Influence of climate change on crop growth and soil microbial functional potential

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Abstract

Climate change is expected to reshape agroecosystems by altering temperature and precipitation patterns, with cascading effects on soil processes, microbial communities, herbicide behavior, and crop performance. This study examined two field experiments conducted in a temperate agricultural system in Quebec, Canada: one investigating the effects of elevated soil temperature (+2.5 °C, TECHS), and the other testing ±30% altered rainfall treatments (DART). The study assessed responses in soil physicochemical properties, microbial abundance, greenhouse gas emissions, herbicide degradation, and plant physiological traits.

In the temperature experiment, elevated temperature led to modest reductions in soil moisture and a general trend toward increased CO_2 flux, although statistical significance was observed only mid-season. Soil pH remained stable throughout the season, and warming had no significant effect on nitrous oxide (N_2O) flux. Bacterial (16S rRNA) and fungal (28S rRNA) gene abundances showed no statistically significant changes under warming, although 16S rRNA abundance trended upward over time in heated plots. These results suggest moderate warming can influence microbial respiration and carbon cycling but may not significantly impact microbial abundance or nitrogen gas fluxes within a single season.

In the rainfall experiment, altered rainfall did not result in statistically significant differences in soil moisture, crop physiological performance, indicating strong physiological resilience of common beans under ±30% precipitation changes. Glyphosate degradation followed expected time-dependent declines, while AMPA concentrations remained relatively stable over time. However, neither glyphosate nor AMPA concentrations were significantly affected by rainfall treatments. Similarly, gene abundances of *goxA*, 16S rRNA, and 28S rRNA remained unaffected by rainfall, suggesting microbial degradation capacity and population size were stable under short-term rainfall manipulation.

Collectively, the findings from both experimental systems suggest that short-term, moderate environmental changes did not lead to statistically significant shifts in soil chemical properties, microbial abundance, herbicide degradation, or plant traits. These results highlight the potential resilience of temperate agroecosystems to moderate climatic variability and underscore the importance of long-term studies to assess cumulative impacts under ongoing climate change.

Résumé

Les changements climatiques ont supposés d'avoir un grand impact sur les agroécosystèmes en modifiant les régimes de température et de précipitations, avec des effets en cascade sur les processus du sol, les communautés microbiennes, le comportement des herbicides et la performance des cultures. Cette thèse a examiné deux expériences de terrain menées dans un système agricole tempéré au Québec, Canada: l'une portant sur les effets d'une élévation de la température du sol (+2,5 °C), et l'autre testant des traitements de précipitations modifiées de ±30 % (DART). Les deux études ont évalué les réponses des propriétés physico-chimiques du sol, de l'abondance microbienne, des émissions de gaz à effet de serre, de la dégradation des herbicides et des traits physiologiques des plantes.

Dans l'expérience sur la température, l'élévation thermique a entraîné une légère réduction de l'humidité du sol et une tendance générale à l'augmentation du flux de CO_2, bien que la différence n'ait été significative qu'à mi-saison. Le pH du sol est resté stable tout au long de la saison, et le réchauffement n'a pas eu d'effet significatif sur le flux de protoxyde d'azote (N_2O). Les abondances des gènes bactériens (16S rRNA) et fongiques (28S rRNA) n'ont montré aucun changement statistiquement significatif en réponse au réchauffement, bien qu'une tendance à l'augmentation de 16S rRNA ait été observée dans les parcelles chauffées. Ces résultats suggèrent qu'un réchauffement modéré peut influencer la respiration microbienne et le cycle du carbone, sans pour autant modifier de manière significative l'abondance microbienne ou les flux d'azote gazeux sur une seule saison.

Dans l'expérience sur les précipitations, les modifications des régimes pluviométriques n'ont entraîné aucune différence significative en matière d'humidité du sol ou de performance physiologique des haricots, indiquant une forte résilience physiologique à des variations de ±30 % de précipitations. La dégradation du glyphosate a suivi une baisse temporelle attendue, tandis que les concentrations d'AMPA sont restées relativement stables. Cependant, ni le glyphosate ni l'AMPA n'ont été significativement affectés par les traitements de précipitations. De même, les abondances des gènes *goxA*, 16S rRNA et 28S rRNA sont restées inchangées, ce qui suggère une stabilité de la capacité de dégradation microbienne et de la taille des populations microbiennes en conditions de manipulation hydrique à court terme.

Dans l'ensemble, les résultats des deux volets expérimentaux suggèrent que des changements environnementaux modérés à court terme n'ont pas entraîné de modifications significatives des propriétés chimiques du sol, de l'abondance microbienne, de la dégradation des herbicides ou des caractéristiques des plantes. Ces résultats mettent en lumière la résilience potentielle des agroécosystèmes tempérés face à une variabilité climatique modérée, et soulignent l'importance d'études à long terme pour évaluer les impacts cumulatifs dans un contexte de changement climatique continu.

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Contribution of Authors

Following the Guidelines Concerning Thesis Preparation issued by McGill University Graduate and Postdoctoral Studies. this thesis is written in manuscript format by Kangxu He. The author was responsible for experimental design and conducting all experiments, data collection, analysis, interpretation of data, and writing of the original draft. Prof. Shiv Prasher as thesis supervisor, and Dr. Mary-Cathrine Leewis as thesis co-supervisor, were actively involved at every stage of the study and provided scientific and technical advice. Dr. Ali Mawof and Dr. Jaskaran Dhiman were involved in this paper for thesis reviewing and data analysis guidance.

Prof. Shiv Prasher, Department of Bioresource Engineering, McGill University: Funding acquisition, Conceptualization, Project administration, Methodology, Resources, Supervision, Writing - review and editing.

Dr. Mary-Cathrine Leewis, Agriculture and Agri-Food Canada: Project administration, Methodology, Supervision, Writing - review and editing.

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Table of Contents

List of Tables

List of Figures

List of Equations

Acronyms

16S rRNA: 16S Ribosomal Ribonucleic Acid

28S rRNA: 28S Ribosomal Ribonucleic Acid

AMPA: Aminomethylphosphonic acid

CAM: Crassulacean Acid Metabolism

CH_4: Methane

CO_2: Carbon Dioxide

DART: Drought and Altered Rainfall Treatment

DAS: Days After Sowing

ECD: Electron Capture Detector

EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase

FID: Flame Ionization Detector

GBH: Glyphosate-Based Herbicides

GHG: Greenhouse Gas

goxA: Glyphosate Oxidoreductase A Gene

HR: Herbicide-Resistant

NDVI: Normalized Difference Vegetation Index

N_2O: Nitrous Oxide

NOAA: National Oceanic and Atmospheric Administration

qPCR: Quantitative Polymerase Chain Reaction

RCP: Representative Concentration Pathway

sarc: Sarcosine Oxidase Gene

SSP: Shared Socioeconomic Pathway

THFA: Tetrahydrofolate

VWC: Volumetric Water Content

WHC: Water Holding Capacity

1. Introduction

Scientists have observed unprecedented global warming and climate change in recent decades (Abbass et al., 2022). According to the National Oceanic and Atmospheric Administration (NOAA) 2023 Annual Climate Report, the land and ocean temperature has increased at an average rate of 0.06℃ per decade since 1850, or about 1.1°C in total. The rate of warming since 1982 is more than three times as fast, i.e., 0.20°C per decade (NOAA, 2023). Anthropogenic emissions of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) have made significant contributions to global warming since the pre-industrial period (Jones et al., 2023).

Global warming can also influence the intensity and frequency of precipitation. Increased atmospheric heating leads to higher rates of evaporation and transpiration, adding more moisture to the atmosphere, which can result in an overall rise in precipitation (Trenberth, 2011). It is estimated by Trenberth (2011) that for every one degree Celsius increase in temperature, the air's capacity to hold water vapour rises by approximately 7%. This increased evaporation can result in increases in extreme events such as floods or droughts, depending on the specific geographic areas (Pizzorni et al., 2024). For instance, in the southwestern United States, regions like California have experienced shifts from consistent seasonal rainfall to irregular heavy downpours, followed by prolonged dry periods (Center for Climate and Energy Solution, 2024).

Increased temperatures, shifting precipitation patterns and an increase in frequency and intensity of extreme events associated with climate change will bring both challenges and opportunities to the agricultural sector (Government of Canada, 2020). Mirzabaev et al. (2023) noted that climate change could reduce the productivity of crops by modulating water availability and quality, causing heat stress, shifting phenology, and altering the pests and disease environment. However, in some regions, farmers can cultivate warmer-weather crops and benefit from a longer growing season with fewer cold weather events that could damage crops (Government of Canada, 2020). Studies show that without adaptation, climate change can reduce crop yields by an average of 11% (Hasegawa et al., 2022). However, adaptation strategies - such as improved irrigation techniques, drought-resistant crop varieties, optimized planting schedules, and soil management practices—can mitigate these losses, reducing the decline to 4.6% (Hasegawa et al., 2022). Several independent studies have claimed that drought and heat are the major abiotic stresses that reduce

crop yields by as much as 50% and weaken regional as well as global food security (Lamaoui et al., 2018; Reza and Sabau, 2022).

Changes in temperature and rainfall significantly influence soil microbial activity, which in turn affects herbicide degradation and greenhouse gas (GHG) emissions. Elevated temperatures generally enhance microbial activity, potentially accelerating the breakdown of organic compounds, including herbicides (Goiun et al., 2013). However, low soil moisture can restrict microbial growth and activity, slowing biodegradation, while excessive moisture can limit oxygen availability, hindering aerobic degradation (Howard and Howard, 1993). Since soils act as both a source and a sink for GHGs, and microbial processes regulate trace gas production and consumption, understanding how temperature and moisture fluctuations impact microbial activity is essential for sustainable agriculture and climate resilience (Tariq et al., 2024; Wang et al., 2022).

Given the profound impact of climate change on temperature, precipitation, and soil microbial processes, a deeper understanding of these interactions is essential for predicting plant growth and soil health under future environment conditions. While previous research has predicted some aspects of climate-induced changes in crop productivity, soil microflora, and greenhouse gas emissions, significant gaps remain - these projections are often only based on crop models, driven by a limited number of climate scenarios, and thus may not have considered the wide range of realistic uncertainties associated with both climate and crop models (Qian et al., 2019). To address these gaps, this study aims to provide a comprehensive assessment of how elevated temperatures and altered rainfall patterns influence crop growth, soil microbial abundance, herbicide degradation, and greenhouse gas emissions in agricultural systems. Furthermore, it seeks to elucidate the connections between microbial communities and soil greenhouse gas emissions, as well as the link between herbicide concentrations and the abundance of pesticide-degrading genes. By conducting controlled field experiments and detailed microbial and chemical analyses, this research will generate valuable empirical data to improve our understanding of climate-driven changes in agricultural soil systems.

The objectives of this study were to:

(1) Assess the effects of elevated temperature (+2.5 °C) on soil greenhouse gas (GHG) emissions, soil bacterial and fungal abundance, and key soil physicochemical properties (e.g., pH, moisture content, and organic carbon); and

(2) Evaluate the effects of varying rainfall patterns (−30%, ambient, and +30%) on plant growth, glyphosate degradation, and microbial community abundance.

The corresponding hypotheses of this study were:

(1) H_0: elevated temperature (+2.5 °C) has no effects on soil GHG emissions, microbial abundances, and other physicochemical properties.

H_1: elevated temperature (+2.5 °C) has effects on soil GHG emissions, microbial abundances, and other physicochemical properties.

(2) H_0: Varying rainfall patterns (−30%, ambient, and +30%) have no effects on plant growth, glyphosate degradation, and microbial community abundance.

H_1: Varying rainfall patterns (−30%, ambient, and +30%) have effects on plant growth, glyphosate degradation, and microbial community abundance.

2. Literature Review

2.1 Climate Change Scenarios and Food Safety

Global temperature change predictions vary depending on the global climate model and the shared socioeconomic pathway (SSP) scenario used for simulations (Scafetta, 2016). For example, Scafetta (2016) stated that based on the most likely SSP according to the current policies reported by the International Energy Agency, global surface warming in the 21st century will likely be moderate, staying below 2.5–3.0°C and averaging under the 2.0°C threshold compared to pre-industrial levels. Nevertheless, Song et al. (2023) indicated that if no action is taken to curb anthropogenic greenhouse gas emissions, the global average temperature would rise to an estimated 3.28 °C (2.46–4.10 °C) above pre-industrial levels.

The impacts of climate change are also influenced by region due to geographic and environmental differences. As shown in Figure 1, the northwest and northern regions of Canada—which include Yukon, Northwest Territories, northern British Columbia, and Alberta, as well as Arctic areas like Nunavut and northern Quebec—experienced the most warming, with annual temperatures rising over 3°C from 1948 to 2016 (the Government of Canada, 2019). This accelerated warming in northern regions is attributed to the phenomenon of Arctic amplification, where the loss of snow and ice cover reduces surface albedo, leading to greater absorption of solar radiation and further warming (Bush and Lemmen, 2019). In contrast, the southeast, including Ontario, Quebec, and the Atlantic provinces, experienced the least warming, with increases below 1°C (the Government of Canada, 2019). According to climate models, future warming in Canada is largely dependent on greenhouse gas emissions, with Representative Concentration Pathways (RCPs) outlining different scenarios. Under RCP2.6, a low-emission scenario with strong mitigation efforts, annual mean warming is expected to stabilize at 1.8°C above the 1986–2005 reference period by 2050, assuming stringent climate policies, a shift to renewable energy, and carbon sequestration (Bush and Lemmen, 2019). In contrast, RCP8.5, a high-emission scenario where fossil fuel use continues unchecked, projects ongoing warming throughout the century, reaching 6.3°C by 2100 (Bush and Lemmen, 2019). Northern Canada is expected to continue experiencing stronger warming than the rest of Canada, especially in the winter (the Government of Canada, 2019).

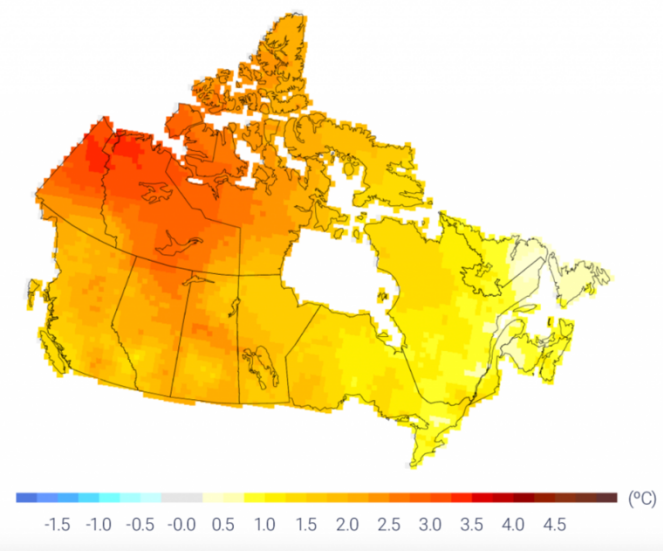

Figure 1. *Observed changes (°C) in annual temperature between 1948 and 2016 (the Government of Canada, 2019).*

Precipitation changes also vary significantly across Canada. The Prairie provinces, such as Alberta, Saskatchewan, and Manitoba, have experienced increased variability in precipitation, with more frequent droughts and reduced soil moisture (Mekis et al., 2020). In contrast, the Atlantic provinces have seen a rise in annual precipitation and more intense rainfall events, leading to increased flooding and coastal erosion (Environment and Climate Change Canada, 2023). Similarly, British Columbia has faced a combination of heavier winter precipitation in coastal areas and prolonged droughts in interior regions, exacerbating risks of wildfires and water scarcity (Zhang et al., 2019). Future projections indicate that by 2081–2100, precipitation will increase across most of Canada, particularly in the North, where annual precipitation is expected to rise by 9.4% under a low emission scenario (RCP2.6) and up to 33.3% under a high emission scenario (RCP8.5) (Government of Canada, 2019). In southern Canada, precipitation is generally projected to increase, but under a high emission scenario, summer precipitation may decline in some southern regions, increasing drought risks (Government of Canada, 2019).

According to Janni et al. (2024), global agricultural production must double by 2050 to meet the demands of an increasing world human population, while changes in temperature and precipitation patterns due to climate change are expected to significantly affect crop quality and quantity (Mirzabaev et al., 2023). Canada presently plays a crucial role in global food supply, acting as the world's fifth largest exporter of agriculture and agri-food products after the EU, the US, Brazil,

and China (Agriculture and Agri-Food Canada 2016). Rising temperatures, shifting growing seasons, and extreme weather events such as droughts and floods pose significant challenges to agricultural productivity in Canada. Therefore, it is essential to investigates the impacts of temperature and precipitation changes on crop growth in Canada, aiming to identify region-specific vulnerabilities and opportunities to enhance agricultural resilience, ensuring the stability of global food systems in a changing climate.

2.2 How Elevated Temperature Effect Crop Growth

Rate of plant growth and development is dependent upon the temperature surrounding the plant and each species has a specific temperature range represented by a minimum, maximum, and

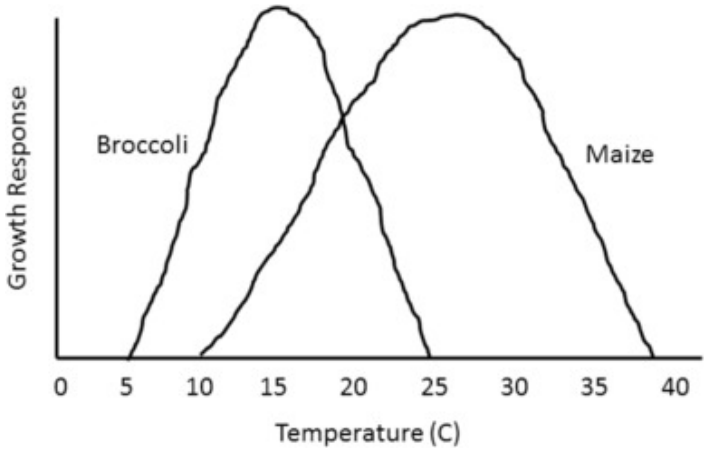

Figure 2. *Temperature response for maize and broccoli plants showing the lower, upper and optimum temperature limits for the vegetative growth phase (Hatfield and Prueger, 2015).*

optimum (Hatfield and Prueger, 2015). If temperatures exceed the species-specific maximum, plant productivity declines due to a concert of reasons, including disrupted pollination, reduced grain yield, accelerated senescence, and increased vulnerability to water stress (Hatfield and Prueger, 2015). Different crop species and genotypes vary in their optimal growth temperatures and heat tolerance throughout their lifecycles (Chen et al., 2021). For example, as shown in Figure 2, an extreme temperature event for maize will be warmer than for a cool season vegetable (broccoli) where the maximum temperature for growth is 25 °C compared to 38 °C.

The optimal temperature for plant growth not only varies across different species but also fluctuates throughout various growth stages and is influenced by geographic and environmental conditions. For example, in spring wheat, the optimal temperatures for heading, anthesis, and grain

filling are approximately 16 ± 2.3°C, 23 ± 1.75°C, and 26 ± 1.53°C, respectively (Khan et al., 2020). Similarly, in soybeans, optimal temperatures range from 15–22°C during emergence, 20–25°C at flowering, and 15–22°C at maturity (Liu et al., 2007). However, these values are not absolute and can vary based on latitude, altitude, soil moisture, and local climate conditions, as plants adapt their thermal thresholds to their growing environment.

As two of Canada's most economically significant crops, spring wheat and soybeans play a crucial role in global food security and trade. Spring wheat, predominantly grown in the Prairie provinces, is essential for bread and pasta production, while soybeans, cultivated mainly in Ontario, Quebec, and Manitoba, is a major source of protein, livestock feed, and biofuel, and contributes to soil fertility through nitrogen fixation (Statistics Canada, 2023; Agriculture and Agri-Food Canada, 2023). Given ongoing climate shifts, understanding how these crops respond to rising temperatures is essential, as temperature extremes could alter their growth cycles, reduce yield stability, and affect overall crop quality.

2.2.1 Temperature Impacts on Crop Physiological Traits

Temperature plays an important role in plant physiological responses by influencing changes in the hydraulic system, turgor pressure, water potential, and stomatal opening. These changes, in turn, regulate transpiration, photosynthetic activity, and other essential physiological processes (Asaari et al., 2022). Previous studies revealed that heat stress decreases photosynthetic efficiency while increasing respiration and photorespiration rates and can affect reproductive development (Moore et al., 2021).

Photosynthesis is highly sensitive to temperature fluctuations, as it directly influences enzymatic activity, carbon assimilation, and energy transfer within chloroplasts (Moore et al., 2021). Heat stress can disrupt chloroplast structure and thylakoid membranes, inhibiting electron transport and reducing photosynthetic pigment biosynthesis, ultimately lowering photosynthetic efficiency (Efeoglu and Terzioglu, 2009; Zhao et al., 2020). The impact of elevated temperature on photosynthesis also varies among plant species due to differences in their photosynthetic pathways—C3, C4, and Crassulacean acid metabolism (CAM) (Sage and Kubien, 2007). C3 plants, such as wheat and soybeans, fix CO_2 into a 3-carbon compound via Rubisco, making them more sensitive to temperature-induced Rubisco inefficiency. In contrast, C4 plants, such as maize and sorghum, use an additional carbon-concentrating mechanism that enhances photosynthetic

efficiency at higher temperatures (Yamori et al., 2013; Wang et al., 2012). CAM plants, including orchids and pineapples, adopt a water-conserving CO_2 fixation strategy at night, making them better adapted to arid conditions (Yamori et al., 2013). As shown in Figure 3, it was indicated that C4 plants exhibit a higher optimal temperature for photosynthesis and greater maximum photosynthetic rates compared to C3 species, while CAM plants typically function at lower optimal temperature values (Sage and Kubien, 2007; Yamori et al., 2013; Moore et al., 2021).

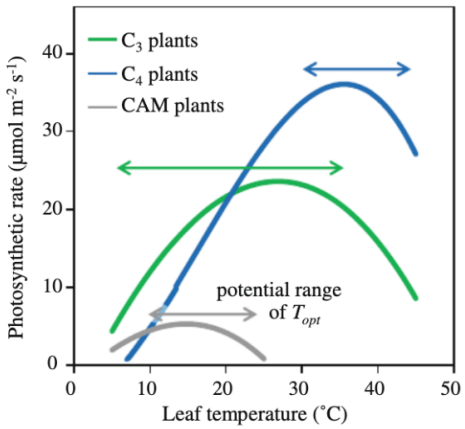

Figure 3. *Typical temperature responses of photosynthesis in C3, C4, and CAM plants (Yamori et al., 2013).*

Transpiration is a vital physiological process in plants where a portion of net radiation energy is converted into latent heat, regulated by stomatal aperture dynamics (Mathur et al., 2014). Stomatal responses to elevated temperatures are complex, as temperature not only influences stomatal conductance but also affects photosynthesis, vapor pressure deficit (VPD), transpiration rates, and plant water status, all of which interact to regulate stomatal behavior (Urban et al., 2017). Moore et al. (2021) described the non-linear relationship between temperature and stomatal response as "bell-shaped," where stomatal conductance initially increases with temperature up to a tipping point before sharply declining at higher temperatures. At extremely high temperatures, stomata may then reopen, potentially as a protective mechanism to facilitate evaporative cooling and mitigate excessive leaf heating, though this can also lead to increased water loss and dehydration stress (Lawson & Blatt, 2014).

Elevated temperatures significantly impact photosynthesis rate and stomatal conductance in spring wheat and soybeans, leading to physiological stress and potential yield reductions. As C3 crops,

both rely on Rubisco for CO_2 fixation, making them highly vulnerable to heat-induced inefficiencies such as increased photorespiration, reduced carbon assimilation, and lower water-use efficiency (Djanaguiraman et al., 2011; Yang et al., 2006). In spring wheat, photosynthesis rates decline sharply above 25–30°C due to Rubisco deactivation, impaired RuBP regeneration, and excessive transpiration (Wahid et al., 2007). Similarly, soybeans exhibits an optimal photosynthesis temperature of 28–30°C, beyond which chlorophyll degradation, reduced electron transport, and heat-induced oxidative stress limit photosynthetic capacity (Zhang et al., 2016). Heat stress also disrupts stomatal conductance in both crops, initially causing an increase in evaporative demand but leading to stomatal closure at temperatures above 30°C in wheat and 35°C in soybeans, restricting CO_2 uptake and reducing photosynthesis (Orr et al., 2021; Gilbert et al., 2011). However, under prolonged heat exposure (e.g., after 60 to 90 minutes at 37°C), wheat stomata may partially reopen to prevent excessive leaf overheating (Yang et al., 2006), whereas soybeans exhibits greater sensitivity to vapor pressure deficit (VPD), leading to more pronounced stomatal closure and increased drought susceptibility (Djanaguiraman et al., 2011).

While the physiological traits mentioned above are intrinsic characteristics of plants that regulate their growth, water use, and adaptation to environmental conditions, advancements in remote sensing and precision agriculture have introduced new ways to externally assess plant health. Vegetation indices, such as the Normalized Difference Vegetation Index (NDVI), is widely utilized as an indicator of vegetation cover, providing a precise assessment of vegetation growth, biomass, photosynthetic activity, and coverage (Tuoku et al., 2024). More specifically, NDVI is a measure of the ratio of reflectance in the near infra-red (NIR) and red wavebands as following formula, with NIR and red being reflectance values that vary between 0 and 100% (Stamford et al., 2023, equation [1]):

$$NDVI = \frac{NIR-RED}{NIR+RED} \quad [1]$$

NDVI values range from − 1 to 1, with higher values indicating greater vegetation vigor and greenness (Huang et al., 2021). NDVI responses to temperature stress vary across crop species, reflecting their distinct physiological adaptations and tolerance thresholds. For example, Cai et al. (2012) stated that increasing temperatures generally enhance NDVI in spring wheat by promoting growth and chlorophyll accumulation, but excessive heat stress (>32°C) accelerates senescence and chlorophyll degradation, leading to NDVI decline. The study also highlights a time-lag effect,

where temperature changes impact NDVI with a delay about one month (Cai et al., 2012). Unlike wheat, research on how elevated temperatures under climate change impact soybeans NDVI remains limited, probably because soybeans has a more complex leaf structure and canopy architecture than wheat, which can affect light absorption, shadowing, and makes NDVI trends in soybeans more difficult to standardize (Gilbert et al., 2011).

2.2.2 Temperature Impacts on Agronomic Traits

Rising temperatures associated with climate change will impact plant phenology, growth stage development, and ultimately crop yield. While various aspects of crop growth are affected, yield remains the primary concern for both producers and consumers (Sharma & Anandhi, 2020; Hatfield & Prueger, 2015). The response of crop productivity to temperature varies by species, depending on their cardinal temperature thresholds and geographic location. Research consistently shows that grain yields decline as temperatures rise, posing a significant challenge for global food production (Hatfield et al., 2011).

This decline in yield is closely linked to disruptions in key developmental stages, particularly during reproduction. The growth cycle of most crops includes germination, vegetative development, transition to flowering, reproduction, and senescence (Lippmann et al., 2019). Among these, the flowering and reproductive stages are the most vulnerable to elevated temperatures, as heat stress can impair pollen viability, shorten flowering duration, and increase sterility (Figure 4; Hatfield and Prueger, 2015; Mustahsan et al., 2024; Silva et al., 2025). One major factor in temperature-induced pollen decline is the premature degradation of the tapetum, which prevents the formation of the exine layer, a protective structure essential for pollen integrity (Lippmann et al., 2016). Without this layer, pollen protoplasts become highly susceptible to rupture, leading to severe fertility reductions (Lippmann et al., 2016). For example, Salem et al. (2007) found that elevated temperatures (38/30°C vs. 30/22°C) reduced pollen production by 34%, pollen germination by 56%, and pollen tube elongation by 33% in soybeanss, highlighting the detrimental effects of heat stress on reproductive success. Similarly, in wheat, temperatures exceeding 30°C during microsporogenesis impair pollen viability, disrupt tapetum function, and accelerate pollen sterility, ultimately reducing fertilization success and grain set (Khan et al., 2022).

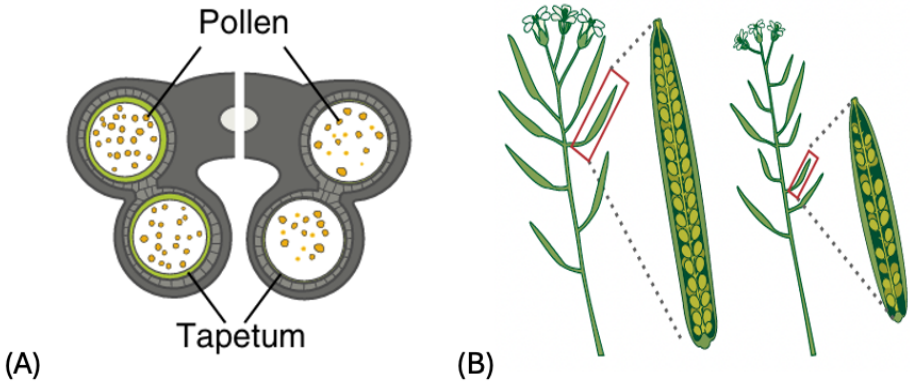

Figure 4. *(A) Pollen development under optimum (left) and warm (right) ambient temperature, resulting in correct (left) and incomplete (right) formation of the tapetum (green cell layer); (B) High temperature effects on fertilization displayed by fruit size and number of seeds per plant (Lippmann et al., 2016).*

High temperatures can lead to early maturity, reducing the plant life cycle duration, limiting the accumulation of biosynthetic products, and ultimately impairing grain development, yield, and overall productivity, as shown in Figure 4B (Khan et al., 2020). At the global scale, it was estimated that without crop improvement strategies, including genetic engineering and adaptation under CO_2 increases, substantial yield declines per °C of warming have been projected for the major cropping systems of maize (7.4%), wheat (6.0%), and soybeans (3.1%) (Zhao et al., 2017). However, future projections of global surface temperature vary significantly across different geographical regions, leading to distinct environmental conditions for plant growth. For example, elevated temperatures (0.8 ℃ higher than the ambient) are expected to cause 2.4% yield losses of soybeans (Glycine max) growing in the Southern U.S., but a 1.7% increase in yield in the Midwestern U.S. (Hatfield et al., 2011). Lobell and Asner (2003) found that temperature variations had distinct effects on soybeans yields in the Midwestern U.S. compared to the Northern Great Plains. In areas where yield declined with increasing temperatures, they estimated that for every 1°C rise in growing season temperature, the soybeans yields decreased by 17% (Lobell and Asner, 2003).

These region-specific impacts of climate warming are also evident in Canada, where its vast landmass spans multiple climate zones, leading to contrasting effects on agricultural productivity. The Prairie provinces (Alberta, Saskatchewan, Manitoba) face the greatest risk of yield losses for wheat due to rising temperatures, particularly at warming levels exceeding 2.5°C, which

accelerates heat stress, soil moisture depletion, and shortened growing periods (Qian et al., 2019). In contrast, Ontario and Quebec may experience some yield benefits for soybeans, driven by longer growing seasons and earlier planting dates, allowing for increased biomass accumulation and grain fill under moderate warming scenarios (Qian et al., 2019; McGinn et al., 2018). These findings emphasize the need for region-specific adaptation strategies to mitigate heat stress risks while leveraging potential benefits in temperate regions.

2.3 How Changing Soil Moisture Affects Crop Growth

Climate change not only influences temperature patterns but also significantly alters precipitation regimes, including drought and flood, which can impact crop growth by altering soil moisture availability. In the crop growth environment, soil water is essential for releasing, transforming, moving, and delivering nutrients to plants (Li et al., 2024). When soil moisture levels are optimal, crops can more easily absorb and utilize nutrients, promoting healthy growth and development. Figure 5 illustrates the effects of different soil moisture levels on plant health, ranging from severe drought conditions to optimal moisture levels and excess water stress. Changes in rainfall distribution, frequency, and intensity can either enhance or hinder crop growth, depending on the region and the crop's water requirements.

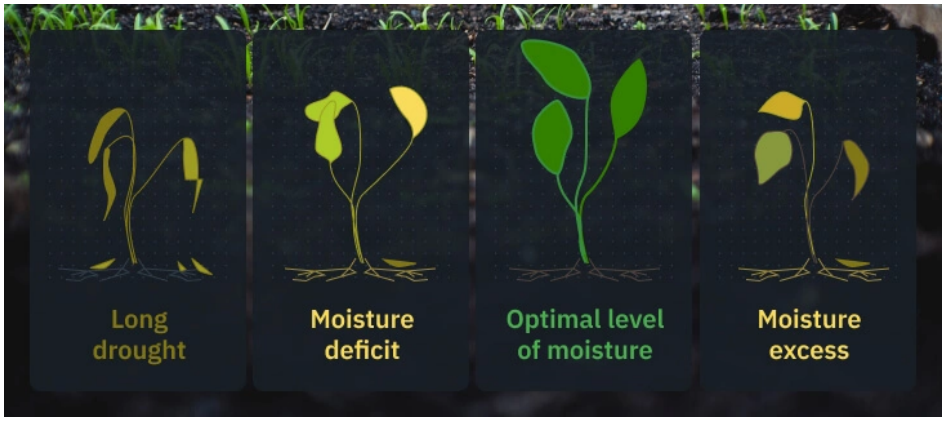

***Figure 5**. Effects of Soil Moisture Levels on Plant Health and Growth (Cherlinka, 2024).*

2.3.1 Soil Moisture Impacts on Crop Physiological Traits

Under water-deficient conditions, crops experience reduced soil water potential, limiting nutrient uptake and impairing physiological functions. Water-stressed plants typically exhibit stunted

growth, reduced leaf water content, lowered turgor pressure, and decreased transpiration rates (Figure 6; Zia et al., 2021). Prolonged drought further disrupts critical cellular processes, including protein synthesis, nitrogen assimilation, and cell membrane stability, ultimately compromising plant metabolism and productivity (Saneoka et al., 2004). Additionally, under drought conditions, crops adjust their physiology by reallocating resources to sustain water uptake. Wheat increases its root-to-shoot ratio by up to 40% to access deeper soil moisture, while soybeans enhances lateral root growth for improved water absorption (Fang et al., 2017; Prince et al., 2021). However, this shift reduces aboveground biomass, limits leaf area development, and delays flowering, ultimately decreasing photosynthetic capacity and grain yield (Vandoorne et al., 2012; Blum, 2011). Figure 6 illustrates the morphological, physiological, and molecular effects of drought stress on plants.

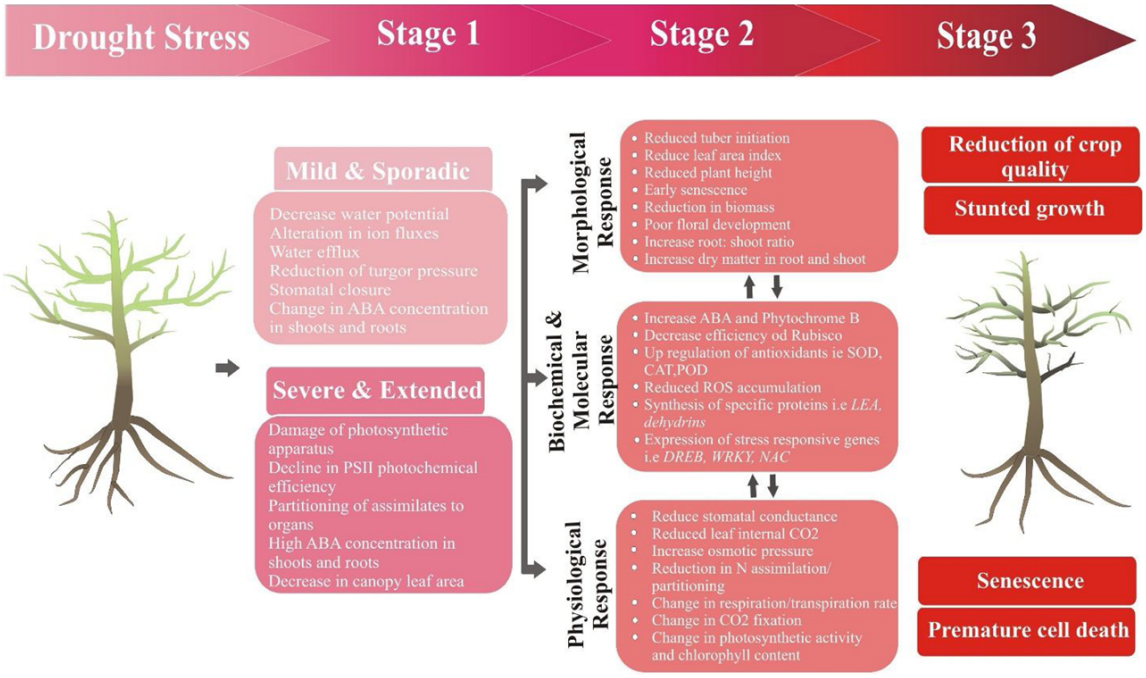

***Figure 6**. Effect of drought stress on morphological, biochemical and physiological functioning of the plant (Zia et al., 2021).*

Conversely, the prolonged rainfall or poor drainage can also cause soil to be waterlogged. Excess moisture conditions, including waterlogged soils, in the crop root-zone might limit the canopy cover due to poor aeration, reduced root and mitochondrial respiration (Ashton & Asante, 2021; Manghwar et al., 2024). Waterlogged soils also exhibit altered redox potential, resulting in the conversion of essential nutrients into forms that are less available for plant uptake, such as iron

and manganese toxicity or nitrogen loss due to denitrification (Kaur et al., 2019). Additionally, hormonal imbalances can occur, particularly with reduced cytokinin and increased ethylene production, which can delay reproductive development and impair photosynthesis (Manghwar et al., 2024). These effects are particularly detrimental during early vegetative and reproductive stages when root growth is crucial for yield formation.

Soil moisture variability influences key physiological traits in wheat and soybeans, including stomatal conductance, leaf water potential, and root development. For example, Wang et al. (2024) reported that, compared to soil with a volumetric water content (VWC) of 25.61%, wheat stomatal conductance decreased by 21% under moderate drought (18.01% VWC) and by 43% under severe drought (12.03% VWC), resulting in reduced photosynthetic efficiency. Wheat leaves adapt stomatal regulation strategies from anisohydric to isohydric in response to reduced soil water content (Wang et al., 2024). Similarly, Ohashi et al. (2006) found that soybeans (Glycine max) under drought stress, with soil moisture reduced from 35% to 15%, exhibited a 50% decline in stomatal conductance and a significant reduction in transpiration rate, leading to impaired carbon assimilation and overall growth inhibition.

Same as temperature, soil moisture plays a critical role in crop growth, affecting various physiological and biophysical properties that can be monitored using vegetation indices. For example, Rajanna et al. (2022) reported that wheat NDVI remained above 0.75 when soil moisture was maintained at 60% field capacity but declined to 0.45 when moisture dropped below 40%, indicating early senescence and chlorophyll degradation. Similarly, Crusiol et al. (2017) observed that soybeans NDVI decreased by 25% under drought conditions (30% field capacity) compared to well-watered plants (80% field capacity), corresponding with reduced leaf area index and biomass accumulation. The effects of soil moisture variability on NDVI are particularly pronounced during critical growth stages. For instance, soybeans NDVI reductions are more severe during reproductive stages, where water stress accelerates leaf senescence and limits pod development (Crusiol et al., 2017). In wheat, the impact of drought on NDVI is most evident during heading and grain-filling stages, where chlorophyll degradation and limited CO_2 assimilation reduce overall yield potential (Rajanna et al., 2022).

2.3.2 Soil Moisture Impacts on Agronomic Traits

Soil moisture is a key environmental factor regulating plant phenology, physiological processes, and final yield. It influences critical growth stages, including germination, vegetative growth, flowering, grain filling, and maturation, with drought stress and waterlogging significantly affecting nutrient uptake, biomass accumulation, and reproductive success. Understanding its impact on crop development is essential for optimizing irrigation strategies, enhancing crop resilience, and ensuring global food security under climate change.

The effects of drought stress on crop phenology and final yield are initially observed during germination and seedling establishment (Fahad et al., 2017). Reduced soil moisture availability delays or inhibits germination, resulting in poor seedling vigor, reduced root elongation, and lower shoot biomass accumulation (Kaya et al., 2006). Several studies have demonstrated that germination potential, early seedling growth, root and shoot dry weight, hypocotyl length, and overall vegetative development are significantly impaired under drought conditions (Fahad et al., 2017). For example, in common beanss (Phaseolus vulgaris L.), Geleta et al. (2024) stated that moisture deficits accelerate flowering by 5–7 days, shorten grain filling by up to 30%, and reduce seed size, leading to a 20–50% decline in yield.

In spring wheat, McMaster and Wilhelm (2003) also found that the general response of wheat to water stress was to reach growth stages earlier (i.e. to hasten development). The most significant response was for the grain filling period, while water stress had little effect on jointing and flag leaf complete/booting growth stages (McMaster and Wilhelm,2003). In addition, Tataw et al. (2016) found that future rainfall scenarios, characterized by less frequent but heavier rain events, significantly reduce wheat yield. Moreover, the interaction between rainfall and soil type influences early wheat development and harvest index, with sandy soils exhibiting the most pronounced negative effects due to lower water retention (Tataw et al., 2016), as shown in Figure 7. These results highlight the importance of soil type in determining crop's resilience to changing precipitation patterns.

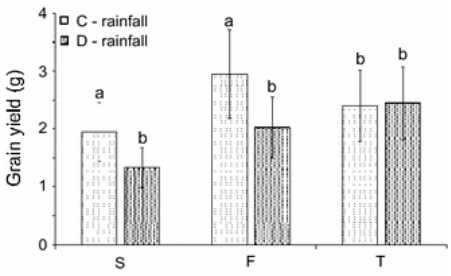

Figure 7. *Wheat grain yield (in g per plant) under current (C) and predicted (D) rainfall patterns on sandy calcaric phaeozem (S), gleyic phaeozem (F) and calcic chernozem (T) soil types. Different letters above bar pair indicate significantly different rainfall effects (P < 0.05) with a particular soil type. Mean ± SD, n = 3 (Tataw et al., 2016).*

Unlike drought stress, excessive soil moisture prolongs the vegetative phase by delaying flowering, grain filling, and overall crop maturity, disrupting the natural progression of plant development. During the grain-filling stage, poor assimilate translocation and premature leaf senescence under excess moisture conditions result in lower grain weight and reduced final yield (Liu et al., 2023). Studies have shown that wheat under prolonged waterlogging can experience a 20–50% reduction in grain yield, depending on the severity and duration of saturation (Malik et al., 2002). Likewise, it is reported that the excess moisture delays flowering by 4–10 days, prolongs the reproductive phase, and decreases pod set and seed number per plant by 15–40% for common beanss (Geleta et al., 2024).

2.4 Glyphosate in Agriculture

Glyphosate (N-Phosphonomethyl-glycine), first commercially introduced in the 1970s, quickly became one of the most popular herbicides due to its broad-spectrum weed control capabilities (Kanissery et al., 2019). Glyphosate-based herbicides (GBH), such as Roundup™, are now the most widely used pesticides globally (Panzacchi, 2018). They accounted for 58% of pesticides used in Canada's agricultural sector in 2017 (Madani & Carpenter, 2022; Bacon et al., 2023). Duke and Powles (2008) described glyphosate as a "once-in-a-century" herbicide, highlighting its unparalleled impact on modern agriculture. Glyphosate's herbicidal activity, effective against weeds and nearly all growing plants, is attributed to its ability to block the shikimic acid pathway by inactivating the key enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Myers et al., 2016; Godínez et al., 2021). This inhibition halts the biosynthesis of essential aromatic amino

acids (phenylalanine, tyrosine, and tryptophan), ultimately disrupting protein production and causing plant death within 1–3 weeks (Godínez et al., 2021). Around 1995, the technology of genetically modified herbicide-resistant (HR) crops was developed, mainly by encoding *cp4 epsps genes* for glyphosate-resistant EPSPS, which allows crops to tolerate glyphosate applications (Green, 2016). Almost 90% of all transgenic crops grown worldwide are glyphosate-resistant, and the adoption of these crops is increasing at a steady pace (Duke and Powles, 2008). This compatibility has significantly simplified weed management for farmers, making glyphosate an essential tool for crop production.

In addition to its prowess as an effective farming tool, glyphosate had long been considered the safest herbicide in the market as well (Gandhi et al., 2021). In the past few decades, it was perceived as a less toxic weed control alternative, safe for agricultural workers and non-target organisms (Godínez et al., 2021). However, research has increasingly shown that the accumulated glyphosate in the soil can adversely affect soil fauna that are involved in biogeochemical cycles, the mineralization of organic remains, and the degradation of other xenobiotics (Godínez et al., 2021). For instance, Pochron et al. (2020) observed that when 26.3 mg of pure glyphosate per kg of soil was applied, compost worms in the treated soil experienced a 14.8–25.9% reduction in biomass and survived a stress test for 22.2–33.3% less time compared to worms in uncontaminated soil. Additionally, humans exposed to this compound have presented multiple organ toxicity, nephrotoxicity, hepatotoxicity, gastrointestinal, cardiovascular, and respiratory effects, such as gastrointestinal disorders, cardiovascular diseases, and chronic degenerative diseases (Godínez et al., 2021). In 2015, the International Agency for Research on Cancer (IARC) classified glyphosate as a probable human carcinogen (Acquavella, 2023).

The environmental degradation of glyphosate can occur through both abiotic and biotic processes, such as absorption, photolysis, thermolysis, and biodegradation facilitated by catabolic enzymes (Singh et al., 2020). Understanding glyphosate degradation is important from environmental and health perspectives, as it provides insight into its persistence, transformation products, and associated risks. Soil microbial activity plays a crucial role in glyphosate breakdown, making it essential to explore how environmental factors influence this process (Chen et al., 2022). Climate variability, including rising temperatures, prolonged droughts, and increased rainfall, can significantly alter soil properties, affecting the ability of microorganisms to degrade glyphosate.

These changes may slow degradation rates, leading to greater glyphosate persistence in soils and an increased risk of environmental contamination. Addressing these challenges requires a deeper understanding of the interactions between climate variables and glyphosate-degrading microbes.

2.5 Glyphosate Chemical Composition and Degradation Pathways

2.5.1 Composition and Persistence

As shown in Figure 1, glyphosate is an amphoteric compound which contains a basic 2° amino group in the centre of the molecule with dibasic-phosphonic and monobasic-carboxylic acidic sites at the two ends (Knuuttila and Knuuttila, 1979). The formed zwitterionic structure can be reflected in high water solubility (11.6 g/L at 25℃) and poor solubility in organic solvents. Glyphosate has a distinct molecular structure compared to most herbicides. Unlike most (95%) herbicides that feature aromatic ring structures, glyphosate has a linear carbon chain with a relatively weaker bond, as illustrated in Figure 8 (NCBI, 2020; Gandhi et al., 2021). The glyphosate contained a direct carbon-to-phosphorus (C-P) bond, which is chemically stable (Wiersema et al., 1977). Therefore, the chemical process of degradation is less effective than microbiological degradation for glyphosate (Manogaran et al., 2018).

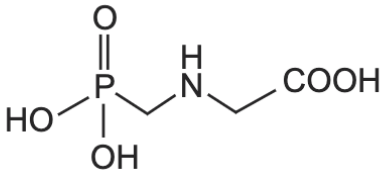

Figure 8. *The chemical structure of glyphosate (Patocka, 2018).*

2.5.2 Microbial Degradation

Glyphosate in the soil is predominantly catabolized by soil microorganisms, including bacteria, actinomycetes, and fungi; however, bacteria are the most frequently reported as involved in degradation (Godínez et al., 2021). The biodegradation of glyphosate by bacteria can generate the formation of metabolites that are used as a source of carbon, nitrogen, and phosphorus, which are essential for bacteria's development (Jensen et al., 2014; Sviridov et al., 2014; Godínez et al., 2021).

Bacteria can degrade glyphosate through two metabolic pathways. In the first pathway, the chemically inert C–P bond is cleaved by an intricate multienzyme complex known as C–P lyase with narrow specificity, yielding sarcosine and inorganic phosphorus (Sviridov et al., 2014). The sarcosine will be subsequently oxidized into the amino acid glycine, which is used directly for metabolism and microbial biosynthesis, and formaldehyde then enters the tetrahydrofolate (THFA) cycle (Godínez et al., 2021). For the second pathway, the herbicide molecule is first attacked by the enzyme known as glyphosate oxidoreductase (GOX), yielding stoichiometric quantities of AMPA and glyoxylate (Sviridov et al., 2014). AMPA can be further transformed with the help of the enzyme aminotransferase to phosphonoformaldehyde, which in turn is transformed by the enzyme phosphonatase into phosphate and formaldehyde which is also entered into the THFA pathway (Godínez et al., 2021). Another degradation pathway was observed in *Achromobacter* sp. Kg16, which utilized glyphosate as the sole phosphorus source, resulting in the production of acetylglyphosate, although the physiological role of this pathway remains unknown (Shushkova et al., 2016; Feng et al., 2020). The three biodegradation pathways of glyphosate in bacteria are shown in Figure 9.

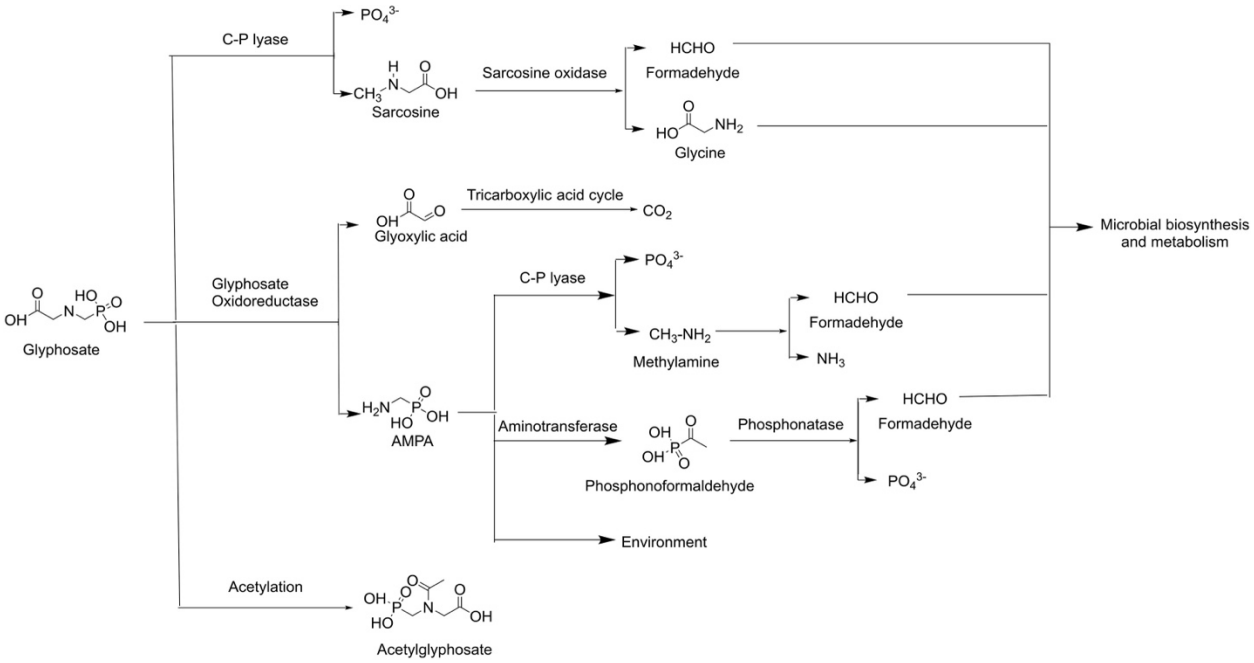

Figure 9. *Biodegradation pathways of glyphosate in bacteria (Feng et al., 2020).*

Current methods for quantifying glyphosate degradation rely on chromatography-based techniques (e.g., High-performance liquid chromatography coupled with mass spectrometry), which are labour-intensive and require costly equipment (Morales et al., 2020). In contrast, real-time quantitative polymerase chain reaction (qPCR) provides an affordable alternative to quantify gene copies (DNA) or their transcriptional expression (RNA), serving as a cost-effective proxy to determine glyphosate degradation potential in a given environmental sample (Morales et al., 2020). The 16S ribosomal RNA (16S rRNA) gene is a small subunit of rRNA that can be found in all bacteria and archaea, while the large subunit of rRNA, the 28S rRNA gene, is widely found in the study of algae and fungi (Tedersoo et al., 2024). The 16S rRNA and 28S rRNA genes are widely utilized in qPCR to quantify the abundance of bacterial, fungal, or algal populations. In contrast, functional genes such as *goxA* and *sarc* are directly involved in glyphosate degradation. The *goxA* gene encodes a glyphosate oxidoreductase, which forms AMPA as the primary metabolite of glyphosate, while the *sarc* gene encodes a sarcosine oxidase, which degrades sarcosine to glycine after its formation from glyphosate by a C-P lyase complex (Mäder et al., 2024). By quantifying the abundances of the two functional genes *goxA* and *sarc*, we were able to determine the genetic potential for glyphosate degradation (Wirsching et al.,2022).

2.6 Impact of Temperature on Microbial Activity and Glyphosate Degradation

The fate of glyphosate, including degradation, can be affected by soil physicochemical (texture, organic material content, pH) and biological properties (microbial community) or climatic conditions (Muskus et al., 2019). Under the trend of irreversible global warming, it is necessary to understand the influence of increasing temperatures on the environmental fate of at elevated temperatures. Additionally, learning how temperature affects glyphosate metabolism can also develop bioremediation-related technology. In general, previous research showed that glyphosate degrades faster in a warmer temperature (Benito et al., 2018).

Mineralization is the main mechanism of breakdown of glyphosate herbicide in the soil to basic inorganic components, such as carbon dioxide (CO_2), phosphate (PO_4^{3-}), and other simple compounds, and soil temperature is the main factor controlling the mineralization kinetics (Muskus et al., 2019; Rampoldi et al., 2008). An experiment conducted by Rampoldi et al. (2008) showed that for soybeans and corn crop residues, average glyphosate mineralization after 56 days of incubation at 15 and 28 °C was 3.9 and 9.9%, respectively, of the ^{14}C-glyphosate initially applied.

Wee et al. (2021) revealed that the half-life of glyphosate was 2.38 days at 20 °C and 1.69 days at 25 °C. Muskus et al. (2019) found that rising temperatures enhanced the mineralization of 13C glyphosate and $^{13}C_3{}^{15}N$ -glyphosate in both agricultural and tropical soils. In agricultural soils (21% clay, 68% silt, 11% sand), mineralization increased from 12–22% at 10 °C to 43–54% at 30 °C, while in tropical soils, it rose from 13–20% to 41–51% over the same temperature range (Muskus et al., 2019).

Various bacterial species have been identified as key contributors to this process, with their activity and efficiency closely tied to specific temperature ranges. Bacterial taxa implicated in the degradation of glyphosate are primarily mesophiles, which grow at moderate temperatures between 20 °C and 45 °C and with an optimum growth temperature in the range of 30–39 °C (Manogaran et al., 2018; Singh and Walker, 2006; Godínez et al., 2021; Schiraldi & Rosa, 2015). For instance, *Pseudomonas putida* and *Ochrobactrum* sp. GDOS, which are recognized as microorganisms capable of degrading glyphosate, were reported to have the highest bacterial growth at 30 °C (Hadi et al., 2013; Benslama and Boulahrouf, 2013). Meanwhile, Fan et al. (2012) noted that *Bacillus cereus* CB4 showed optimum degradation rates at 35 °C. Moreover, *Burkholderia vietnamiensis* strain AQ5-12 achieved at least 80% glyphosate degradation within 48 hours, with 32 °C identified as the most effective temperature for both biodegradation and bacterial growth, while no bacterial growth and degradation was observed at 50 and 60 °C (as shown in Figure 10).

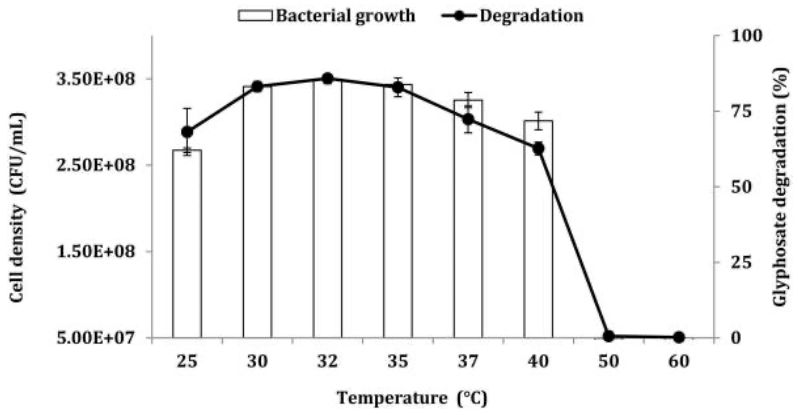

Figure 10. *Effects of temperature on biodegradation of 50 ppm glyphosate by resting cells of Burkholderia vietnamiensis strain AQ5-12. Error bars represent mean ± standard deviation (n = 3) (Manogaran et al., 2018).*

Research on glyphosate degradation under extreme temperatures is relatively limited, possibly due to their impracticality for agriculture and lower relevance to regions of significant glyphosate use. To date, *Geobacillus caldoxylosilyticus* T20 is the only bacterial strain found which capable of utilizing glyphosate as phosphorus at 60 °C (Obojska et al. 2002). Stenrød et al. (2005) simulated winter regimes by constant thaw (+5 °C), constant freezing (−5 °C), unstable conditions with short fluctuations (24 h of −5 °C followed by 24 h of +5 °C), and long duration fluctuations (3 weeks of −5 °C followed by 3 weeks of +5 °C). They found that the constant freezing treatment exhibited the lowest amount of glyphosate mineralization, the constant thawed treatment and the treatments with fluctuating temperature exhibited significantly increased mineralization (Stenrød et al., 2005).

Temperature not only affects the degradation rate but also influences the preference for degradation products (Moller et al., 2024). As previously mentioned, glyphosate degradation follows two distinct pathways: one leading to the production of sarcosine (which will be further transformed into the amino acid glycine) and inorganic phosphorus, and the other yielding AMPA and glyoxylate (Godínez et al., 2021). According to the findings of Moller et al. (2024), the AMPA and glycine pathways compete during initial reaction times, but as temperature and reaction time increase, the glycine pathway becomes dominant. This research highlights that glyphosate degradation primarily occurs at the C−N bond under higher temperatures, favouring the formation of glycine—a less toxic by-product compared to AMPA (Moller et al., 2024).

2.7 Impact of Soil Moisture on Microbial Activity and Glyphosate Degradation

Climate change influences not only temperature but also the intensity and frequency of precipitation, which directly impacts soil moisture dynamics (US Environmental Protection Agency, 2024). Warmer temperatures enhance evaporation, which reduces surface water and drying out soils and vegetation (Center for Climate and Energy Solution, 2024). Simultaneously, higher evaporation and transpiration rates add moisture to the atmosphere, which can lead to rising overall precipitation (Trenberth, 2011). It is estimated by Trenberth (2011) that for every degree Celsius increase in temperature, the air's capacity to hold water vapour rises by approximately 7%. Moreover, climate change is linked with more substantial uncertain precipitation episodes due to the augmented capacity of the air to retain moisture. For instance, in the southwestern United States, regions like California have experienced shifts from consistent seasonal rainfall to irregular heavy downpours followed by prolonged dry periods (Center for Climate and Energy Solution, 2024).

The National Climate Assessment (2024) also noted that the number of heavy downpours and major hurricanes has increased in the United States, and the strength of these events has increased, too. While greater precipitation generally increases soil moisture, long-term water retention in the soil is moderated by transpiration, runoff, and terrestrial factors such as vegetation, soil properties, meteorology, and topography (Yang et al., 2023).

Soils with smaller particles, such as clay and silt, tend to retain more water due to their larger surface area, which allows for greater adhesion of water molecules (Curell, 2011). Additionally, their higher cation exchange capacity (CEC) enables them to retain more positively charged ions, which can indirectly affect water retention (Curell, 2011). Electrostatic charges in fine-textured soils also contribute to water retention by attracting water molecules (Huang et al., 2011). In contrast, sandy soils, which have larger particles, exhibit lower WHC due to their reduced surface area, lower CEC, and weaker electrostatic interactions, allowing water to drain more quickly (Curell, 2011).

These multifaceted interactions make it challenging to predict the effects of climate and precipitation changes on soil moisture and, consequently, on microbial activity. It has been demonstrated that the mineralization of glyphosate by soil microorganisms generally increases with elevated soil moisture levels; however, in conditions of excessive moisture, such as flooding, anaerobic environments may develop, potentially inhibiting microbial activity and altering the degradation pathways of glyphosate.

Previous studies revealed that water availability significantly influences microbial activity and degradation processes. Schroll et al. (2006) investigated the effect of soil water potential on pesticide mineralization at 20°C. They found that pesticide mineralization was minimal under extreme drought conditions (≤ -20 MPa, equivalent to $\leq 13\%$ WHC), and a linear increase was observed in mineralization between -20 MPa and -0.015 MPa (approximately 40% WHC), but the mineralization was considerably reduced when soil moisture neared water holding capacity (Schroll et al., 2006). However, Bento et al. (2016) found that glyphosate degradation increased consistently with rising soil moisture, from 20% WHC to saturation, challenging the reduction observed by Schroll et al. (2006) when soil moisture was approximating WHC. Furthermore, Grundmann et al. (2007) highlighted the immediate impact of soil moisture fluctuations on glyphosate mineralization rates, emphasizing the critical role of temporal variability in influencing

microbial activity and pesticide degradation (as shown in Figure 11). It was also stated that the glyphosate in cold and dry soil (5 °C, 20% WHC) was 30 times more persistent than in warm and wet soil (30 °C, 60% WHC) (Bento et al., 2016).

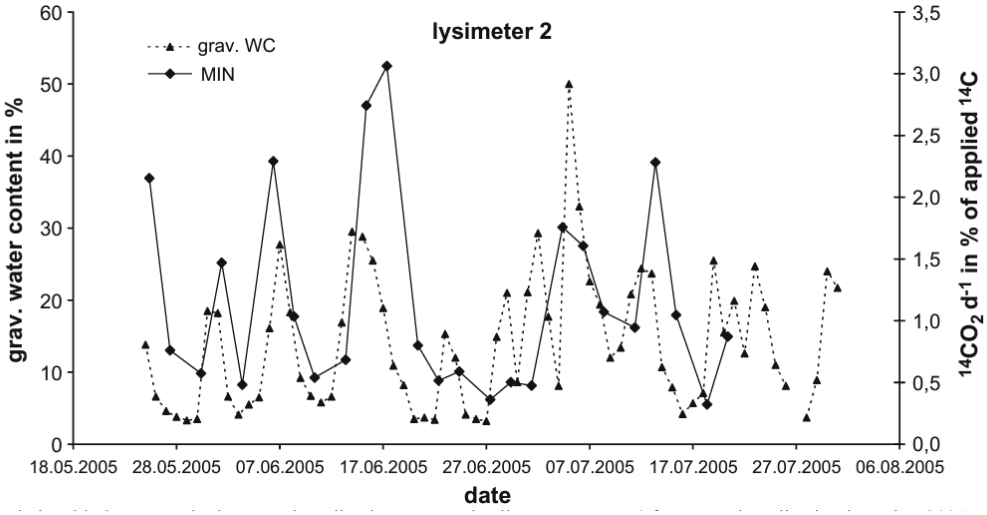

***Figure 11**. Relationship between glyphosate mineralization rates and soil water content (soil water content was measured in a soil depth of 1 cm; grav. WC = gravimetric water content; MIN = mineralization) (Grundmann et al., 2007).*

As Earth's climate changes, it is impacting extreme weather across the planet, including drought and flooding (National Oceanic and Atmospheric Administration, 2024). During drought conditions, the reduced soil moisture content lowers microbial activity due to water stress, limiting enzyme function and cellular processes, including those required for glyphosate degradation (Stark and Firestone, 1995). Schroll et al. (2006) observe that a minimum amount of water is essential to initiate microbial transformation processes in glyphosate degradation. In contrast, flooding conditions create waterlogged environments that restrict oxygen availability, shifting soil microbial communities from aerobic to anaerobic metabolic strategies (Boggis et al., 2017). This transition inhibits aerobic microbes and facultative aerobes, which are typically responsible for glyphosate degradation while promoting anaerobic microbes that may lack the necessary enzymatic pathways for efficient breakdown of the herbicide (Bian et al., 2022). Furthermore, flooding can lead to soil stratification and redox potential changes, impacting nutrient availability and microbial community composition (Rupngam and Messiga, 2024). The limited diffusion of

oxygen in saturated soils hampers oxidative enzyme activities essential for breaking down glyphosate and its byproducts (Schjønning et al., 2003).

Connecting Statement to Chapter 3

The literature review in Chapter 2 provided a comprehensive synthesis of how climate change influences key environmental factors—namely, temperature and precipitation—and their subsequent impacts on crop growth, herbicide degradation, and soil microbial activity. It emphasized the physiological and agronomic responses of crops to thermal and moisture stress, while also exploring microbial processes associated with glyphosate degradation. Key knowledge gaps were identified, including the limited empirical evidence from field-scale studies under natural climate variability. Building upon this foundation, Chapter 3 presents the first experimental investigation focused on the effects of elevated soil temperature (+2.5 °C) on soil health and microbial abundance. The chapter details the design and implementation of a field-based lysimeter experiment, incorporating gas flux measurements, soil physicochemical analysis, and microbial quantification, to evaluate short-term responses of temperate agroecosystems to warming conditions.

3. The Effects of Elevated Temperature on Soil Health and Microbial Abundance

Abstract

Climate change-driven increases in temperature are expected to significantly impact soil health, microbial activity, and greenhouse gas emissions. However, field-based studies evaluating these changes under natural conditions remain limited. This chapter investigates the impacts of moderate soil warming (+2.5 °C) on soil physicochemical properties, greenhouse gas (GHG) emissions, and microbial abundance in a temperate agricultural system. Field experiments using lysimeter plots assessed soil moisture, pH, CO_2 and N_2O fluxes, and bacterial (16S rRNA) and fungal (28S rRNA) gene abundance. Results showed that elevated temperature modestly reduced soil moisture and increased CO_2 flux mid-season without significantly altering soil pH, N_2O flux, or microbial gene abundance. The findings suggest that short-term moderate warming can influence soil respiration but may not immediately disrupt soil microbial communities.

3.1 Introduction

Climate change is driving consistent increases in global surface and soil temperatures, with projections indicating rises of 1.5 °C to over 3 °C by the end of the 21st century (IPCC, 2021). Soil temperature is a master regulator of numerous belowground processes, including nutrient cycling, microbial activity, greenhouse gas (GHG) fluxes, and soil moisture dynamics (Davidson & Janssens, 2006). Even modest warming in the surface layer can significantly alter agroecosystem functioning, particularly when coupled with changes in precipitation. These effects are especially pronounced in coarse-textured soils such as sandy loam, where high porosity and low water-holding capacity can amplify drying under elevated temperatures (Zhou et al., 2013). As climate variability intensifies, understanding how soil warming influences key indicators of soil health—moisture content, pH, and microbial abundance—has become increasingly important for predicting agroecosystem responses and informing sustainable land management.

Elevated soil temperatures typically accelerate evaporation, leading to reduced soil water content even under ambient or elevated precipitation. This can suppress microbial activity and shift community composition, particularly within bacterial and fungal domains (Sheik et al., 2011; Rousk et al., 2009). Temperature can also influence soil pH through changes in biochemical processes such as organic matter mineralization or nitrification (Liu et al., 2017), while simultaneously altering the production and emission of trace gases. Soil CO_2 and N_2O fluxes—two important greenhouse gases released from soil—are closely linked to microbial respiration and nitrogen cycling, both of which are sensitive to temperature and moisture interactions (Butterbach-Bahl et al., 2013). Despite this complexity, field-based studies examining the joint impacts of warming and moisture variability on GHG emissions and microbial abundance remain limited.

Previous research has often relied on laboratory incubations, short-duration trials, or greenhouse experiments with limited environmental realism (Albright et al., 2020; Manzoni et al., 2012). Additionally, many experiments lack temporal resolution or replication under natural climate conditions, making it difficult to capture microbial adaptation, seasonal dynamics, or the interactive effects of temperature and moisture on soil processes. To address these limitations, the present study implemented a field experiment simulating a moderate soil warming scenario (+2.5 °C) under natural weather conditions. The objective was to assess how elevated soil

temperature affects soil health indicators including moisture content, pH, and microbial abundance (16S and 28S rRNA gene copy numbers). In parallel, soil CO_2 and N_2O emissions were monitored to evaluate potential shifts in GHG fluxes linked to temperature-induced changes in microbial function and substrate availability. By integrating microbial, chemical, and gaseous measurements over the growing season, this study provides new insights into the short-term impacts of climate warming on soil ecosystem processes in temperate agroecosystems.

The objective of heating project is to investigate the effects of moderate soil warming (+2.5 °C) on key soil health indicators in a temperate agricultural system. This includes assessing changes in soil physicochemical properties such as moisture content and pH, evaluating greenhouse gas emissions (CO_2 and N_2O), and quantifying shifts in soil microbial abundance using 16S and 28S rRNA gene markers. The study aims to provide insight into the short-term biological and chemical responses of soils under elevated temperature conditions.

3.2 Materials and Methods

3.2.1 Field Setup

The temperature study was established at a lysimeter research site on the Macdonald Campus of McGill University in Sainte-Anne-de-Bellevue, Quebec, Canada (latitude 45°24′48.6″ N, longitude 73°56′28.1″ W). A total of 24 plots (2 m × 2 m each) were laid out with buffer zones between them to minimize edge effects. Of these, twelve plots were randomly assigned to receive a +2.5 °C soil warming treatment, maintained continuously via in-ground heating systems (Figure 12). The warming system consisted of insulated Styrofoam chambers and zigzag-patterned heating coils buried at ~30 cm depth. Power supply and output were monitored regularly to ensure a consistent temperature differential between heated and ambient plots. All plots were filled with sandy loam soil, sourced from Excavation Pierre Daoust Inc. (814 g sand kg^{-1}, 147 g silt kg^{-1}, and 38 g clay kg^{-1}), classified as sandy loam under the USDA Soil Classification System.

A preliminary field trial was conducted in summer 2023 to evaluate the effects of three factors on soybean growth and glyphosate degradation: elevated temperature (+2.5 °C), increased rainfall (+30%), and the presence or absence of a cover crop. The experiment followed a full factorial design with 2 levels for each factor (elevated vs. ambient temperature, increased vs. ambient rainfall, and with vs. without cover crop), resulting in 8 treatment combinations. A total of 24 plots were used, with each treatment combination replicated in 3 plots. However, due to logistical delays,

the temperature and rainfall treatments were not implemented until September 1, 2023—after the peak growing season. Consequently, only limited data were collected, and these were considered insufficient for inclusion in the main analysis. While the 2023 dataset was excluded from the primary results, selected observations are presented in the supplementary material for reference.

The main experiment was conducted from August 2024 to September 2024, during which temperature treatments were applied consistently from the outset. Glyphosate was also used to manage weed pressure, applied on May 30 at 10:30 AM at a rate of 1.7 L ha^{-1}, along with the surfactant Li-700 at 0.25% (v/v), using a tractor-mounted sprayer. Fertilizer was applied on June 14 at rates of 175 kg/ha of 46% urea and 270 kg/ha of 60% potash to ensure adequate nutrient availability. Despite these preparatory efforts, the experimental plants were lost to herbivory before they could establish, resulting in the exclusion of plant growth parameters from the analysis. Consequently, the study shifted focus toward evaluating the effects of elevated temperature on soil moisture, soil greenhouse gas (GHG) emissions, and microbial community dynamics.

Figure 12. *Layout of the experiment site (left). Schematic layout of the pots by treatments (heated and non-heated) and site dimension (right).*

3.2.2 Gas and Soil Sampling

Soil greenhouse gas (GHG) sampling was conducted on August 6, August 28, and September 24, 2024. These dates were selected based on logistical constraints associated with vial reuse; since

the same Exetainers were required for GHG analysis, soil and gas collections were scheduled only after the vials were returned and ready for reuse.

Gas fluxes of CO_2 and N_2O were measured using manual non-steady-state chambers installed in all plots. Each chamber system consisted of a clear acrylic box (50 cm × 50 cm × 15 cm) that was placed on a PVC collar embedded 5 cm into the soil, with 10 cm protruding above the ground to ensure a tight seal. At each sampling event, the chamber lid was sealed onto the collar, and headspace gas was sampled at four time points (0, 15, 30, and 45 minutes) using 20 mL syringes. The collected gas was immediately transferred into pre-vacuumed 12-mL glass Exetainers and stored for subsequent analysis.

Soil sampling was conducted simultaneously with GHG collection to capture matched environmental and microbial conditions. During each event, five surface soil subsamples (approximately 5–10 g each) were randomly collected within a 5 cm radius of each chamber collar. These subsamples were combined into a single sterile plastic Ziploc bag per plot and thoroughly mixed to ensure homogeneity. From this mixture, three separate composite samples were prepared: one sample was stored at 4 °C for subsequent moisture and pH analysis, another was frozen at −20 °C for microbial DNA extraction, and a third was retained as a backup to mitigate the risk of sample loss or contamination during transport or processing. A sterilized metal spoon, disinfected with 70% ethanol and dried between plots, was used to prevent cross-contamination during collection.

Figure 13. *Manual Non-Steady-State Chamber Setup for Soil Greenhouse Gas Flux Measurements (left). The author is taking the sample (right).*

3.2.3 Gas Analysis

Gas samples were analyzed for N_2O and CO_2 concentrations using a gas chromatograph (Bruker 450-GC, Bruker Corporation, Billerica, MA, USA) fitted with a flame ionization detector (FID) for CO_2 analysis, a ^{63}Ni electron capture detector (ECD) for N_2O analysis and using high-purity helium as a carrier gas (Njoku et al., 2022). The concentrations of N_2O and CO_2 were estimated using the Hutchinson-Mosier R (HMR) software package (v1.0.1; Pedersen, 2020), considering the relative molecular mass of carbon and nitrogen (Njoku et al., 2022).

3.2.4 Soil Analysis

Physiochemical Analysis

Temperature, moisture, and pH in the top layer of soil are key parameters in the generation of soil GHGs. Temperature was measured by inserting a thermometer probe 10 cm into the soil for each plot when collecting the GHG. Soil moisture content was determined using the gravimetric method. A subsample of the mixed soil (approximately 10 g) was weighed and then oven-dried at 105°C for 48 hours. The gravimetric soil moisture content (%) was calculated as:

$$Moisture\ Content\ (\%) = \frac{Fresh\ Soil\ Weight - Dry\ Soil\ Weight}{Dry\ Soil\ Weight} \times 100\% \quad [2]$$

Soil pH was measured using the water method as per Hendershot et al. (2007). A 1:1 soil-to-water ratio was used, where 10 g of fresh soil was added to 15 mL of deionized water in a clean beaker. The suspension was thoroughly mixed and allowed to settle for 30 minutes, then decanted for another hour before measuring the pH of the supernatant with a calibrated pH meter (Fisher Scientific Accumet AB15, Fisher Scientific, Waltham, MA, USA). The pH meter was calibrated with standard buffer solutions (pH 4.0, 7.0, and 10.0) before each measurement session.

Microbial Analysis

Soil samples collected for microbial analysis (described in section 3.2.1) were sent to Agriculture and Agri-Food Canada (AAFC), Quebec Research and Development Centre (Quebec, QC, Canada) for DNA extraction and qPCR analysis. Total DNA was extracted from soil samples using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. DNA concentration was determined using a Quantifluor dsDNA System (Promega North America, Madison, WI) and diluted 100 times prior to subsequent steps.

The abundance of bacterial and fungal taxonomic marker genes in soil was quantified with qPCR on purified DNA (diluted 100 times). The bacterial abundance (16S rRNA gene) was assessed using primers and conditions adapted from Fierer et al. (2005): forward Eub338 (5′-GCTGCCTCCCGTAGGAGT-3′) and reverse Eub518 (5′-ATTACCGCGGCTGCTGG-3). Fungal community abundance (28S rRNA gene) was assessed with primers and conditions detailed in White et al. (1990): cTW13 (5′-CGTCTTGAAACACGGACC-3′) and TW14 (5′-GCTATCCTGAGGGAAACTTC-3′). For both 16S and 28S rRNA quantification assays, the total reaction volume was 10 µL and contained: 5 µL of PowerTrack SYBR Green Master Mix (2X) for qPCR (ThermoFisher Scientific, Mississauga, ON), 0.25 µL of each forward and reverse primers (10 µM), 0.5 µl BSA (10 mg/ml), 2 µL of template DNA, and PCR-grade water. All standards were run in triplicate, samples were run in duplicate with 10% in triplicate.

3.2.5 Statistical Analysis

To evaluate the effects of soil warming (+2.5 °C) on soil physicochemical properties, greenhouse gas (GHG) emissions, and microbial abundance, independent two-sample t-tests at a level of significance $p \leqslant 0.05$ were conducted at each of the three sampling dates to compare heated and non-heated treatments. Variables analyzed included gravimetric soil moisture content, pH, CO_2

and N_2O fluxes, and gene copy numbers of 16S rRNA and 28S rRNA. All statistical analysis was performed in SAS-JMP 16 Pro (Copyright 2016, SAS Institute Inc.).

3.2 Result and Discussion

To improve our understanding of how projected climate warming may influence belowground ecological processes, this study investigated the short-term effects of moderate soil temperature elevation on soil physicochemical properties and microbial communities within a temperate agroecosystem. A sustained +2.5 °C warming treatment was applied under field conditions to assess its impact on soil moisture content, pH, microbial abundance (as indicated by 16S and 28S rRNA gene copy numbers), and greenhouse gas emissions. The results presented in the following sections provide insight into how elevated soil temperatures alter biological activity and chemical balance in sandy loam soils. These findings contribute to a broader understanding of the potential consequences of climate-induced soil changes on agroecosystem function and resilience.

3.2.1 Effects of Temperature on Soil Moisture and pH

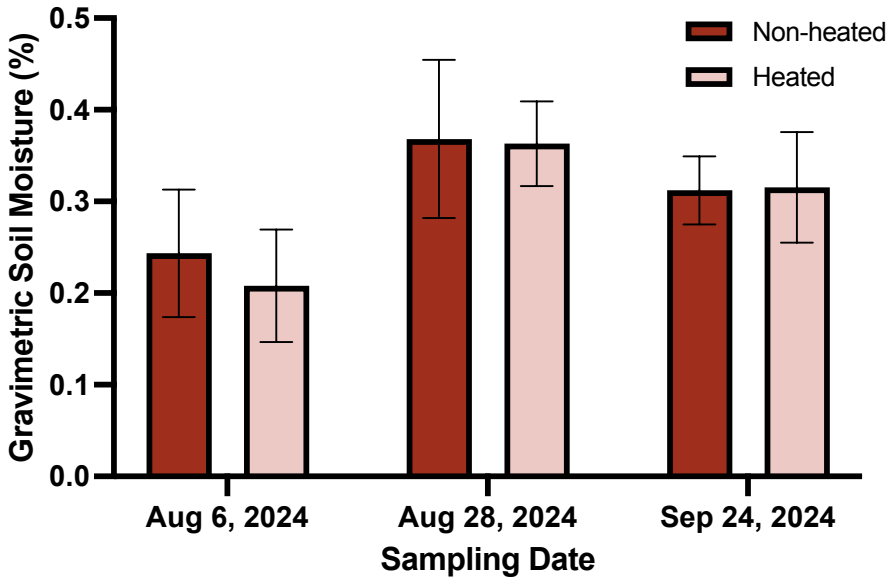

Figure 14. *Temporal dynamics of soil moisture content (%) in heated (+2.5 °C) and non-heated plots across three sampling points. Error bars represent standard deviation (n = 12).*

The average gravimetric soil moisture content, expressed as Soil Moisture Content (%), showed some differences between heated and unheated plots across the three sampling times (Figure 14). While no formal statistical test was applied here, consistent patterns suggest that soil warming reduced moisture in heated plots. At Sampling Time 1 (August 6, 2024), soil moisture was lower

in heated plots (mean = 20.8%) compared to unheated plots (mean = 24.3%). This gap narrowed slightly after 22 days at Sampling Time 2 (August 28, 2024), where both treatments exhibited higher moisture values due to seasonal rainfall, yet moisture content in heated plots (mean = 36.3%) still trailed slightly that of un-heated plots (mean = 36.8%). By Sampling Time 3 (September 24, 2024), moisture levels declined across both treatments, with heated plots (mean = 31.5%) again showing marginally higher content than unheated plots (mean = 31.2%).

These findings align with established mechanisms linking elevated soil temperatures to increased evaporative loss and reduced surface moisture. Even a modest temperature increases of +2.5 °C can enhance both evaporation and plant root water uptake, thereby lowering soil moisture content (Wan et al., 2002). In addition to direct thermal effects, prolonged soil warming may also alter physical properties such as soil structure and infiltration capacity, or influence plant transpiration rates, all of which contribute to long-term reductions in moisture availability (Borken & Matzner, 2009; Song et al., 2019).

Despite the consistent trend, differences between heated and unheated plots remained modest—typically within 2–4 percentage points—suggesting some degree of resilience or buffering in soil water dynamics under moderate thermal stress. This limited response could reflect the coarse texture and high porosity of the sandy loam soil used in this experiment, which may facilitate rapid drainage and reduce the accumulation of temperature-induced moisture deficits. Additionally, the absence of a crop canopy due to herbivory likely minimized plant-driven differences in transpiration between treatments. These findings are consistent with previous studies in temperate agroecosystems where warming-induced drying was evident but moderated by soil properties, vegetation cover, or precipitation variability (Zhou et al., 2016; Suseela et al., 2012).

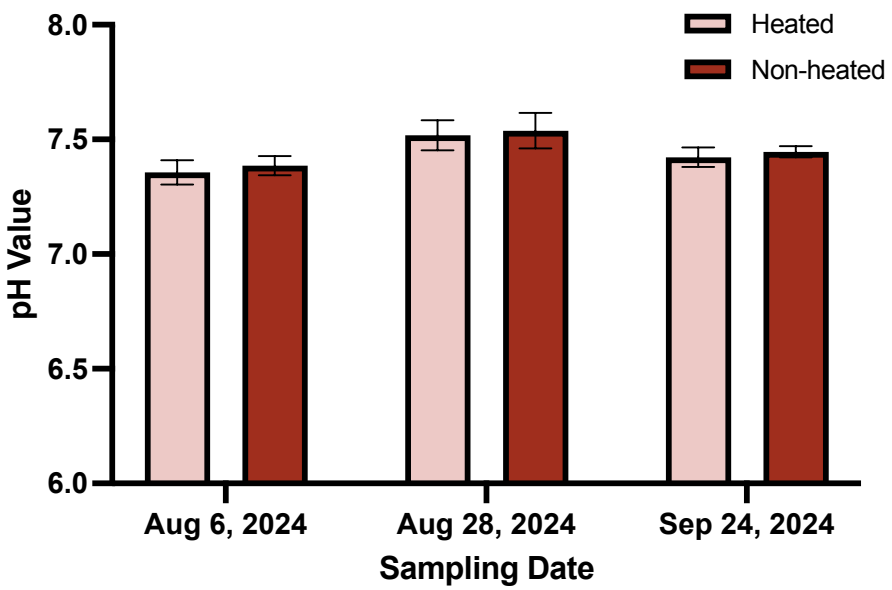

***Figure 15**. Temporal dynamics of soil moisture content (%) in heated (+2.5 °C) and non-heated plots across three sampling periods. Error bars represent standard deviation (n = 12).*

Soil pH levels were compared between heated and non-heated plots at each sampling time to assess whether increased temperature (+2.5 °C) influenced soil buffering capacity. Across the 50 days of the experiment, no statistically significant differences were found between treatments. Specifically, t-tests revealed $p = 0.286$ (August 6, 2024), $p = 0.633$ (August 28, 2024), and $p = 0.098$ (September 24, 2024) (Figure 15).

The slight pH reduction observed in heated plots at Time 3 (September 24, 2024) may reflect delayed or cumulative effects of soil warming on microbial and biochemical processes. Potential mechanisms include the increased mineralization of organic matter, which can release organic acids, and enhanced nitrification, which produces hydrogen ions and acidifies the soil (Rustad et al., 2001; Schindlbacher et al., 2012). Additionally, warming may promote greater root respiration and rhizodeposition, both of which can lower soil pH through carbonic acid formation or exudate-induced acidification.

Despite these possible processes, the lack of statistically significant differences suggests that the soil system—characterized by a neutral baseline pH (7.3–7.6)—may be buffered against short-term thermal changes. Soil texture, carbonate presence, and microbial community resilience may all contribute to stabilizing pH under warming conditions (Rousk et al., 2010; Zhou et al., 2016).

These findings align with prior research indicating that while temperature influences many soil biological processes, pH may remain relatively stable in the short to medium term, especially in well-buffered agroecosystems.

3.2.2 Effects of Temperature on Soil GHG Emissions

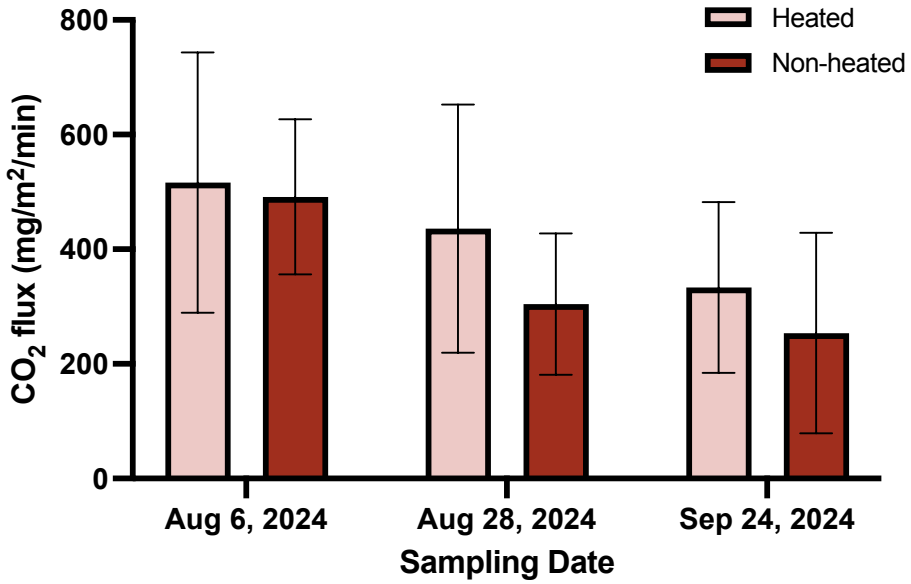

Figure 16. *Temporal dynamics of soil CO_2 flux in heated (+2.5 °C) and non-heated plots across three sampling periods. Error bars represent standard deviation (n = 12).*

As shown in Figure 16, CO_2 flux declined gradually over the three sampling periods, with the highest values observed in early August and lower emissions recorded in late August and late September. This trend likely reflects the seasonal reduction in ambient and soil temperatures, which can strongly influence microbial and root respiration (Davidson & Janssens, 2006; Subke & Bahn, 2010). In Montreal, the mean daily temperatures were approximately 18.7 °C, 17.4 °C, and 15.0 °C on the three respective sampling dates. As temperatures drop, enzymatic activity in soil microorganisms slows, and plant root respiration typically declines, leading to reduced overall soil CO_2 emissions (Davidson & Janssens, 2006; Subke & Bahn, 2010).

When considering the effects of the warming treatment, CO_2 flux was consistently higher in heated plots than in non-heated controls across all three time points (Figure 53). While this trend was not statistically significant at Time 1 (August 6, 2024) ($p = 0.38$) or Time 3 (September 24, 2024) ($p = 0.14$), a significant difference was observed at Time 2 (August 28, 2024) ($p = 0.04$), indicating

that soil warming enhanced respiration during mid-season conditions when temperature and moisture were likely optimal. The overall trend suggests that +2.5 °C warming has the potential to stimulate CO_2 flux, especially when environmental constraints are minimal. This is consistent with findings from other studies where warming increased microbial and root respiration under favorable soil conditions (Carey et al., 2016; Melillo et al., 2002).

Although the warming effect was only statistically significant at one time point, the consistent direction of the response—higher fluxes in heated plots at all dates—suggests a potential cumulative or threshold-driven impact of warming. Mechanistically, this could be attributed to increased microbial enzymatic activity, accelerated decomposition rates, or enhanced root respiration in warmer soils (Davidson & Janssens, 2006; Luo & Zhou, 2006). However, the short duration of the experiment—spanning less than two months—likely limited the ability to detect more definitive or progressive shifts in microbial processes and gas fluxes. Subtle or time-lagged responses in soil microbial communities often require longer observation periods to become apparent. If the warming treatment had been sustained over an entire growing season, or across multiple seasons, it is plausible that clearer trends would have emerged, particularly as soil carbon pools, substrate quality, and microbial community structure evolved under continued thermal stress. These findings underscore both the sensitivity and temporal complexity of soil biological responses to warming and highlight the need for longer-term field studies to fully characterize climate impacts on belowground processes.

In summary, seasonal temperature decline drove the overall reduction in CO_2 flux, while the warming treatment had a modest but positive effect, particularly during putative periods of high biological activity. These findings emphasize the importance of considering both seasonal variability and climatic treatment interactions when assessing soil respiration responses to environmental change.

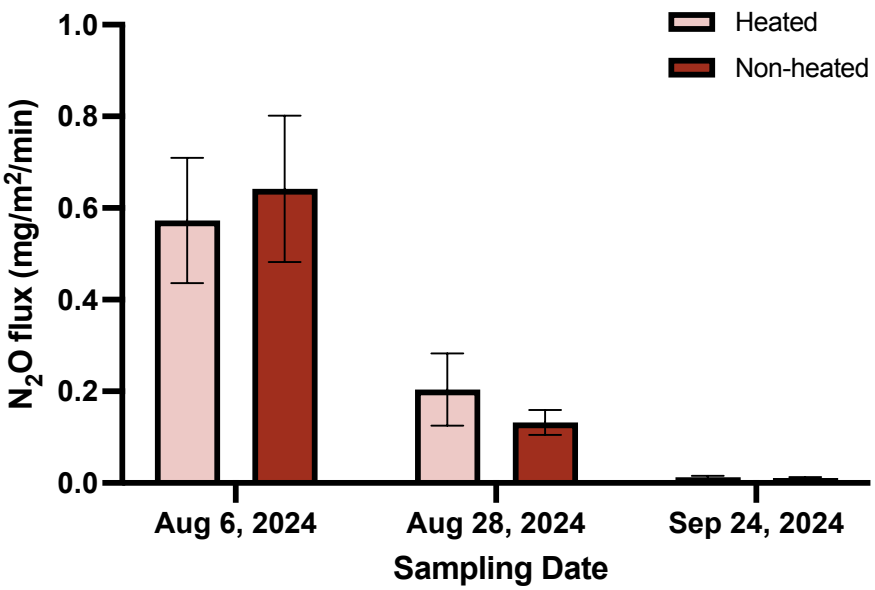

Figure 17. *Temporal dynamics of soil N_2O flux in heated (+2.5 °C) and non-heated plots across three sampling periods. Error bars represent standard deviation (n = 12).*

Nitrous oxide (N_2O) flux showed a clear seasonal decline over the course of the growing season, with the highest emissions observed in early August and substantially lower values recorded in late August and September (Figure 18). This temporal pattern was statistically significant ($p < 0.001$), suggesting strong environmental controls on N_2O emissions. The initial peak in N_2O flux may be partially attributed to the nitrogen fertilizer applied on June 14, which likely enhanced substrate availability for microbial nitrification and denitrification. As the season progressed, emissions declined markedly, a trend that can be linked to both biogeochemical and environmental shifts. The pool of readily available nitrogen in the soil may have become depleted over time, reducing the substrate necessary for continued microbial N_2O production (Butterbach-Bahl et al., 2013). Simultaneously, declining temperatures toward the end of the growing season likely suppressed microbial activity, further limiting enzymatic processes involved in nitrogen transformation. Together, these changes in nitrogen availability and microbial dynamics contributed to the sharp seasonal decline in N_2O flux.

In contrast to the strong seasonal trend, soil warming (+2.5 °C) had no significant effect on N_2O flux at any of the three sampling periods. Independent t-tests comparing heated and non-heated plots yielded non-significant results at all time points (August 6: $p = 0.746$; August 28: $p = 0.407$; September 24: $p = 0.720$). Although warming can increase microbial activity and potentially

enhance N_2O production under certain conditions, the lack of observed effect in this study may be attributed to stable moisture conditions, low levels of mineral nitrogen, or spatial variability in denitrifier abundance (Dijkstra et al., 2012; Kool et al., 2011). These results suggest that in this field context, moderate soil warming alone was not sufficient to significantly alter N_2O emissions, particularly under relatively balanced nutrient and moisture conditions.

3.2.3 Effects of Temperature on Soil Microbial Abundance

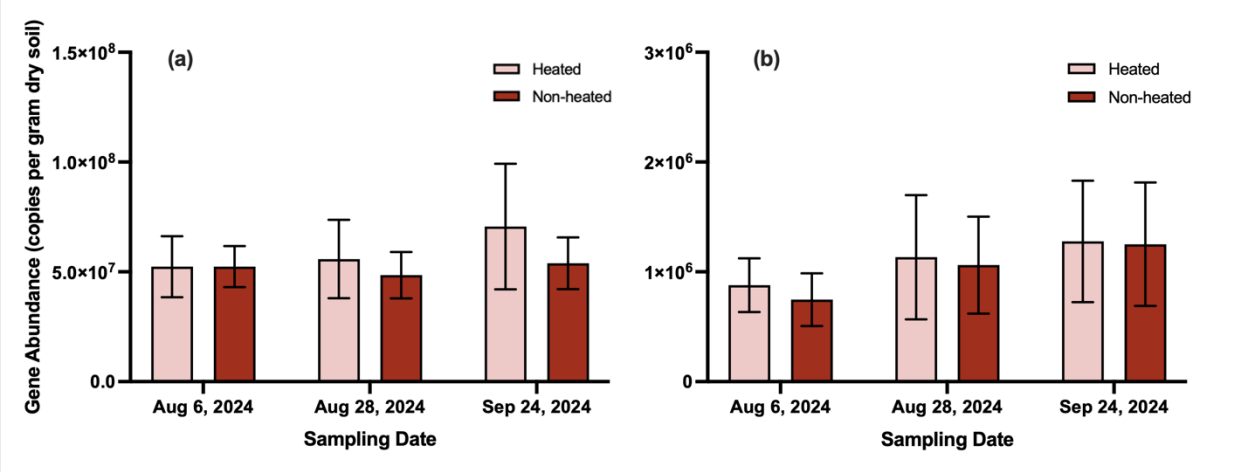

Figure 18. *Temporal dynamics of (a) bacterial (16S rRNA gene) abundance, and (b) fungal (28S rRNA gene) abundance in heated (+2.5 °C) and non-heated plots across three sampling periods. Error bars represent standard deviation (n = 12).*

Microbial abundance, assessed via 16S rRNA (bacterial marker) and 28S rRNA (fungal marker) gene copy numbers per gram of dry soil, remained relatively stable across the growing season and was not significantly affected by soil warming (Figure 18). In the case of 16S rRNA abundance (Figure 18a), non-heated plots showed relatively consistent gene copy numbers across all three sampling dates, indicating stable bacterial abundance throughout the season. In contrast, heated plots exhibited a gradual increase in bacterial abundance, with higher gene copy numbers observed toward the end of the season (September 24). Although this trend was not statistically significant ($p = 0.072$ for time effect across all plots), it may suggest that bacterial populations in warmed soils became more active or proliferated slightly under elevated temperature conditions. However, no significant differences were detected between heated and non-heated plots at individual time points ($p > 0.08$), suggesting that warming-induced changes in bacterial abundance were modest and within the range of natural variability.

Similarly, 28S rRNA gene abundance (Figure 18b) showed no statistically significant differences over time ($p = 0.280$) or between treatments ($p > 0.19$ at all time points). Although gene copy numbers tended to be slightly higher in heated plots throughout the season, the variation fell within overlapping error margins. These results indicate that fungal abundance, like bacterial abundance, was relatively insensitive to short-term temperature elevation, at least in terms of bulk gene copy number per unit soil.

Several factors likely contributed to the limited microbial response observed during the experiment. Because the soil was freshly added to the site in the summer of 2023, microbial communities across all plots likely began from a comparable baseline, minimizing pre-existing differences in structure or function. This uniform starting point may have reduced the potential for early divergence in microbial abundance due to environmental history or soil legacy effects. Moreover, the absence of a developing plant community—caused by herbivory—likely constrained belowground microbial differentiation, as root exudates and plant–microbe interactions are critical drivers of microbial activity and community shifts. In addition, the relatively short duration of the experiment—spanning less than two months—may not have allowed sufficient time for measurable shifts in microbial abundance to develop. Microbial responses to warming, particularly in terms of population size and community restructuring, often occur over extended periods as changes in substrate inputs, soil resource availability, and ecological feedback accumulate. Therefore, while this study captures early-season microbial dynamics under warming conditions, longer-term experiments would be necessary to detect sustained or more pronounced effects on microbial abundance and function.

Overall, this study revealed that short-term soil warming resulted in minimal and largely non-significant changes in key soil parameters. Soil moisture content remained statistically indistinguishable between heated and non-heated plots, with only slight, non-significant reductions observed under warming. Similarly, while CO_2 fluxes tended to be higher in heated plots, this pattern was not consistent across sampling dates and lacked strong statistical support. No significant treatment effects were observed for soil pH, N_2O emissions, or microbial gene abundance. These results suggest that moderate temperature elevation over a limited timeframe did not substantially alter soil physicochemical conditions or microbial population sizes. However, the directional consistency of some trends—such as marginal increases in CO_2 flux and bacterial

abundance—indicates that longer-term experimentation may be necessary to detect more robust warming-induced responses in temperate soils.

3.3 Conclusions

This study investigated the short-term effects of moderate soil warming (+2.5 °C) on soil moisture, pH, greenhouse gas emissions, and microbial abundance in a temperate agroecosystem. The main findings indicate that soil warming did not lead to significant changes in most measured parameters over the 50-day experiment. Soil moisture remained largely comparable between treatments, and no statistically significant differences were observed in pH, N_2O emissions, or microbial gene abundances (16S and 28S rRNA). A consistent but non-significant trend of higher CO_2 flux and increased bacterial abundance in heated plots suggests a possible warming effect on microbial respiration and activity, especially under mid-season environmental conditions.

Despite these insights, the study was limited by its short duration, simplified conditions, and herbivory. The experiment lasted less than two months, which may not have been sufficient to capture delayed or cumulative microbial responses to elevated temperature. Additionally, the use of freshly added, homogenized soil and the loss of plant cover due to herbivory likely constrained ecological differentiation and reduced the representativeness of plant–microbe–soil interactions. These constraints limited the ability to fully detect long-term or functionally meaningful shifts in microbial structure and soil health under warming conditions.

Future research should extend the temporal scale of soil warming experiments and include multiple growing seasons to assess legacy effects and inter-annual variability. Integrating plant communities and simulating realistic agricultural management practices will provide more ecologically relevant insights. Moreover, the use of high-resolution molecular techniques (e.g., metagenomics, transcriptomics) could help uncover functional changes in microbial communities that are not captured by gene abundance alone. These approaches will be essential for predicting how warming alters agroecosystem processes and for informing adaptive strategies to sustain soil health under future climate scenarios.

Connecting Statement to Chapter 4

Chapter 3 presented the results of a field experiment assessing the influence of elevated soil temperature on soil health indicators and microbial communities. Findings suggested that moderate warming can affect soil moisture dynamics and greenhouse gas fluxes without causing major shifts in microbial abundance within a single season. Building on this, Chapter 4 explores the impacts of altered rainfall patterns on crop growth, glyphosate degradation, and soil microbial functions. A complementary field study with controlled precipitation treatments was conducted to evaluate plant physiological responses, herbicide persistence, and microbial resilience under variable moisture conditions, providing a broader understanding of climate variability effects on agroecosystems.

4. The Effects of Precipitation on Crop Growth and Soil Microbial Functions

Abstract

Climate change is expected to increase variability in rainfall patterns, which may alter soil moisture availability and impact crop production and microbial processes. Understanding the resilience of agricultural systems to these changes is critical for future food security and soil sustainability. This chapter examines the influence of altered rainfall patterns (±30% from ambient) on common beans growth, glyphosate degradation, and soil microbial community dynamics. Field trials manipulated precipitation levels using rainout shelters and irrigation systems. Plant physiological traits, crop yields, herbicide breakdown, and microbial abundances (16S rRNA, 28S rRNA, goxA genes) were measured. Despite rainfall changes, soil moisture, plant growth, and herbicide degradation showed minimal significant differences, reflecting strong resilience of the crop and soil microbial functions to short-term moisture variability. The results underscore the stability of agroecosystems under moderate shifts in precipitation in the short term.

4.1 Introduction

Global climate change is fundamentally altering precipitation patterns by amplifying both the intensity and variability of rainfall. As rising temperatures increase atmospheric water-holding capacity, some regions face more intense downpours, while others experience prolonged droughts (Trenberth, 2011; Center for Climate and Energy Solution, 2024). This dual trend of wetter and drier extremes has direct implications for agricultural systems, influencing not only crop productivity but also the chemical and biological dynamics of soils.

One of the most immediate effects of changing rainfall patterns is on soil moisture, which governs the physiological responses of crops. In water-limited conditions, reduced soil water availability impairs stomatal conductance and transpiration, limiting photosynthesis and nutrient uptake. In contrast, excessive moisture often leads to waterlogged soils, restricting root respiration and suppressing plant development (Ohashi et al., 2006; Manghwar et al., 2024). These stress responses can be effectively captured using remote sensing tools such as the Normalized Difference Vegetation Index (NDVI), a widely used indicator of plant health and chlorophyll content. NDVI typically declines under both drought and flood conditions due to reduced photosynthetic activity and canopy greenness (Crusiol et al., 2017; Rajanna et al., 2022).

Rainfall-induced soil moisture variability also significantly influences the biodegradation of glyphosate, a widely used herbicide in global crop production. Glyphosate is primarily broken down by soil microorganisms through metabolic pathways that yield either aminomethylphosphonic acid (AMPA) or glycine. The efficiency of this degradation is highly sensitive to moisture levels: microbial degradation tends to increase under moderate soil moisture but declines in both excessively dry and saturated soils due to inhibited enzymatic activity or oxygen limitation (Bento et al., 2016; Schroll et al., 2006).

Critically, the capacity of soils to degrade glyphosate is not only a function of moisture but also of microbial abundance and community structure, which are shaped by precipitation patterns. Microbial taxa responsible for glyphosate degradation—such as bacteria and fungi—are tracked through genetic markers like 16S rRNA (for bacteria), 28S rRNA (for fungi), and *goxA*, a gene encoding glyphosate oxidoreductase. These organisms and their functional genes tend to decline in abundance during droughts, due to water stress, or in flooded conditions, where anaerobic environments hinder aerobic microbial processes (Godínez et al., 2021; Wirsching et al., 2022).

Thus, shifts in rainfall indirectly affect glyphosate persistence by modulating the abundance and activity of its microbial degraders.

This intricate relationship illustrates how climate-driven precipitation changes cascade through soil-plant-microbe systems. Reduced or increased rainfall not only influences plant physiological responses—such as transpiration rate and NDVI—but also alters microbial community structure, which in turn affects the degradation rate of agrochemicals like glyphosate. Understanding these interconnected processes is critical for predicting agroecosystem responses under future climate conditions and for managing the sustainability of food production systems.

The objective of rainfall study is to evaluate the impact of altered rainfall patterns (−30%, ambient, and +30%) on crop performance, herbicide behavior, and soil microbial dynamics. Specifically, the study assesses how changes in precipitation affect plant physiological traits, crop yield, glyphosate degradation, and the abundance of microbial genes (16S rRNA, 28S rRNA, and goxA) associated with microbial activity and herbicide breakdown. This chapter aims to understand how short-term moisture variability influences agroecosystem stability and function.

4.2 Methodology

4.2.1 Field Setup

The Diversity and Precipitation Treatment (DART) project, initiated in the summer of 2022 and led by Dr. Cynthia Kallenbach, was conducted at the McGill Emile A. Lods Agronomy Farm (Latitude 45°25′33.7″ N, Longitude 73°55′43.4″ W). The soils were classified as Gleysolic loam, with 190 g kg^{-1} clay, 490 g kg^{-1} sand, and 320 g kg^{-1} silt. The bulk density was measured at 1.21 g cm^{-3}, and the volumetric field capacity was 0.24 cm^3 cm^{-3}. The study followed a full-factorial design with two main factors: crop diversity and precipitation treatments, with four replicates per treatment. The experiment followed a full-factorial design, examining two primary factors: crop diversity and precipitation treatments, with four replicates per treatment. Crop diversity treatments included various combinations of species, such as Kernza (perennial wheat), cover crops (Rye, White clover), cereal rye, wheat, soybeans (dry beans), cereal (spring wheat), birdsfoot, and white clover (as shown in Figure 19).

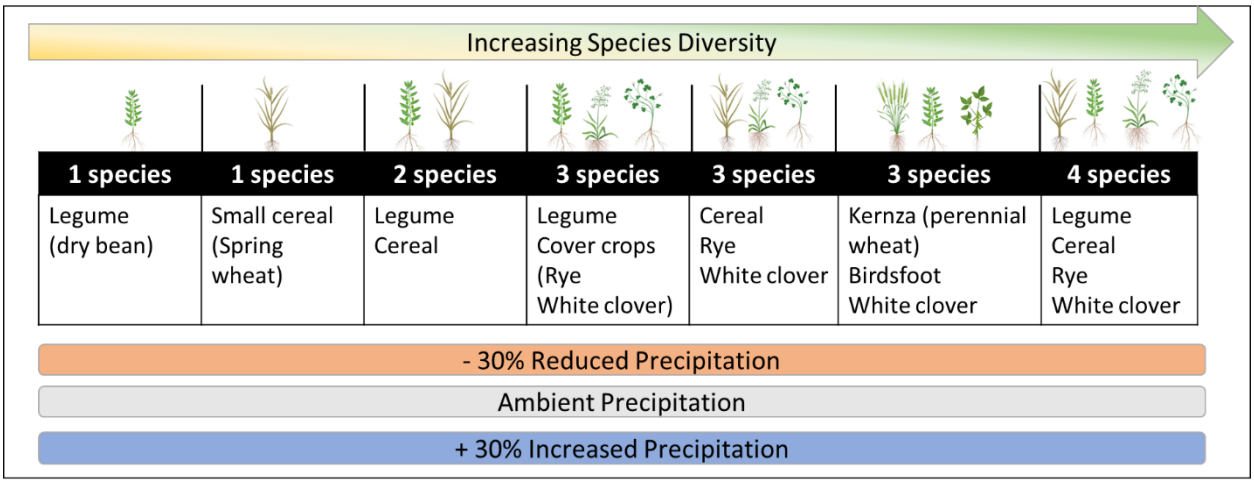

Figure 19. *Experimental design of the Diversity and Precipitation Treatment (DART) project.*

Precipitation treatments comprised three levels: reduced (-30% precipitation), ambient (control), and increased (+30% precipitation). These precipitation manipulations were implemented using rainout shelters and an irrigation system (shown in Figure 20). The precipitation levels remained fixed throughout the study, while crop species or varieties were adjusted based on prior-year performance and research objectives.

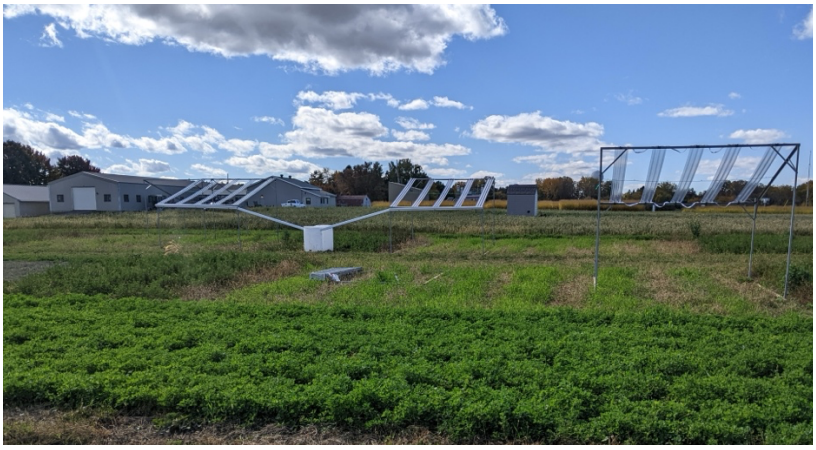

Figure 20*. Rainout shelters and the irrigation system in DART.*

4.2.2 Plant Physiological Parameters Analysis

To assess plant physiological responses, measurements were conducted exclusively on beans monoculture plots and beans-wheat mixed plots. This selection was made because beanss have broad leaves, which facilitate more accurate and consistent physiological measurements, such as photosynthetic rate, stomatal conductance, and chlorophyll content. In contrast, other species in

the experiment, such as wheat and kernza, have narrow leaves, making physiological assessments more challenging and less comparable across treatments.

Plant photosynthetic activity, stomatal conductance and transpiration rates were measured every week with a Li-COR 6800 (Li-COR, Nebraska, US). The canopy reflectance at the leaf level was measured every week from 9 a.m. –10 a.m. with a PolyPen RP 410 (Phyton Systems Instruments, spol. s r.o., Czech Republic) across a wavelength range of 300 nm - 800 nm. All measurements of leaves (PolyPen, reflectance, photosynthesis, conductance and transpiration) were taken on the fully expanded third trifoliate leaf from the top of the plant, as it represents stable photosynthetic activity and is less affected by aging or shading. For each plot (e.g., 1B-R), five soybeans plants were randomly selected for measurement, and the results were averaged to obtain a representative value for that subplot.

4.2.3 Soil Sampling and Analysis

Herbicide application was restricted to beans, beans–wheat, and wheat plots, as the other plant species were perennial and not re-seeded. Glyphosate was applied on Friday, May 31 at 10:30 AM at a rate of 1.7 L ha^{-1}, along with the surfactant Li-700 (0.25% v/v), using a tractor-mounted sprayer. Soil samples were collected at multiple time points to track glyphosate degradation, as outlined in Table 1. Pre-application sampling was conducted to assess any residual herbicide levels prior to treatment (All dates are presented in MM/DD/YY format). At 2 and 6 days after application, only surface soil (0 cm) was sampled due to the absence of rainfall and expected lack of vertical herbicide movement. For all subsequent sampling dates, soil was collected from two depths—surface (0 cm) and subsurface (10–12 cm)—to evaluate potential translocation of glyphosate and its metabolites. Samples were extracted using a 3 cm diameter auger, which was thoroughly cleaned between plots to prevent cross-contamination.

Table 1. *Soil sampling schedule, target analytes, depths, and number of samples collected.*

Sampling Date	Time after Application (days)	Sampling Depth (cm)	Number of Samples
05/24/2024	0 (pre-application)	2	36
06/01/2024	2	2	36
06/06/2024	6	2	36
06/12/2024-06/13/2024	12 & 13	2	36
		10-12	36
06/27/2024-06/28/2024	27 & 28	2	36
		10-12	36

During each plot, five surface soil subsamples (approximately 5–10 g each) were randomly collected, then combined into a single sterile plastic Ziploc bag per plot and thoroughly mixed to ensure homogeneity. From this mixture, three separate composite samples were prepared: one was for glyphosate/AMPA concentration analysis, one was for microbial DNA extraction, and a third was retained as a backup to mitigate the risk of sample loss or contamination during transport or processing. All samples were frozen at −20 °C.

Soil Microbial Analysis

Soil samples collected for microbial analysis (described in section 3.2.1) were sent to Agriculture and Agri-Food Canada (AAFC), Quebec Research and Development Centre (Quebec, QC, Canada) for DNA extraction and qPCR analysis. Total DNA was extracted from soil samples using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. DNA concentration was determined using a Quantifluor dsDNA System (Promega North America, Madison, WI) and diluted 100 times prior to subsequent steps.

The quantification assays 16S and 28S rRNA have been described in Section 3.2.4. Quantitative PCR (qPCR) assays were designed to assess the microbial potential for glyphosate (*goxA*, sarcosine oxidase gene / *sarc*) degradation. The primer sets were developed based on previously established methodologies for detecting functional genes involved in phosphonate metabolism (Morales et al., 2020; Hernández-Alomia et al., 2022). The qPCR protocol was optimized for

environmental DNA extracted from soil samples to quantify the abundance of glyphosate-degrading microbial populations. Primers for *goxA* quantification were: *goxA*fd (5'ATC GGC TTY GAR ACT GAA GG 3') and *goxA*rd (5' CCR TTT CCA TMG GBG TWG CG 3') (Wirsching et al., 2022). The reaction mixture for qPCR assays was 10 µLand contained; 5 µL of PowerTrack SYBR Green Master Mix (2X) for qPCR (ThermoFisher Scientific, Mississauga, ON), 1.0 µL of each forward and reverse primers (10 µM), 0.5 µl BSA (10 mg/ml), 2 µL of template DNA (5 ng/µl) and PCR-grade water. Thermocycling conditions were as follows: initial denaturation at 95°C for 10 min, followed by 45 cycles of (95°C 30 sec, 56°C 30 sec, 72°C 30 sec), followed by a 72 °C final elongation step. Minimum Each 10 µL PCR reaction for *goxA* contained: All standards were run in triplicate, samples were run in duplicate with 10% in triplicate, with minimum accepted reaction efficiency of 90% and standard curve r^2 of 1.

We determined the presence/absence of sacrosine oxidase (*sarc)* using primers SarcF (5'CGT GTG AAA CCT GGA AAA CGT GGT 3') and SarcR (5' TAG CGG CTA CAT GAA CAC CTG CT 3'; González-Valenzuela and Dussán 2018) in an end-point PCR and subsequent gel visualization. Each 10 µL PCR reaction for Sarc contained: 10 µL of PowerTrack SYBR Green Master Mix (2X) for qPCR (ThermoFisher Scientific, Mississauga, ON), 0.5 µL of each forward and reverse primers (10 µM), 4 µL of template DNA (5 ng/µL) and PCR-grade water. PCR conditions 95 °C (10 min); [95 °C (15 sec), 60 °C (30 sec), 72 °C 30 sec)] × 40; 72 °C (10 min). PCR products were run on a 1.5.% agarose gel to visualize presence or absence of a band at 100 bp, and verified against a positive *sarc* control plasmid.

Soil Herbicide Concentration Analysis

Soil samples were analyzed for glyphosate and its primary degradation product, aminomethylphosphonic acid (AMPA), at Pathogenia (Montreal, QC, Canada) using a standardized derivatization and LC-ESI-MS/MS protocol. Briefly, 2.0 g of homogenized soil was extracted with 10.0 mL of an aqueous sodium tetraborate buffer containing disodium EDTA. The mixture was vortexed, subjected to ultrasonic extraction, and centrifuged at 4500 rpm. A 5.0 mL aliquot of the supernatant was derivatized with FMOC-Cl (6 mg/mL in acetonitrile) at 60°C for one hour. The reaction was terminated by acidifying the solution to pH 2–3 with hydrochloric acid (6 M), followed by a second centrifugation. A 10 µL portion of the final extract was then injected into an LC-ESI-MS/MS system for analysis.

The instrumentation used included an Agilent 1290 Infinity II UHPLC coupled with an Agilent 6470 triple quadrupole mass spectrometer. Chromatographic separation was performed using a Poroshell 120 EC-C18 column (3.0 × 100 mm, 2.7 µm, 120 Å) maintained at 40°C. The mobile phases consisted of 5 mM ammonium formate with 0.1% formic acid in water (A) and methanol (B), delivered in a linear gradient from 20% to 95% B at a flow rate of 0.4 mL min^{-1}. Quantification was achieved using an eight-point calibration curve ranging from 1 to 200 µg/L, matrix-matched against a non-contaminated soil extract. The method had a limit of quantification (LOQ) of 2 µg/kg for both glyphosate and AMPA. Multiple reaction monitoring (MRM) transitions included m/z 390.1 → 167.9 and 392.0 → 214.0 for glyphosate, and m/z 334.0 → 156.0 and 334.0 → 111.8 for AMPA, using both positive and negative electrospray ionization modes. This method provided robust, reproducible measurements of glyphosate and AMPA concentrations across diverse soil samples.

4.2.4 Statistical Analysis

To assess the effects of altered precipitation regimes (−30%, ambient, +30%) on crop physiology, herbicide degradation, and microbial abundance, one-way analyses of variance (ANOVA) were performed for each variable of interest across treatments. The response variables included volumetric soil moisture content, crop phenological stages, transpiration rate, NDVI, beans yield, glyphosate and AMPA concentrations, and gene copy numbers of 16S rRNA, 28S rRNA, and goxA. Statistical comparisons were conducted independently at each sampling date or developmental stage, with a significance threshold set at $p \leq 0.05$. All statistical analyses were conducted using SAS-JMP 16 Pro (Copyright 2016, SAS Institute Inc.).

4.3 Result & Discussion

Building on the experimental framework of the DART project, which imposed three levels of rainfall manipulation (−30%, ambient, and +30%), this section investigates how altered precipitation affects key plant and soil responses. The study focuses on crop physiological performance—such as transpiration rate and NDVI—and soil microbial abundance and herbicide degradation potential. By integrating these indicators, the analysis provides insight into how rainfall variability shapes agroecosystem dynamics and contributes to our understanding of climate resilience in field-based agricultural systems.

4.3.1 Effects of Rainfall on Soil Moisture

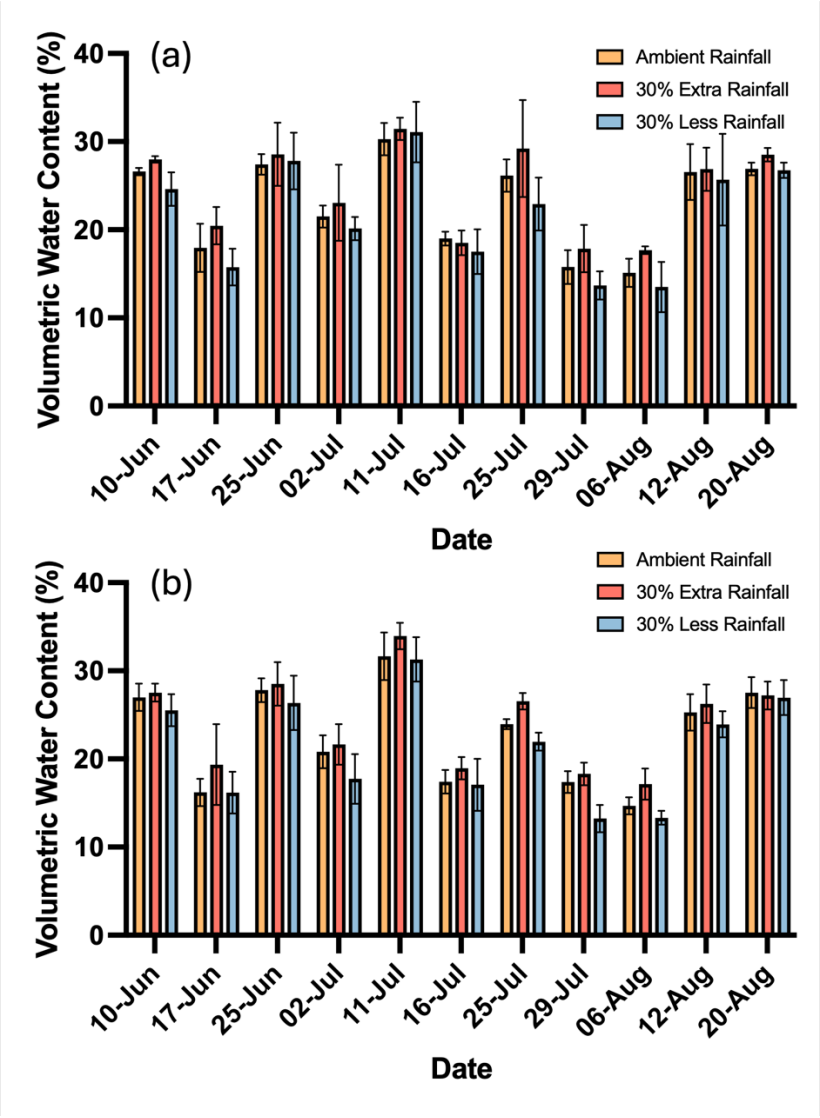

Figure 21. *Volumetric Water Content (%) in different rainfall treatments over time in (a) plots only grown beans and (b) plots grown beans intercropped with wheat. Each error bar represents ± one standard deviation (n = 4).*

As shown in Figure 21, no statistically significant differences in soil moisture content were observed among the precipitation treatments at either depth. This outcome suggests that the long-term precipitation manipulations—applied consistently over three growing seasons (2022–2025) at levels of −30%, ambient, and +30%—had limited measurable effects on soil water availability in the current season. Nevertheless, a consistent directional trend was observed across the 2024

growing season, with volumetric water content generally following the pattern: +30% rainfall > ambient > −30% rainfall. While this trend indicates some persistent influence of the rainfall treatments, the magnitude of the differences remained too small—relative to natural variability and soil hydraulic properties—to yield statistical significance.

Several factors may have contributed to the absence of statistically significant differences in soil moisture across precipitation treatments. Figure 22 shows that both precipitation and temperature during the study period in the Montreal region were highly variable. Most days received minimal rainfall, interspersed with a few high-intensity events. This irregular distribution could have weakened the contrast between treatments, as natural rainfall may have intermittently replenished moisture in reduced-rainfall plots or caused rapid leaching in increased-rainfall plots. Additionally, the persistently warm temperatures throughout the season likely sustained high evapotranspiration rates, further limiting soil water retention across all treatments.

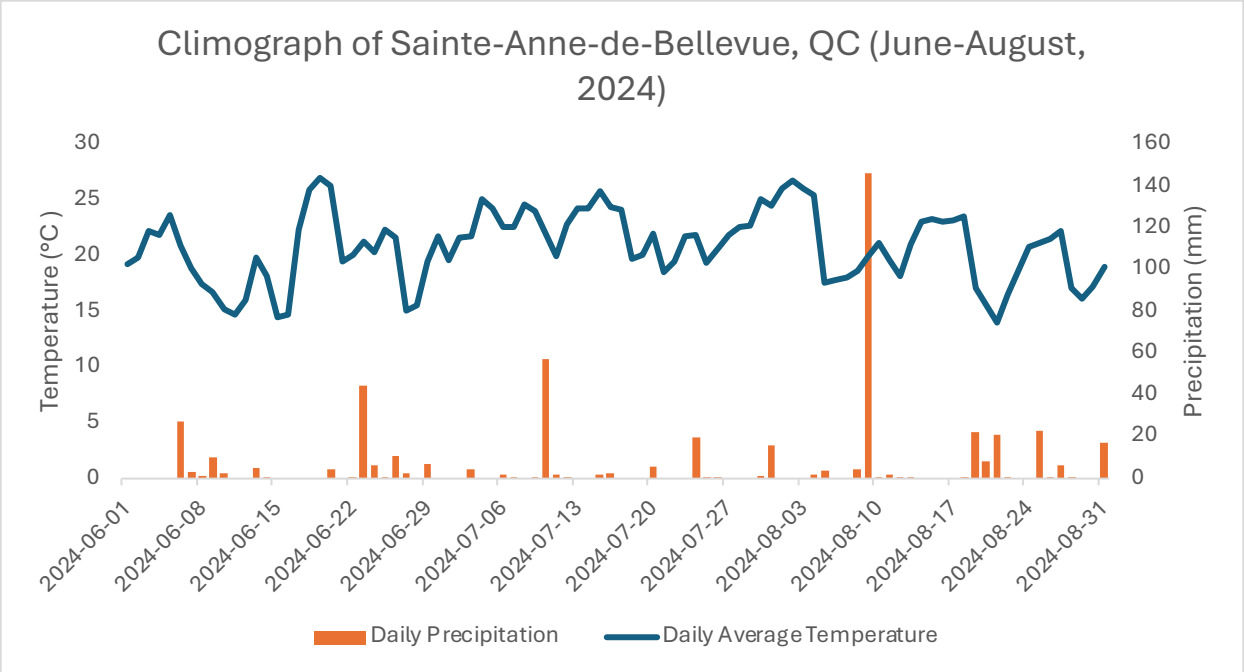

Figure 22. *Mean Air Temperature and Daily Precipitation in Sainte-Anne-de-Bellevue, QC (June–August 2024). Data source: Government of Canada.*

Beyond climatic variability, soil physical properties may have also played a role. Texture analysis at the site indicated predominantly sandy loam soils, with sand content ranging from 34.6% to 48.1% and clay content between 16.2% and 27.4%. These soils are known for their rapid drainage

and low water-holding capacity (Rawls et al., 1982; Hillel, 1998), which may have reduced the effectiveness of the +30% rainfall treatment by allowing added water to percolate below the root zone. Conversely, the −30% treatment may not have markedly lowered soil moisture due to periodic natural rainfall events. Taken together, these climatic and soil characteristics may have limited the soil's sensitivity to moderate, long-term precipitation manipulations, even after three consecutive growing seasons (2022–2025).

4.3.2 Effects of Rainfall on Crop Phenology

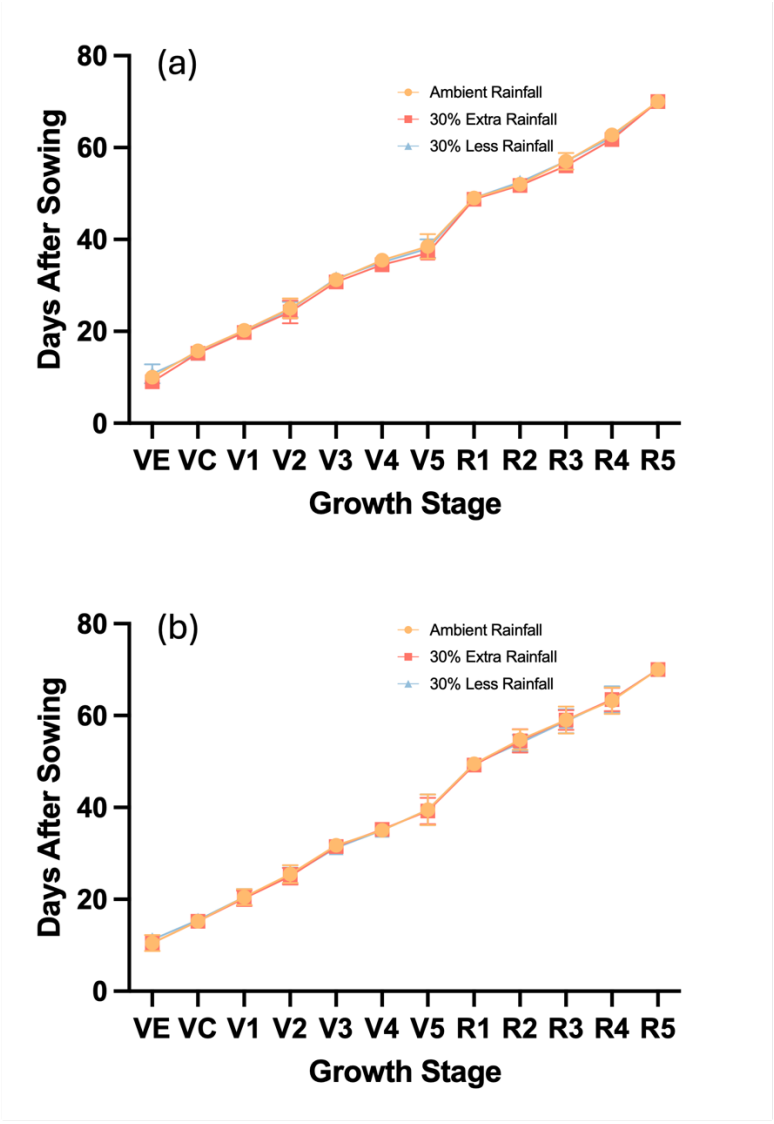

Figure 23. *Effect of Rainfall Treatments on Growth Stage Progression on (a) plots only grown beanss and (b) plots grown beans intercropped with wheat. Each error bar represents ± one standard deviation (n = 4). Sowing date: June-04.*

To evaluate how precipitation variability influenced crop development, the timing of key phenological stages was assessed across rainfall treatments in both monoculture and intercropped systems. No statistically significant differences were observed in the timing of phenological stages among precipitation treatments for either mono-cropped beanss or beans-wheat intercrops (Figure 23). Developmental milestones—including emergence (VE), vegetative stages (V1–V5), and reproductive phases (R1–R5)—occurred within a narrow temporal range across treatments, suggesting a relatively low sensitivity of phenological development to moderate precipitation variability. However, a consistent directional trend was evident in both cropping systems: plants in the elevated rainfall treatment reached phenological stages slightly earlier than those in the ambient and reduced rainfall treatments (Table 2). This trend was most pronounced during early vegetative stages, where mean differences between treatments ranged from 0.5 to 1.5 days, indicating that enhanced soil moisture availability may modestly accelerate phenological development even in the absence of statistically significant effects.

Table 2. *Mean days after sowing (± SD) to reach key phenological stages in beans monoculture and beans–wheat intercropped systems under different rainfall treatments.*

Crop	Rainfall	VE	VC	V1	V2	V3	V4	V5	R1	R2	R3	R4	R5
Bean	A	10.0 ± 1.4	15.8 ± 0.5	20.3 ± 1.5	25.0 ± 2.9	31.3 ± 1.0	35.5 ± 1.3	38.5 ± 2.4	49.0 ± 1.2	52.0 ± 1.4	57.0 ± 1.8	62.8 ± 1.0	70.0 ± 0.0
	E	9.0 ± 0.0	15.3 ± 0.5	19.8 ± 1.3	24.3 ± 2.6	30.8 ± 0.5	34.5 ± 0.6	37.0 ± 0.0	48.8 ± 0.5	51.8 ± 0.5	56.0 ± 1.4	61.8 ± 1.5	70.0 ± 0.0
	R	10.8 ± 2.2	15.3 ± 0.5	20.0 ± 1.4	24.8 ± 1.9	31.5 ± 0.6	35.0 ± 0.0	38.0 ± 2.0	49.0 ± 0.8	52.5 ± 0.6	57.0 ± 1.2	62.3 ± 0.5	70.0 ± 0.0
Bean + Wheat	A	10.5 ± 1.7	15.3 ± 0.5	20.5 ± 1.9	25.5 ± 2.4	31.8 ± 1.5	35.0 ± 1.4	39.5 ± 3.4	49.5 ± 1.0	54.8 ± 2.6	59.0 ± 3.2	62.5 ± 3.0	70.0 ± 0.0
	E	10.3 ± 1.5	15.3 ± 0.5	20.8 ± 1.0	24.0 ± 1.4	31.8 ± 1.0	35.3 ± 1.0	39.3 ± 3.1	49.5 ± 1.0	54.3 ± 3.1	58.8 ± 2.6	64.5 ± 2.1	70.0 ± 0.0
	R	10.8 ± 1.5	15.5 ± 1.0	20.5 ± 1.7	25.8 ± 1.9	31.5 ± 1.0	35.0 ± 0.0	39.0 ± 3.6	49.3 ± 0.8	54.3 ± 2.2	59.3 ± 2.4	63.3 ± 1.5	70.0 ± 0.0

The observed advancement under elevated rainfall is consistent with literature indicating that soil moisture status plays a critical role in modulating early phenological transitions in legumes (Kazan & Lyons, 2016).). In particular, water deficits during seedling establishment have been shown to delay radicle emergence and leaf initiation (Fahad et al., 2017), whereas improved water availability can enhance seed imbibition, germination rate, and leaf expansion (Cherlinka, 2024). Although the intercropping system exhibited a slight delay in early growth stages relative to

monocultures—potentially due to interspecific competition for soil resources or canopy-induced microclimate modification (Tataw et al., 2016)—the relative ranking of rainfall treatments remained consistent. Moreover, the convergence of phenological timing at later stages, particularly R5, across all treatments suggests that photoperiodic control or genotypic determinism may dominate over moisture-driven variability during reproductive development (Manghwar et al., 2024; Geleta et al., 2024).

Collectively, these results imply that while moderate precipitation alterations (±30%) may not induce statistically detectable shifts in crop phenology, elevated rainfall can subtly advance developmental progression, particularly in early stages. This response reflects a complex interaction between soil hydraulic properties, environmental buffering in intercropped systems, and crop physiological plasticity.

4.3.3 Effects of Rainfall on Crop Transpiration Rate and NDVI

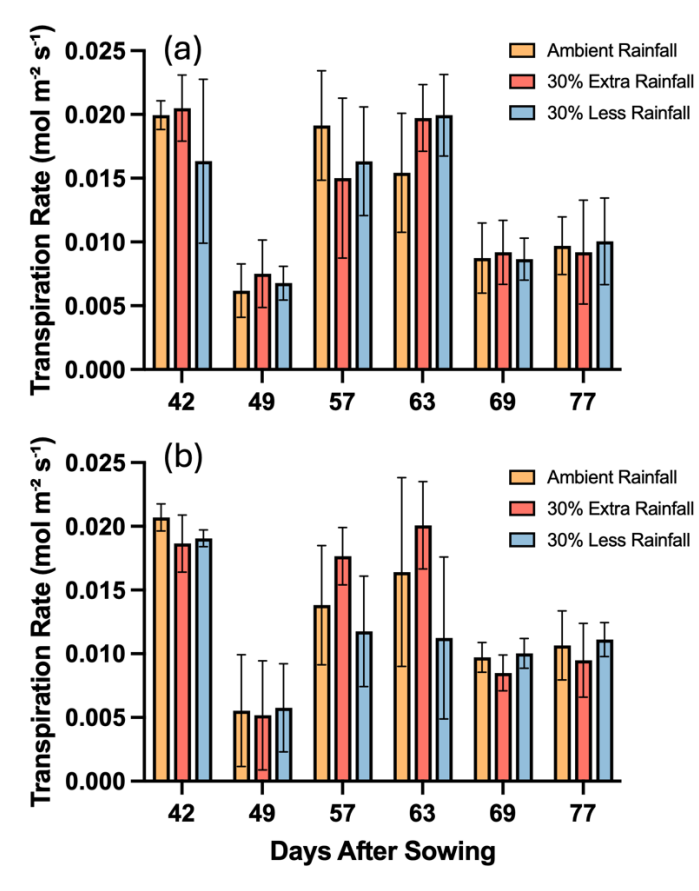

Figure 24. *Changes in leaf transpiration rate of beans plants in (a) beanss monoculture and (b) beanss intercropped with wheat during the growing season under different rainfall treatment. Each error bar represents ± one standard deviation (n = 4).*

Transpiration rate measurements were taken at multiple time points throughout the growing season to evaluate the physiological response of beans plants to different rainfall treatments. While some temporal variation was observed, the overall transpiration rates did not differ significantly among the reduced, ambient, and increased rainfall treatments (Figure 24). The lack of statistically significant differences in transpiration rate among treatments suggests that beans plants demonstrated physiological stability in their water regulation, despite moderate changes in water availability. This outcome is consistent with the notion that transpiration is often tightly regulated by stomatal behavior in response to both internal hydraulic status and external environmental conditions. Under moderate drought or water surfeit, many legume species—including common beans—can maintain stable stomatal conductance and transpiration through osmotic adjustment

and partial stomatal closure, avoiding both excessive water loss and photosynthetic disruption (Fahad et al., 2017; Sinclair et al., 2005).

Moreover, the error bars in Figure 24 reflect substantial biological variability in different days after sowing (DAS), particularly around peak growth stages (e.g., 49–63 DAS), suggesting that individual plant responses to rainfall treatments may have been heterogeneous, potentially due to microenvironmental variation or genotype-specific plasticity. Notably, transpiration rates decreased uniformly across all treatments toward the end of the growing season (69–77 DAS), likely reflecting natural senescence and reduced canopy activity as the crop approached maturity.

The similarity of response patterns in both monoculture and intercropped systems further supports the hypothesis that moderate changes in precipitation do not elicit strong shifts in transpiration dynamics under field conditions where compensatory mechanisms and soil buffering effects may moderate plant-level water stress (Brooker et al., 2015). These findings suggest that the transpiration trait in common beans is relatively resilient to precipitation variation within ±30% of ambient, reinforcing the observed phenological stability and pointing to the potential of this species for climate-resilient cropping systems.

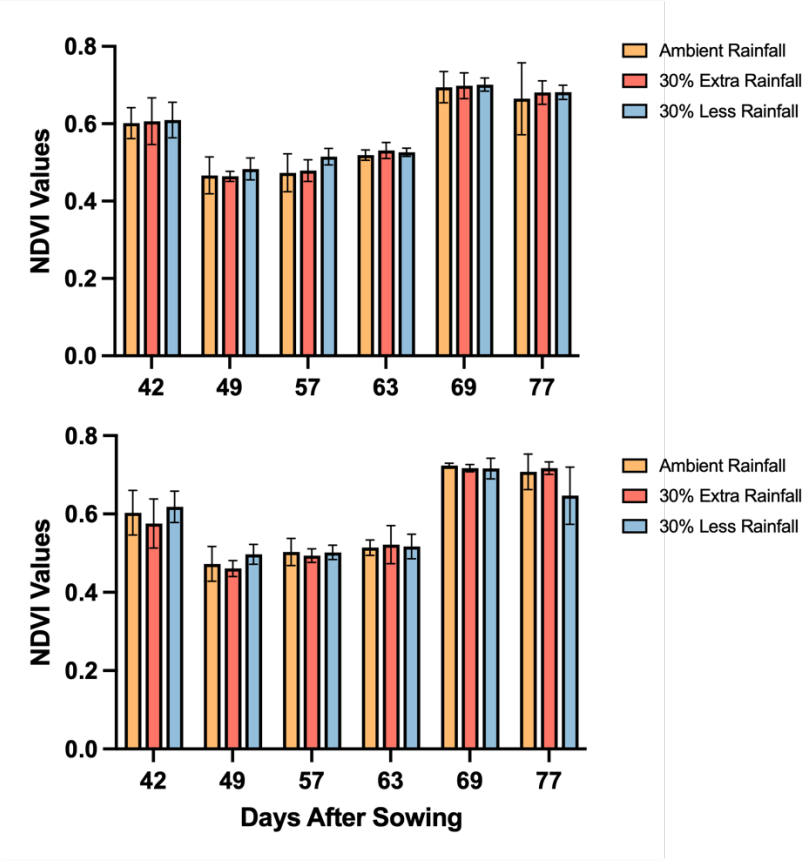

Figure 25. *Changes in crop NDVI of beans plants in (a) beanss monoculture and (b) beanss intercropped with wheat during the growing season under different rainfall treatment. Each error bar represents ± one standard deviation (n = 4).*

NDVI values across the growing season revealed consistent patterns in both cropping systems (Figure 10). Contrary to conventional phenological expectations, NDVI did not peak during the flowering stage (around 57–63 DAS). Instead, the lowest NDVI values occurred during midseason (49–57 DAS) under all rainfall treatments. This suggests that canopy greenness—as sensed by NDVI—was not maximized during reproductive transitions but rather increased toward the late seed-filling and early maturity stages (69–77 DAS). This delayed NDVI peak may reflect continued greenness in the upper canopy or delayed senescence traits, rather than total biomass accumulation. Such "stay-green" phenotypes have been associated with extended canopy photosynthetic activity in legume crops under favorable late-season conditions (Fahad et al., 2017).

Statistical analysis confirmed that NDVI differences between rainfall treatments were not statistically significant at any measured stage ($p > 0.05$; Figure 10). In beans monoculture plots,

reduced rainfall occasionally produced slightly higher NDVI values during mid-season, possibly due to mild water stress triggering adaptive responses such as deeper rooting, better stomatal control, or delayed senescence (Fahad et al., 2017). In contrast, the extra rainfall treatment did not consistently enhance NDVI. In some cases (e.g., 49 DAS), NDVI values were slightly lower, which could be attributed to temporary soil saturation or reduced oxygen availability, impeding nutrient uptake during early reproductive phases (Zhang et al., 2016).

The intercropped beans–wheat system exhibited greater NDVI stability across all rainfall treatments and stages, with lower variability and more uniform trajectories. This suggests a buffering effect of intercropping, possibly due to complementary root systems, improved soil water regulation, or altered microclimatic conditions that mitigate rainfall extremes (Brooker et al., 2015). Even during mid-season NDVI declines, values remained tightly grouped across treatments, indicating that intercropping enhanced physiological resilience under fluctuating water conditions.

In summary, while moderate rainfall variation induced subtle differences in NDVI, none were statistically significant, and values converged late in the season. This supports the conclusion that NDVI is a robust canopy trait under moderate climate variation, especially when common beans is intercropped with wheat. The delayed NDVI rise toward maturity suggests prolonged canopy activity, which may offer adaptive advantages in climate-resilient cropping systems.

4.3.4 Effects of Rainfall on Crop Yield

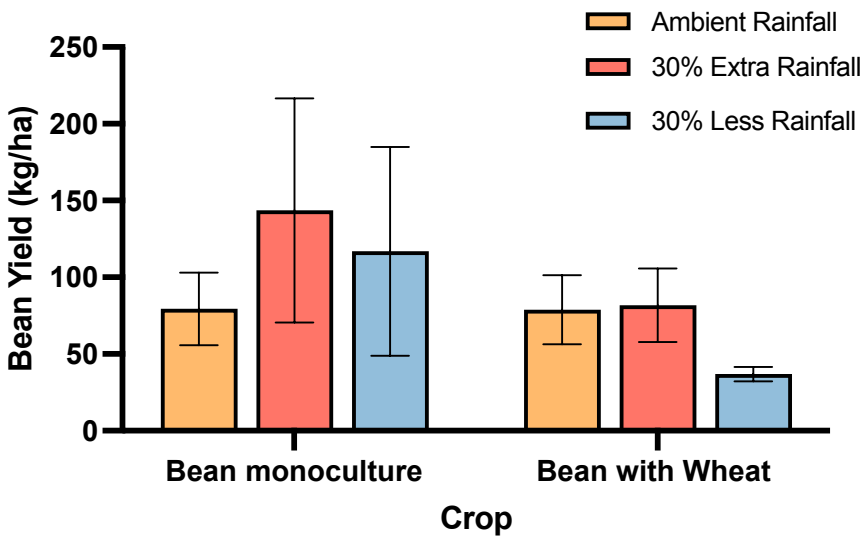

Figure 26. *Mean yield of beans in monoculture plots and beans intercropped with wheat plots. Each error bar represents ± one standard deviation (n = 4).*

To evaluate how rainfall variation may influence crop productivity, beans yields were compared across different precipitation treatments in both monoculture and intercropped systems. As shown in Figure 26, no statistically significant differences in yield were detected among rainfall treatments ($p > 0.05$), indicating that precipitation manipulation alone did not exert a strong influence on final productivity during the study period.

Despite this, the observed yield patterns suggest that other environmental or site-specific factors may have influenced crop performance. In the monoculture plots, yields tended to be higher under elevated rainfall, whereas reduced rainfall generally corresponded with lower yields, particularly in intercropped plots. These trends, though not statistically significant, may reflect the influence of soil variability across plots. For instance, some replicates appeared to benefit from heavier clay soils that retained water more effectively or suppressed weed growth after surface cracking. Others were situated on better-quality soils that likely supported more consistent water availability during dry spells. While rainfall treatments did not independently explain yield variation, the results underscore the complexity of agroecosystem responses and the importance of local soil conditions in modulating the impacts of precipitation.

4.3.5 Effects of Rainfall on Glyphosate Degradation

Surface Soil – Glyphosate

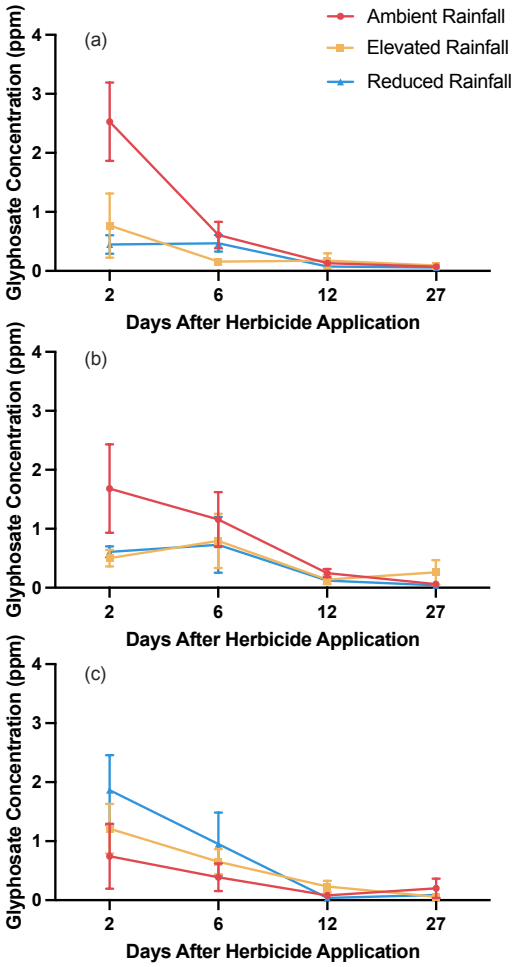

Figure 27. *Temporal dynamics of glyphosate degradation under three rainfall treatments across crop types in surface soil. (a) Beans monoculture, (b) Wheat monoculture, and (c) Beans–wheat intercropping. Each error bar represents ± one standard deviation (n = 4).*

To assess how glyphosate residues changed shortly after application, total soil glyphosate concentrations were measured at multiple time points following herbicide treatment. Despite the absence of precipitation within 2- and 6-days following glyphosate application on May 30 (Figure 27), substantial variability in total soil glyphosate concentrations was observed during these early sampling intervals. As no rainfall events occurred during this period, the observed fluctuations are unlikely to be attributed to leaching or surface runoff processes. A plausible explanation is sample

heterogeneity, wherein the non-uniform distribution of glyphosate in the soil matrix results in significant variation among replicate cores (Feng and Thompson, 1989). Factors such as localized differences in herbicide deposition, soil texture, organic matter content, and moisture retention likely contributed to this spatial variability.

To further evaluate the consistency of residue levels within each time point, one-way ANOVA analyses were conducted independently for four key sampling days (Days 2, 6, 12, and 27). In all cases, no statistically significant differences were detected among replicates ($p > 0.05$), indicating that the observed variability was random rather than systematic. While variability on Days 2 and 6 may be attributed to spatial heterogeneity, the lack of significant variation on Days 12 and 30 is likely due to the substantial reduction in glyphosate concentrations over time. By these later stages, glyphosate residues were near or below detection thresholds in many samples, leaving minimal variation to detect statistically.

When assessed across the full sampling period, total glyphosate concentrations demonstrated a clear and statistically significant temporal decline, consistent with progressive degradation and/or sorption to soil components. This pattern aligns with previous findings showing that glyphosate is strongly adsorbed and gradually degraded by soil microbial communities under a range of environmental conditions (Hadi et al., 2013; Benslama and Boulahrouf, 2013; Fan et al., 2012). A two-way ANOVA further confirmed that sampling day had a significant effect on glyphosate concentration ($p < 0.05$) in all cropping systems (beans, wheat, and beans–wheat intercrop), whereas rainfall treatment and its interaction with time were not significant ($p > 0.05$). These results suggest that glyphosate degradation is primarily a function of time and soil biological activity, with rainfall playing a negligible role under the conditions tested.

Surface Soil – AMPA

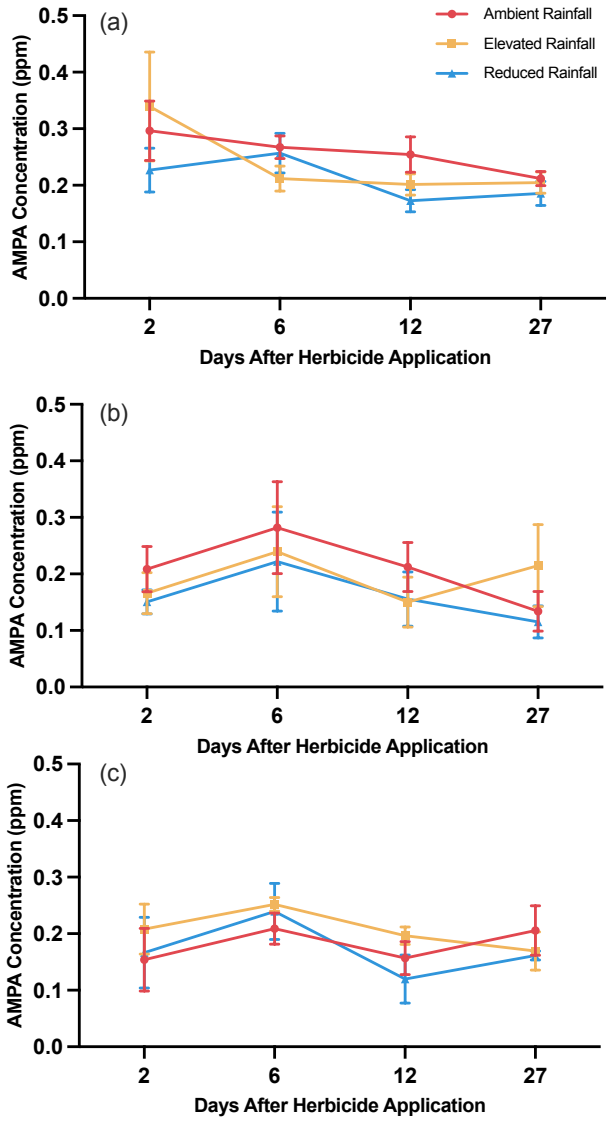

Figure 28. *Temporal dynamics of AMPA concentration under three rainfall treatments across crop types in surface soil. (a) Beans monoculture, (b) Wheat monoculture, and (c) Beans–wheat intercropping. Each error bar represents ± one standard deviation (n = 4).*

To investigate the degradation pathway of glyphosate in response to varying precipitation treatments, AMPA concentrations were monitored over time in surface soil across three crop systems. Figure 28 presents the temporal dynamics of AMPA concentrations in the surface soil (0–5 cm) following glyphosate application under three rainfall scenarios—ambient, elevated (+30%), and reduced (−30%)—across three crop systems: beans (a), wheat (b), and beans–wheat intercrop

(c). AMPA was measured at four time points—2, 6, 12, and 30 days after herbicide application, to examine patterns of glyphosate degradation under different moisture conditions.

Across all treatments, AMPA concentrations remained relatively stable over the 30-day period. A modest peak in AMPA levels was observed around Day 6 across most treatments, particularly under ambient and elevated rainfall conditions. This transient rise likely corresponds to the microbial degradation of glyphosate via the glyphosate oxidoreductase (GOX) pathway, which cleaves the C–N bond to yield AMPA and glyoxylate as intermediate products (Feng et al., 2020; Castrejón-Godínez et al., 2021). The presence of this peak aligns with literature showing that GOX-driven transformation occurs rapidly within days post-application, particularly under moderate temperature and moisture conditions favorable for microbial metabolism (Manogaran et al., 2018; Godínez et al., 2021).

Despite this fluctuation, one-way ANOVA revealed no statistically significant differences ($p > 0.05$) in AMPA concentrations between rainfall treatments at any time point or cropping system. This suggests that short-term alterations in rainfall volume (±30%) did not significantly influence the rate or extent of glyphosate degradation to AMPA. This is consistent with previous findings that AMPA is a relatively persistent metabolite in soil due to its high affinity for soil particles and limited leachability (Sviridov et al., 2014; Mamy et al., 2016). The observed stability across rainfall treatments could be attributed to AMPA's strong sorption to soil organic matter and mineral surfaces, especially in soils with moderate clay and organic carbon content, which limit its mobility regardless of increased or decreased water inputs (Piccolo et al., 1994; Erban et al., 2018).

The consistent presence of AMPA throughout the 30-day period also follows the literature which indicates its intermediate persistence relative to the parent compound. While glyphosate may rapidly dissipate through microbial mineralization, AMPA's transformation occurs more slowly, particularly under ambient field conditions without major shifts in microbial gene expression or environmental stressors (Mäder et al., 2024; Morales et al., 2020).

In summary, the observed AMPA dynamics suggest that microbial degradation of glyphosate occurred efficiently following application, with AMPA accumulation peaking transiently around Day 6. However, the lack of significant differences among rainfall treatments indicates that under the soil texture and rainfall intensity tested, precipitation alone was not a dominant factor influencing AMPA persistence. These findings further emphasize the primary role of microbial

enzymatic activity—rather than rainfall-induced leaching or hydrolysis—in governing early-stage glyphosate degradation and AMPA accumulation under field conditions.

Deeper Soil (10cm – 12cm) – Glyphosate

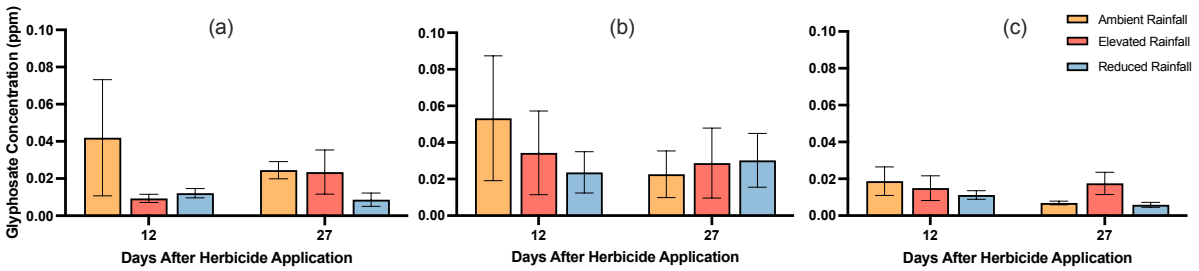

Figure 29. *Glyphosate concentration at 10 cm soil depth across three crop systems (a) Beans monoculture, (b) Wheat monoculture, and (c) Beans–wheat intercropping under different rainfall treatments at 12 and 27 days after herbicide application. Each error bar represents ± one standard deviation (n = 4).*

To evaluate whether different rainfall regimes influenced glyphosate translocation into the subsurface soil layer, glyphosate concentrations at 10 cm depth were analyzed at 12 and 30 days after herbicide application across three rainfall treatments: ambient, elevated (+30%), and reduced (−30%). A one-way analysis of variance (ANOVA) was conducted for each crop system and sampling date. The results indicated that no statistically significant differences ($p > 0.05$) were observed between rainfall treatments at either time point for any of the crop systems (Figure 29). These findings suggest that, under the conditions of this study, rainfall amount alone did not have a measurable effect on glyphosate movement into deeper soil layers within the 30-day post-application window.

This outcome is consistent with the well-documented chemical properties of glyphosate, which exhibits strong adsorption to soil particles—particularly iron and aluminum oxides and organic matter—limiting its leaching potential under most environmental conditions (Borggaard & Gimsing, 2008; Giesy et al., 2000). The lack of detectable translocation despite altered precipitation input implies that vertical movement of glyphosate is restricted primarily to macropore flow or high-intensity rainfall events, which were not prevalent during the sampling period. The variability observed within rainfall treatments, particularly under ambient conditions,

is likely due to natural spatial heterogeneity among field replicates. Microtopographic variation, soil microstructure, and root-zone interactions can all contribute to uneven herbicide infiltration, even under uniform treatment applications (Feng & Thompson, 1989). Additionally, localized microbial degradation near the surface may have limited the amount of glyphosate available for downward movement.

In some cases, glyphosate concentrations in deep soil increased slightly over time, which could be explained by slow infiltration or redistribution due to delayed rainfall events. However, the lack of significant differences, coupled with consistently low glyphosate concentrations across treatments, reinforces the herbicide's low mobility in sandy loam soils. Moreover, biological factors, such as microbial degradation or root uptake, may have further reduced glyphosate availability for vertical transport (Hadi et al., 2013; Benslama & Boulahrouf, 2013).

Deeper Soil (10cm – 12cm) – AMPA

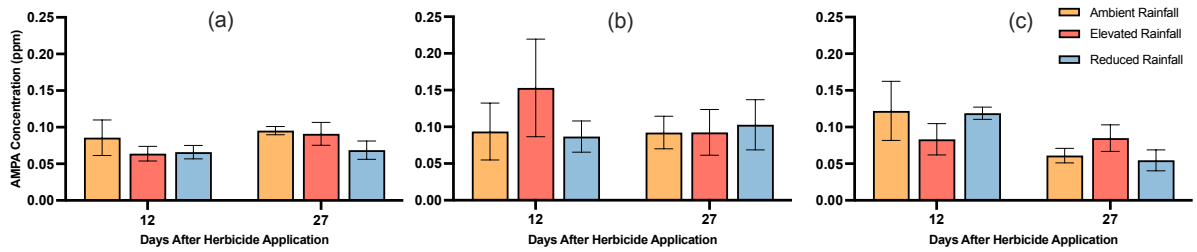

Figure 30. *AMPA concentration at 10 cm soil depth across three crop systems (a) Beans monoculture, (b) Wheat monoculture, and (c) Beans–wheat intercropping under different rainfall treatments at 12 and 27 days after herbicide application. Each error bar represents ± one standard deviation (n = 4).*

Figure 30 displays the AMPA concentrations measured at 10 cm soil depth in beans (a), wheat (b), and beans–wheat intercrop (c) systems under ambient, elevated (+30%), and reduced (−30%) rainfall treatments, sampled at 12 and 30 days after glyphosate application. Across all crop systems and time points, AMPA concentrations remained relatively low and showed no statistically significant differences ($p > 0.05$) among rainfall treatments, based on one-way ANOVA analysis.

The low AMPA levels and lack of treatment effects are consistent with glyphosate's limited vertical mobility and AMPA's even lower leaching potential due to its strong adsorption to soil particles,

particularly in soils with moderate organic matter and iron/aluminum oxides (Sviridov et al., 2014; Mamy et al., 2016). While some increase in AMPA concentrations at depth was noted over time—especially in beans and wheat monocultures—these trends were not statistically robust and likely reflect gradual degradation of glyphosate near the root zone rather than rainfall-induced leaching.

The detection of AMPA in deeper soil layers may suggest slow glyphosate infiltration followed by microbial degradation in situ. However, AMPA itself is not a terminal metabolite; it is not chemically stable in soil and can be further degraded by microbial processes into phosphate, carbon dioxide, and inorganic nitrogen compounds (Borggaard & Gimsing, 2008; Benslama & Boulahrouf, 2013). As a result, the relatively low and variable AMPA concentrations observed at depth may also reflect ongoing transformation into these simpler products, particularly in biologically active soils.

Overall, the data reinforce that both glyphosate and AMPA are largely confined to surface or near-surface zones in the absence of extreme rainfall or macropore flow. The limited accumulation of AMPA at 10 cm depth, coupled with its known susceptibility to further microbial degradation, suggests that rainfall amount alone does not significantly influence AMPA persistence or mobility under the tested field conditions. These findings highlight the critical role of microbial activity in regulating not only glyphosate dissipation but also the fate of its degradation products.

4.3.6 Effects of Rainfall on Soil Microbial Abundance

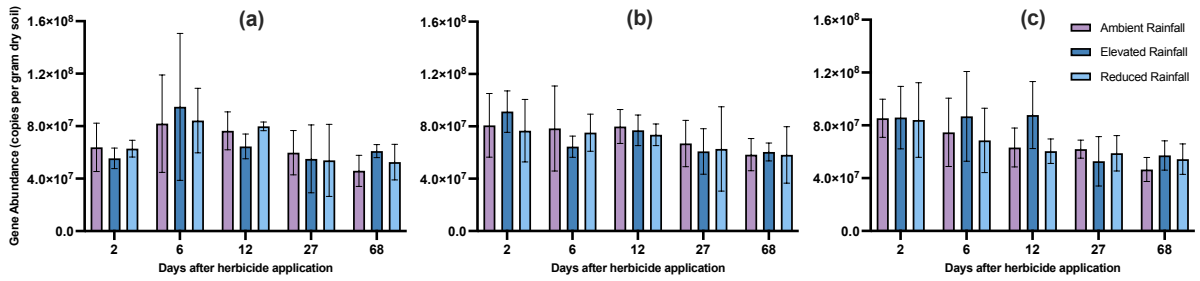

Figure 31. *Dynamics of bacterial 16S rRNA gene abundance in surface soil (0–5 cm) under ambient, elevated (+30%), and reduced (−30%) rainfall treatments across crop systems: (a) beans monoculture, (b) wheat monoculture, and (c) beans–wheat intercrop following glyphosate application. Error bars represent standard error of the mean (n = 4).*

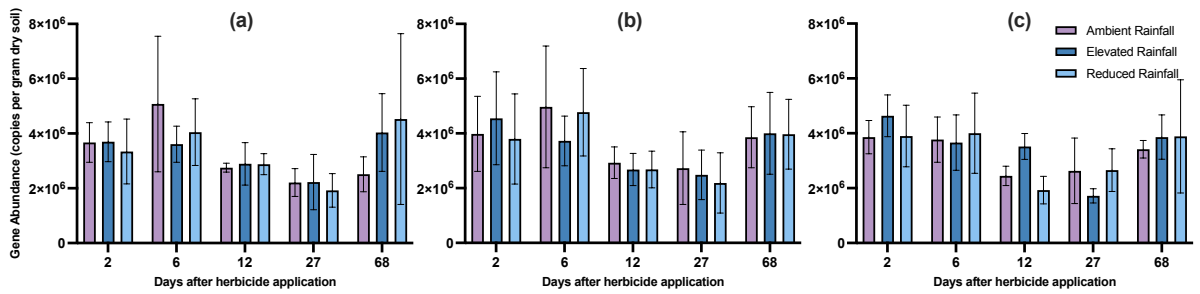

Figure 32. *Dynamics of fungal 28S rRNA gene abundance in surface soil (0–5 cm) under ambient, elevated (+30%), and reduced (−30%) rainfall treatments across crop systems: (a) beans monoculture, (b) wheat monoculture, and (c) beans–wheat intercrop following glyphosate application. Error bars represent standard error of the mean (n = 4).*

The dynamics of microbial populations were examined by quantifying 16S and 28S rRNA gene abundances across three cropping systems (beans, wheat, and beans-wheat intercrop) under different rainfall treatments (ambient, elevated 30%, and reduced -30%) following glyphosate application. Statistical analyses using one-way ANOVA indicated no significant differences ($p > 0.05$) among rainfall treatments for either bacterial (16S rRNA) or fungal (28S rRNA) gene abundances across all sampling dates and cropping systems (Figures 31 and 32). This suggests that moderate alterations in rainfall input alone did not significantly impact soil microbial population abundance at the community level within the observed timeframe.

For bacterial abundance (16S rRNA), the trends were notably fluctuating without a consistent pattern across time points or rainfall treatments. Bacterial gene copy number varied intermittently throughout the season, with no clear correlation to the timing of herbicide application, plant growth, or specific precipitation regimes. This irregularity likely reflects the inherent responsiveness of bacterial communities to short-term shifts in soil microenvironments, such as changes in moisture, substrate diffusion, or nutrient pulses (Fierer et al., 2007; Shade et al., 2012). Although no major disturbance events (e.g., tillage or additional chemical inputs) occurred during most of the study period, minor environmental heterogeneities and microbial succession dynamics may still have contributed to temporal variability. Moreover, the disturbance at the end of the season—including possible shifts due to late-stage root senescence or drying soils—could have introduced additional fluctuations in bacterial abundance.

In contrast, fungal abundance (28S rRNA) exhibited a more discernible temporal trend. A marked decline was observed around 12- and 27-days post-application, while the remaining time points generally showed higher abundance. This decrease in fungal gene abundance may indicate a delayed stress response to glyphosate exposure or transient changes in the rhizosphere environment that disrupt fungal colonization or activity (Zaller et al., 2014). Fungi, due to their filamentous growth and reliance on stable organic substrates, often display slower but more persistent responses to environmental change than bacteria (Treseder, 2008). The subsequent recovery of fungal populations suggests resilience and potential adaptation to herbicide-induced stress and fluctuating moisture levels.

Overall, these results highlight the distinct ecological strategies of bacteria and fungi in response to combined chemical and hydric stress. While bacterial communities appear more opportunistic, fungal communities respond in a more structured and resilient manner. This distinction is critical for understanding and predicting how soil microbial community's mediate nutrient cycling and ecosystem stability under shifting precipitation patterns and agricultural inputs (Allison & Martiny, 2008).

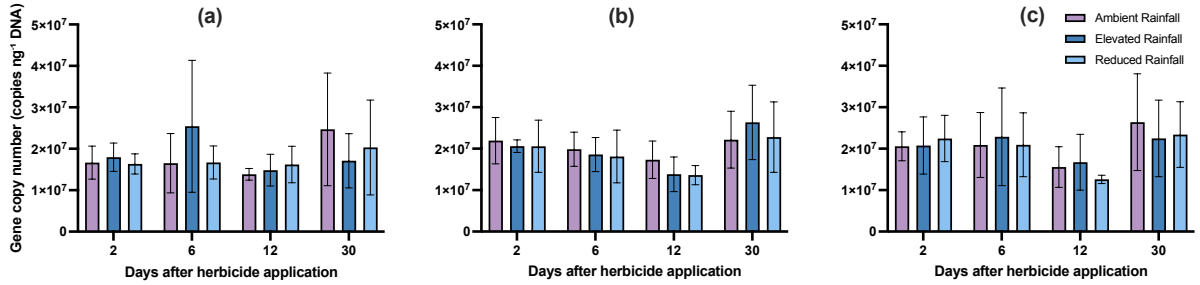

Figure 33. *Dynamics of goxA gene abundance in surface soil (0–5 cm) under ambient, elevated (+30%), and reduced (−30%) rainfall treatments across crop systems: (a) beans monoculture, (b) wheat monoculture, and (c) beans–wheat intercrop following glyphosate application. Error bars represent standard error of the mean (n = 4).*

The abundance of the *goxA* gene (copies ng^{-1} DNA), which encodes glyphosate oxidoreductase responsible for the first step in glyphosate degradation (Godínez et al., 2021), remained relatively stable over time across all rainfall treatments (ambient, elevated, and reduced) following herbicide application (Figure 33). Statistical analysis showed no significant differences in *goxA* abundance among rainfall treatments (one-way ANOVA, $p = 0.826$), and no clear increasing or decreasing

trends were observed during the 30-day monitoring period. This suggests that microbial populations harboring *goxA* genes persisted at a baseline level regardless of fluctuations in soil moisture due to rainfall manipulation.

Although glyphosate concentrations showed a general downward trend over time under all treatments (Figure 27), the correlation between *goxA* abundance and glyphosate concentration was weak and not statistically significant ($r = 0.063$, $p = 0.468$). This indicates that the temporal dynamics of glyphosate dissipation were not directly mirrored by changes in *goxA* gene copy number. Similar findings have been reported in prior studies where *goxA* presence did not always scale with glyphosate input, possibly due to microbial community resilience or functional redundancy (Mäder et al., 2024; Wirsching et al., 2022).

The stable *goxA* levels may reflect constitutive expression or ecological buffering, whereby microbial communities retain degradation potential even after substrate levels decline (Sviridov et al., 2015). Furthermore, environmental factors such as soil structure, pH, carbon availability, or oxygen diffusion—which are indirectly affected by rainfall—may play a larger role in regulating microbial gene abundance than herbicide concentration alone (Grundmann et al., 2008; Bento et al., 2016). Additionally, it is plausible that *goxA*-containing microbes' function more slowly or switch to alternate phosphorus sources under fluctuating moisture conditions (Feng et al., 2020).

Taken together, the results indicate that rainfall treatments influenced the pace of glyphosate dissipation but had minimal effect on the genetic potential for microbial degradation as measured by *goxA* abundance. This highlights the importance of integrating both chemical and genetic indicators to accurately assess the fate of herbicides under changing environmental conditions.

4.4 Conclusions

This study explored the impacts of moderate rainfall manipulation (±30%) on crop physiological responses, herbicide degradation, and soil microbial abundance in a temperate agroecosystem over the 2024 growing season. Despite consistent treatment application over three years, results showed no statistically significant differences in soil moisture content, crop phenology, transpiration rate, NDVI, or yield across precipitation treatments. Similarly, glyphosate and AMPA concentrations, as well as microbial gene abundances (16S rRNA & 28S rRNA genes, and *goxA*), remained largely unaffected by rainfall variation. Nonetheless, subtle trends were observed: elevated rainfall tended to accelerate early phenological stages and slightly increase beans yield, while reduced rainfall

often coincided with lower NDVI and fungal abundance. These trends, although not statistically robust, indicate that plant and microbial systems may exhibit directional but buffered responses to moderate hydrologic perturbations under field conditions.

Several limitations may have constrained the detection of stronger treatment effects. First, the spatial and temporal variability of natural rainfall during the study period—characterized by sporadic high-intensity events—may have obscured treatment-induced differences in soil moisture. Second, the sandy loam soil at the experimental site likely limited water retention, causing added rainfall to drain rapidly and reducing the effective contrast between treatments. Third, site-specific heterogeneity in soil texture—such as higher clay content observed in one block—may have altered water retention, microbial activity, or herbicide dynamics in ways not captured by the treatment design. Lastly, the 30-day monitoring window post-glyphosate application may have been insufficient to fully capture microbial succession or long-term degradation processes, particularly for slow-responding taxa or in deeper soil layers.

5. Scholarly Discussion

This thesis investigated the effects of climate change-induced environmental stressors on agroecosystem dynamics, with a particular focus on soil physicochemical properties, microbial abundance, herbicide degradation, and crop physiological responses. Through two field-based experiments, this work examined the influence of elevated soil temperature (+2.5 °C) and altered precipitation regimes (±30% relative to ambient) in a temperate agricultural system in Quebec, Canada. Although the core experimental chapters addressed these variables separately, this final chapter integrates and reflects upon the overarching themes that emerged, discusses the methodological and conceptual limitations of the research, and proposes pathways for future investigations and practical applications.

5.1 Overarching Themes and Patterns

Across both experimental chapters, the findings revealed relatively muted effects of the imposed treatments on the measured soil, crop, and microbial parameters. In the elevated temperature experiment, the application of in-ground heating led to a consistent increase of ~2.5 °C in soil surface temperature throughout the growing season. However, the responses of soil moisture

content, pH, greenhouse gas fluxes, and microbial gene abundance were generally modest and statistically non-significant. While elevated temperature did appear to marginally reduce volumetric water content (VWC) during mid-season—likely due to enhanced evapotranspiration—this reduction did not translate into significant stress signals for microbial communities or soil chemical parameters. A weak but noticeable increase in CO_2 efflux was observed in July, possibly reflecting temperature-induced stimulation of microbial respiration, yet this effect diminished in later sampling periods.

In the rainfall manipulation experiment, ambient rainfall was systematically altered using a rainout shelter and drip irrigation system to simulate ±30% deviations from normal precipitation. Over the course of the growing season, rainfall treatments had minimal effects on crop phenology, yield, NDVI, or transpiration rate. Glyphosate degradation and AMPA formation followed expected temporal patterns, with concentrations declining over time across all treatments. The relative abundance of microbial genes associated with herbicide degradation (e.g., goxA) and overall microbial biomass (16S and 28S rRNA) remained stable across rainfall conditions, suggesting limited microbial sensitivity to short-term shifts in soil moisture availability. The lack of strong microbial or herbicide-related responses may reflect the system's capacity for ecological buffering, or potentially the relatively moderate magnitude and duration of the imposed treatments.

Taken together, the findings from both experiments underscore an important emergent theme: the resilience of temperate agroecosystems to moderate short-term climate stressors. While environmental manipulations did produce directional trends in some variables (e.g., reduced VWC, elevated CO_2 flux, glyphosate degradation), these effects were often small, inconsistent, or statistically non-significant. This suggests that the combination of plant physiological plasticity, microbial community redundancy, and soil structural characteristics may act in concert to stabilize ecosystem functioning under mild perturbations.

5.2 Interpretation of Microbial and Herbicide Dynamics

One of the central goals of this research was to examine how climate variability influences the fate of glyphosate and its microbial degradation pathways. Across both experiments, glyphosate concentrations declined with time, and AMPA concentrations increased initially before tapering

off, consistent with known degradation kinetics in aerobic surface soils. The goxA gene, a marker of glyphosate oxidoreductase activity, was consistently detected in all samples but did not show significant changes in relative abundance in response to either elevated temperature or altered rainfall. Similarly, 16S and 28S rRNA gene copy numbers remained stable across treatments.

These findings indicate that microbial potential for glyphosate degradation, as represented by goxA gene abundance, may be relatively insensitive to the short-term and moderate environmental changes simulated in this study. It is possible that microbial communities in this agricultural soil were already adapted to variable moisture and temperature conditions and were thus capable of maintaining functional activity under modest perturbations. Alternatively, functional redundancy among microbial taxa may allow ecosystem processes such as glyphosate degradation to persist even as community composition fluctuates. These interpretations align with prior research suggesting that while microbial structure can be sensitive to environmental shifts, microbial function often demonstrates greater resilience due to redundancy and dormancy strategies.

Importantly, the persistence of AMPA—an environmental transformation product of glyphosate—underscores the need for continued monitoring of herbicide degradation products under changing climate conditions. While glyphosate itself degraded predictably, the longer-term dynamics of AMPA accumulation and dissipation, particularly under combined stressors or prolonged exposure, remain poorly understood and merit further investigation.

5.3 Methodological and Conceptual Limitations

While the findings of this thesis offer useful insights, several limitations must be acknowledged. First, the duration of both experiments was limited to a single growing season. Microbial responses to environmental stress often occur over longer timescales as communities shift in composition and function. Therefore, the short duration of treatment exposure may not have been sufficient to capture delayed or cumulative microbial responses.

Second, natural environmental variability, particularly in rainfall, likely diminished the contrast between treatment groups in the precipitation manipulation experiment. Despite the rainout and

irrigation systems, natural precipitation events—especially during stormy periods—led to convergence in soil moisture levels between treatment plots, thereby reducing the experimental power to detect differences in microbial or crop responses.

Third, the use of gene copy numbers as proxies for microbial abundance and functional potential, while informative, does not necessarily reflect gene expression or enzymatic activity. qPCR-based quantification provides a snapshot of microbial potential, but additional methods such as reverse transcription qPCR (RT-qPCR), enzyme assays, or metatranscriptomics would be needed to verify whether elevated temperature or altered moisture regimes directly influence microbial degradation activity.

Fourth, the experimental design, while fully factorial, was constrained by a relatively small number of replicates (n = 3–4 per treatment), which limits statistical power and increases the risk of Type II error. High variability in field conditions—including soil heterogeneity, plant establishment, and microclimatic fluctuations—further complicates interpretation. Future experiments should consider larger sample sizes and more intensive temporal sampling to capture transient responses.

Finally, due to logistic delays, the preliminary trial conducted in 2023 was not fully implemented until after the peak growing season, limiting the quantity and quality of data collected. While select observations from this pilot trial were included in the supplementary material, the results were not integrated into the main analysis, reducing the study's temporal scope.

5.4 Practical Implications

Despite these limitations, the results of this thesis carry several practical implications for climate adaptation in agriculture. The apparent robustness of soybean and wheat cropping systems under moderate climatic variation suggests that such systems may not require immediate modification under current or near-term climate projections. In particular, the stability of glyphosate degradation and microbial gene abundance across treatments indicates that existing pesticide management practices may remain effective under moderately warmer or wetter/drier conditions.

Moreover, the findings suggest that temperate agroecosystems characterized by well-drained, coarse-textured soils may be particularly buffered against hydrological stress, at least in the short term. This has important implications for the design of future farming systems and for selecting management practices that enhance resilience without excessive intervention. For instance, the consistent microbial and herbicide responses observed here imply that land managers may prioritize interventions such as organic matter enhancement, reduced tillage, or cover cropping to address long-term sustainability rather than short-term climate volatility.

Furthermore, the methodological framework developed in this thesis—integrating soil, plant, microbial, and chemical indicators—provides a replicable template for climate impact assessment in agricultural contexts. By adopting a multi-scale, interdisciplinary approach, future studies can build upon this foundation to assess the broader ecological and agronomic consequences of environmental change.

5.5 Future Research Directions

Building on the findings and limitations of this thesis, several avenues for future research can be identified. Longitudinal studies spanning multiple growing seasons and crop rotations are essential to capture slow-developing responses, cumulative impacts, and microbial adaptation processes. Multi-year trials would also allow researchers to distinguish between transient versus persistent effects of climate variables on soil and crop systems.

In addition, future experiments should consider more extreme or compounding stress scenarios. While this study examined moderate warming and rainfall shifts, future research could include acute drought periods, heatwaves, or combined nutrient and water stress to better simulate future climate extremes projected by global circulation models. These conditions may reveal thresholds beyond which system resilience begins to erode.

From a methodological perspective, incorporating functional metagenomics and transcriptomics would enable deeper insight into microbial adaptation mechanisms, gene expression dynamics, and pathway-level responses to stressors. Such approaches could clarify whether stable gene abundance reflects functional stability or simply the presence of dormant or inactive microbial taxa.

Additional research should also explore the interactions between agronomic practices and climate responses. For example, the use of cover crops, biochar, compost amendments, or reduced tillage may modify the sensitivity of microbial and herbicide dynamics to environmental stress. Understanding these interactions would help design integrated management strategies that enhance both climate resilience and agroecological sustainability.

Finally, future work should consider incorporating socio-economic dimensions—such as farmer perceptions, economic trade-offs, and risk thresholds—to contextualize biophysical findings within real-world decision-making. This would enhance the translational value of scientific research and support evidence-based policy and practice.

5.6 Conclusion

In conclusion, this thesis provides empirical evidence that temperate agroecosystems may exhibit substantial resilience to moderate short-term shifts in temperature and precipitation. Despite consistent application of environmental treatments, the observed impacts on soil moisture, microbial gene abundance, glyphosate degradation, and crop physiological performance were limited in magnitude and often statistically non-significant. These findings challenge the assumption that all components of agroecosystems will respond uniformly and rapidly to climate change and instead suggest a nuanced picture of system-specific and scale-dependent responses.

The research also highlights key methodological and interpretive challenges in field-based climate studies, including the need for long-term monitoring, high-resolution microbial analyses, and adaptive experimental designs. By integrating insights across disciplines and temporal scales, future studies can advance our understanding of climate–soil–plant–microbe interactions and inform resilient agricultural management strategies in a changing world.

6. Conclusions

This study investigated the short-term effects of climate change on agroecosystems by evaluating how elevated soil temperature (+2.5 °C) and altered rainfall regimes (−30%, ambient, +30%) influenced soil properties, microbial abundance, herbicide degradation, and crop physiological responses in a temperate agricultural setting in Quebec, Canada. Despite the application of sustained treatments across multiple growing seasons, the findings revealed limited statistically

significant differences across most variables tested. Elevated temperature modestly reduced soil moisture and increased mid-season CO_2 flux but did not significantly affect soil pH, N_2O flux, or microbial abundances. Similarly, rainfall manipulation had no significant impact on crop phenology, transpiration rate, NDVI, yield, or microbial gene copy numbers (16S, 28S rRNA, and goxA). Glyphosate degradation followed expected temporal declines, with AMPA levels remaining stable, and both compounds exhibited no statistically significant differences among rainfall treatments. These results suggest a high degree of ecological buffering in these systems, with temperate crops and microbial communities exhibiting resilience to moderate, short-term climatic perturbations.

These studies encountered several limitations that may have constrained the detection of treatment effects. High natural variability in precipitation, coupled with episodic high-intensity rainfall events, likely reduced the contrast among rainfall treatments and limited their influence on soil moisture. Moreover, the predominance of sandy loam soils at the experimental site—with high porosity and low water-holding capacity—may have diminished the effects of both rainfall addition and retention. Considerable block-level heterogeneity was observed, with some replicates exhibiting higher clay content that may have retained more moisture and influenced plant and microbial responses locally. Additionally, the short duration of temperature treatment may not have been sufficient to elicit measurable shifts in slow-responding variables such as nutrient cycling and microbial abundance. These factors underscore the challenges of detecting subtle climate treatment effects in complex field settings and highlight the importance of replicate and long-term observation.

Future research should consider longer experimental durations and finer-scale assessments of soil texture and microtopography to better isolate treatment effects. Integrating metagenomic or transcriptomic tools could help uncover functional shifts in microbial communities that are not captured through gene abundance alone. Furthermore, incorporating crop varieties with contrasting stress responses, and monitoring additional indicators such as enzyme activity, soil aggregate stability, or root dynamics, could enhance understanding of adaptive responses in agroecosystems. As climate change intensifies, multi-factorial and site-specific research will be essential for developing resilient cropping strategies and sustainable land management practices in diverse agricultural landscapes.

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8. Appendix

To investigate the effects of elevated temperature, increased precipitation, and cover cropping on soil microbial dynamics, the field experiment was established in 2023 at the lysimeter research site on the Macdonald Campus of McGill University (45°24′48.6″ N, 73°56′28.1″ W). Twenty-four 2 × 2 m plots were filled with sandy loam soil (814 g sand kg^{-1}, 147 g silt kg^{-1}, 38 g clay kg^{-1}) and arranged with buffer zones to minimize edge effects. Half of the plots received continuous soil warming of +2.5 °C using buried heating coils at ~15 cm depth, while the others served as ambient controls. A full-factorial design tested the interactions of warming, 30% increased precipitation, and cover crop (CC), resulting in eight treatment combinations with three replicates each (Figure A1). Although a preliminary trial was initiated in 2023, the late application of environmental treatments (starting September 1) limited data collection.

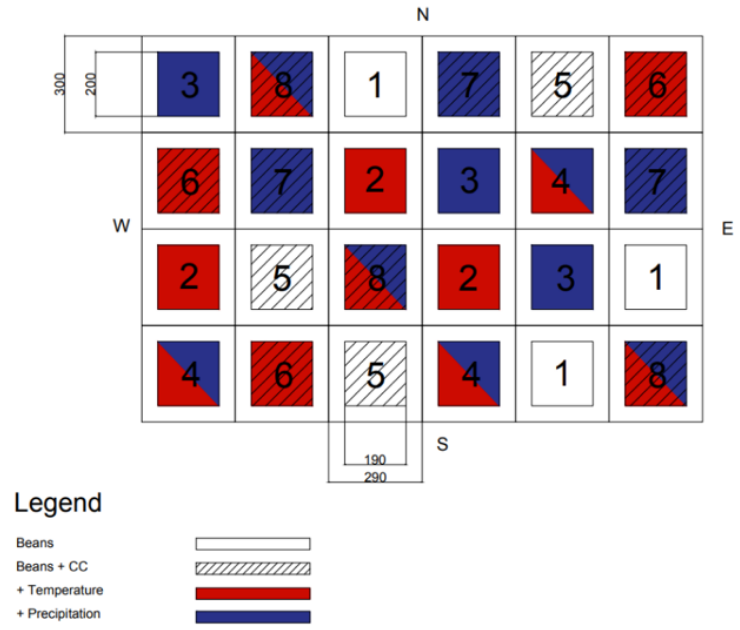

Figure A1: Spatial layout of the 2023 field experiment investigating the effects of elevated temperature, increased precipitation, and cover cropping on soil microbial dynamics. Plots were randomly assigned to eight treatments: (1) Beanss, (2) Beanss + 2.5 °C, (3) Beanss + +30% rainfall, (4) Beanss + 2.5 °C + 30% rainfall, (5) Beanss + CC, (6) Beanss + CC + 2.5 °C, (7) Beanss + CC + 30% Rainfall, (8) Beanss + CC + 2.5 °C + 30% rainfall.

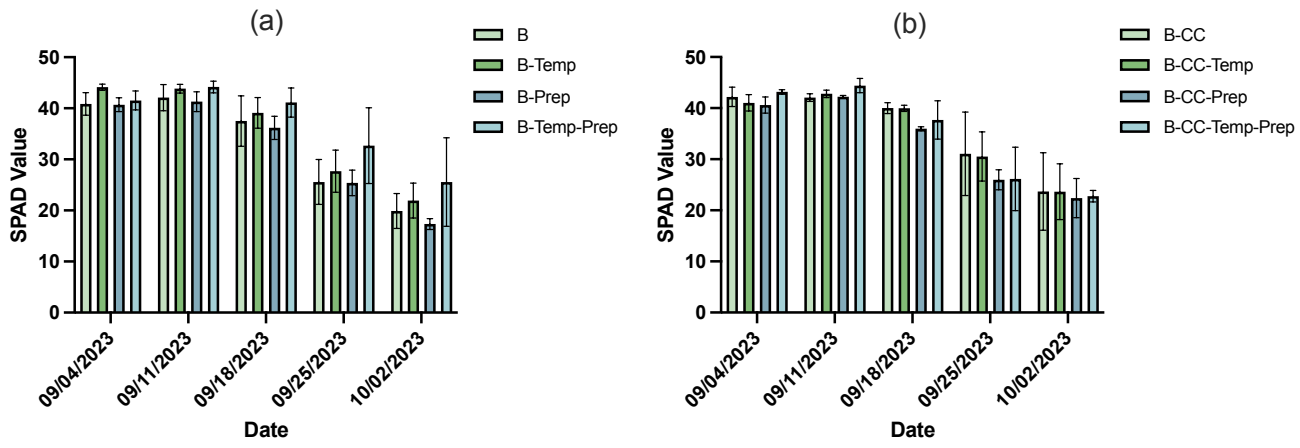

Figure A2. SPAD readings of beans plants under different temperature, precipitation, and cover crop treatments across sampling dates in 2023. (a) Beans monoculture plots (B); (b) Beans + cover crop plots (B-CC). Temperature (Temp) and precipitation (Prep) treatments started on 09/01/2023 (beans and cover crop seeding date: 07/18/2023). Each error bar represents ± one standard deviation (n = 3).

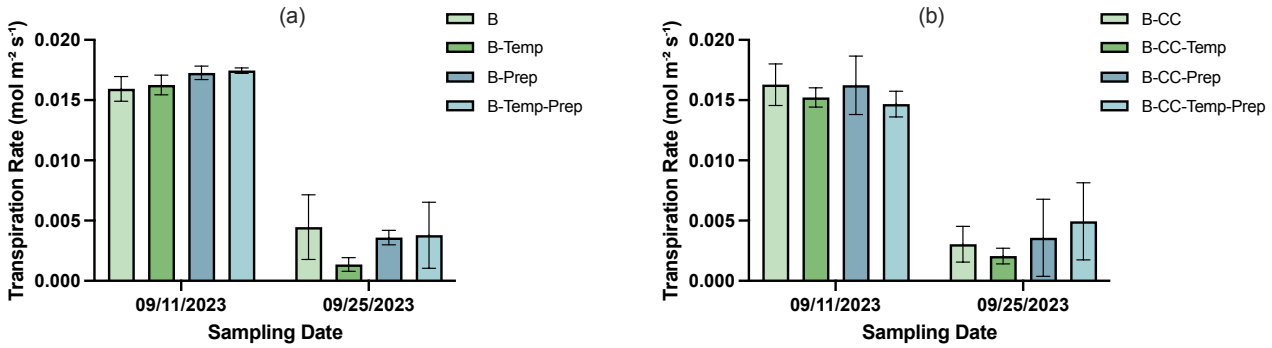

Figure A3. Transpiration rate (mol m^{-2} s^{-1}) of beans plants (n= 3) under different temperature, precipitation, and cover crop treatments during the 2023 growing season. (a) Beans monoculture plots (B); (b) Beans + cover crop plots (B-CC). Temperature (Temp) and precipitation (Prep) treatments started on 09/01/2023 (beans and cover crop seeding date: 07/18/2023). Each error bar represents ± one standard deviation (n = 3).

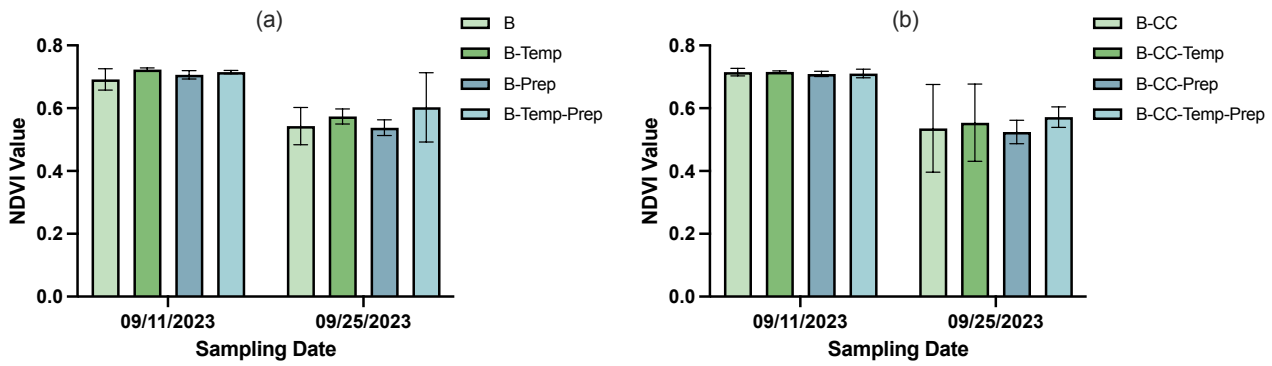

Figure A 4. NDVI values of beans plants (n= 3) under different temperature, precipitation, and cover crop treatments during the 2023 growing season. (a) Beans monoculture plots (B); (b) Beans + cover crop plots (B-CC). Temperature (Temp) and precipitation (Prep) treatments started on 09/01/2023 (beans and cover crop seeding date: 07/18/2023). Each error bar represents ± one standard deviation (n = 3).

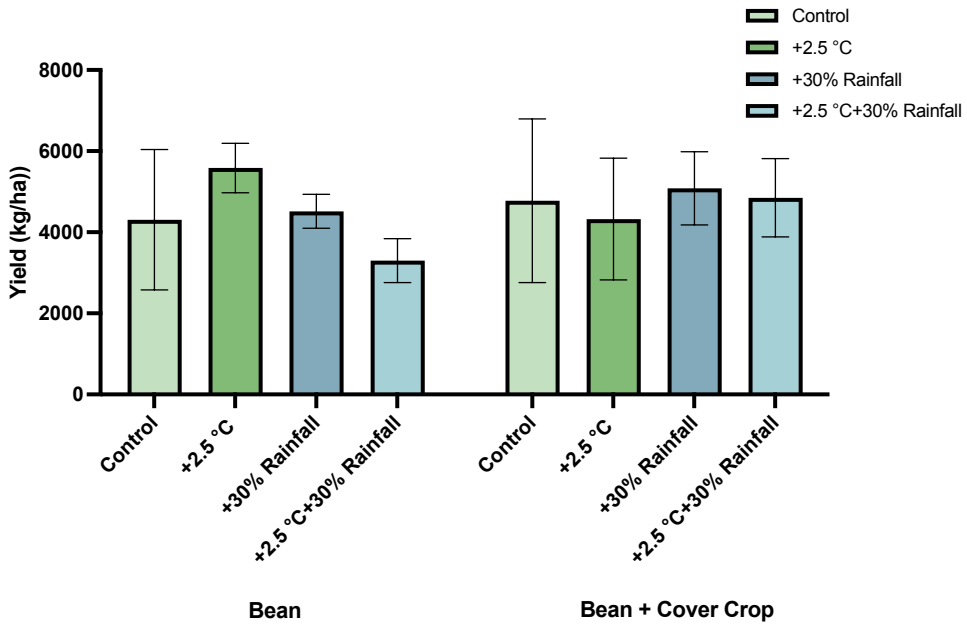

Figure A5 Beans yield (kg ha^{-1}) (n= 3) under different temperature (+2.5 °C), precipitation (+30%), and cover crop treatments in the 2023 field experiment after 86 days of growth. Each error bar represents ± one standard deviation (n = 3).

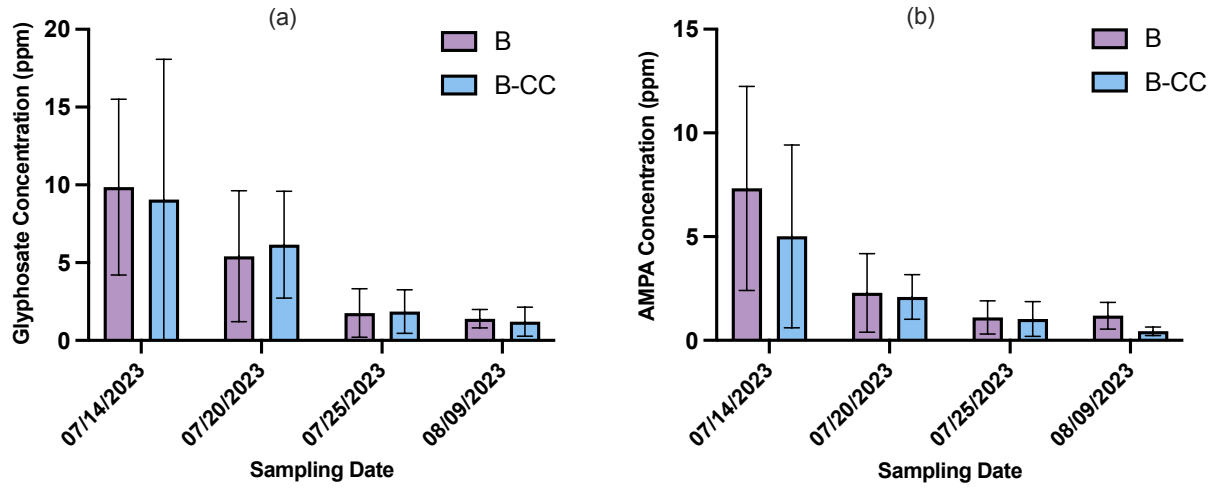

Figure A6 Glyphosate and AMPA concentration (ppm) of beans plants (n= 3) under cover crop treatments across sampling dates in 2023. (a) glyphosate concentration (ppm); (b) AMPA concentration (ppm). Herbicide application date: 07/12/2023. Each error bar represents ± one standard deviation (n = 3).

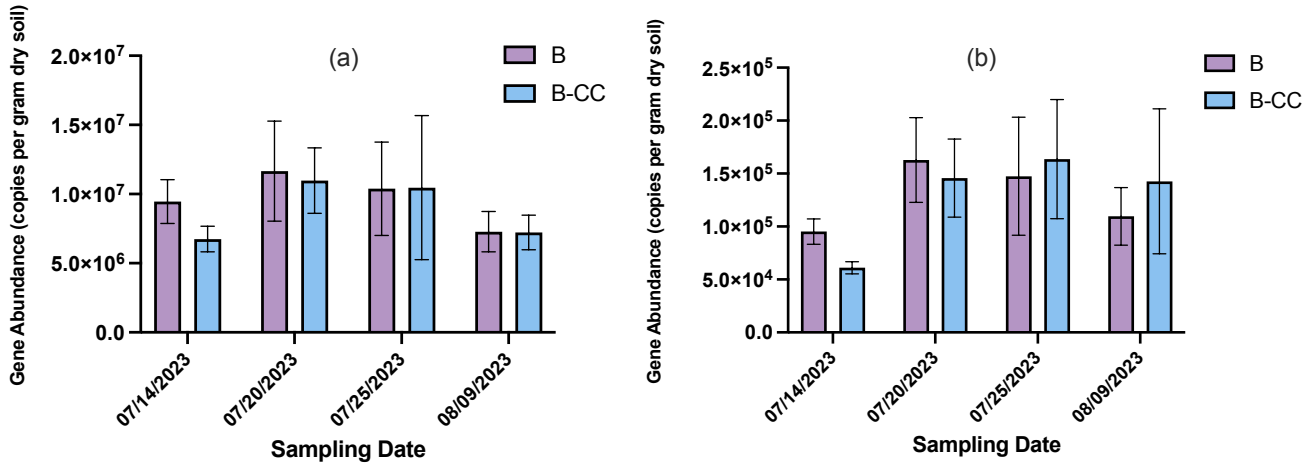

Figure A7 Soil microbial abundance of beans plants under cover crop treatments across sampling dates in 2023. (a)16S rRNA gene abundance; (b)28S rRNA gene abundance. Herbicide application date: 07/12/2023. Each error bar represents ± one standard deviation (n = 3).