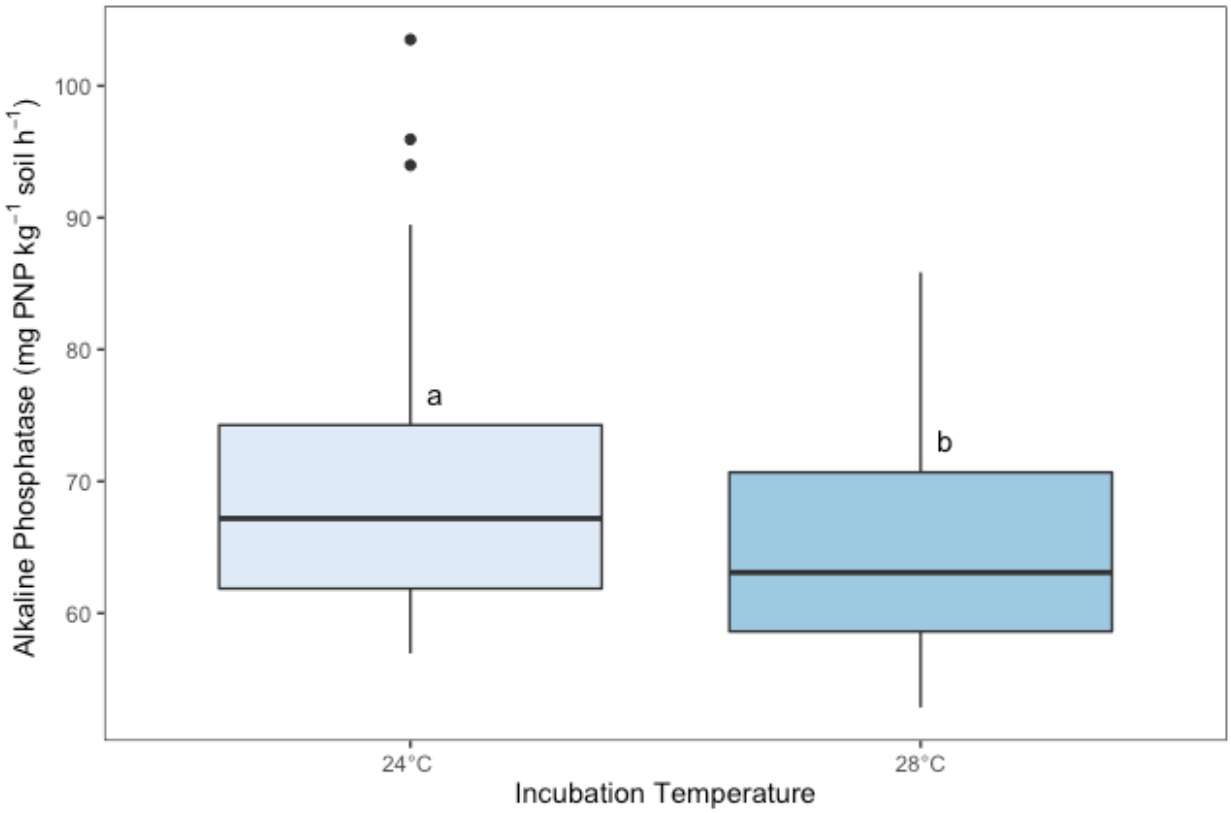

Figure 15 Incubation temperature effects, 24°C and 28°C, on alkaline phosphatase. Error bars represent the standard deviation (n=72).

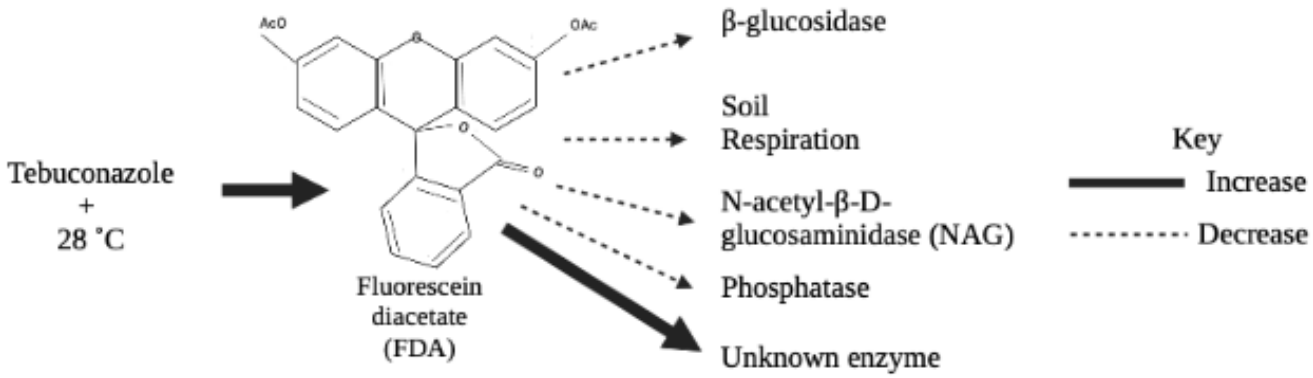

Figure 16 FDA hydrolysis impacts on enzymes from interactive effects of tebuconazole and higher temperatures (28°C). Thick arrows depict stimulative effects on enzymes, while dotted arrows are suppressive effects. Diagram was created with BioRender.

CHAPTER III

CONCLUSIONS

In this study, soil microbial enzymes (Fluorescein Diacetate (FDA) hydrolysis, β-glucosidase, N-acetyl-β--D-glucosaminidase (NAGase), urease, acid phosphatase, and alkaline phosphatase) and respiration were estimated under the Intergovernmental Panel on Climate Change (IPCC) Representative Concentrated Pathway (RCP) 8.5 climate change scenario (2070-2100) coupled with fungicides, azoxystrobin and tebuconazole, in the Lower Rio Grande Valley, Texas, U.S.A. In sum, this study found FDA hydrolysis was enhanced by interactive effects from tebuconazole and 28˚C, however, other indicators were suppressed or unchanged. This may indicate other processes that were enhanced but not measured in this study. In addition, with increments of temperatures, β-glucosidase and phosphatase activities were reduced. Tebuconazole significantly decreased β-glucosidase and phosphatase activities while azoxystrobin drastically reduced urease rates. Enzyme, NAGase, and soil respiration were significantly negatively impacted through interactive effects of higher temperatures, reduced moisture, and fungicide treatments. The decrease of microbial activities due to climatic variables and fungicide may impact nutrient availability to other microbial organisms and plants. Solely moisture reduction had no significant impact on soil microbial activities. Thus, alternatives such as the use of organic fungicides and bio-fungicides, as well as, implementing holistic farming tactics such as the inclusion of crop diversity and composting, should be considered. In addition,

results of this investigation served in delivering an insight on future changes of soil health under changes of climate and fungicides. It is then critical to protect and implement holistic management to aid crop production, the sustainability of agriculture, and healthy soil ecosystems. Future research should consider whether microbial functioning can withstand the long-term exposure to these disturbances, exploring adaptation mechanisms (e.g., shifts in microbial diversity) upon ecosystem-based strategies for prevention of crop diseases.

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BIOGRAPHICAL SKETCH

Armida Rivera graduated from South Texas College with an associate of interdisciplinary studies with Magna Cum Laude in May 2017. She then transferred her credits to the University of Texas Rio Grande Valley, where she obtained a bachelor's degree of science in environmental science with a minor in art May of 2020 with Summa Cum Laude. As an undergraduate student, Armida had the opportunity in working under the Global Change Ecology lab, where she assisted two graduate students on their thesis projects. One project consisted of an endangered endemic plant species (*M. walkerae*), climate change, and geographical distribution, while the other involved avian diversity associations with canals.

In August 2022, Armida earned her Master of Science in Agricultural, Environmental, and Sustainability Sciences at the University of Texas Rio Grande Valley and received the Presidential Graduate Research Assistantship Scholarship. As a graduate student, she focused on assessing the impacts of climate change and fungicide use on soil enzymes and activities in the Lower Rio Grande Valley to contribute to sustainability of food production.

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