Evaluating the role of ecosystem processes in the enhancement of soil carbon stocks and functional resilience

Final report for research project 4.1.005

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PEER REVIEW STATEMENT

The Soil CRC recognises the value of knowledge exchange and the importance of objective peer review. It is committed to encouraging and supporting its research teams in this regard.

The author(s) confirm(s) that this document has been reviewed and approved by the project's steering committee and by its program leader. These reviewers evaluated its:

- originality
- methodology
- rigour
- compliance with ethical guidelines
- conclusions against results
- conformity with the principles of the Australian Code for the Responsible Conduct of Research (NHMRC 2018), and provided constructive feedback which was considered and addressed by the author(s).

KEYWORDS

Drought resilience; Soil organic matter; Soil texture and pH; Soil microbial respiration; Soil microbial functional resilience index; Ecosystem-based management practices

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CONTENTS

EXECUTIVE SUMMARY

Soil health is defined as the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals and humans. This concept attracted significant attention as a potential approach to assess dynamics in soil biological function and resilience to stresses. Many farming systems are interested in using ecosystem-based management practices to enhance soil health and resilience. There is also an increasing interest in better understanding the functionalities of soil organic carbon (SOC) and their role in improvement of soil resilience and productivity. Ecosystem-based management can increase soil carbon storage, nutrient bioavailability and microbial biodiversity. However, the yield responses may also depend on environmental factors and soil initial properties. Therefore, proper understanding of soil functional health and resilience is essential to the sustainability of the agricultural ecosystem. This project assessed the role of ecosystem-based managements on quality and quantity of SOC and soil resilience. The project used the concept of soil resistance (ability of soil to maintain its functional stability after disturbance) and resilience (speed with which soil system can return to its pre-disturbance level) developed in Soil CRC Project 4.1.003 (led by Dr Rezaei Rashti) to assess the changes to soil function and resilience in long-term Soil CRC field sites. Here we provide brief summaries of the key findings from each chapter.

Chapter 1: A Meta-analysis of the Role of Edaphic Variables and Management Practices in Regulating Soil Microbial Resilience to Drought

Disturbances to the environment caused by natural events and human activities can impact the resistance and resilience of soil microbial functional activities. A paucity of information exists on the impact of drought stress on soil and how soil biological systems respond to and demonstrate resilience to stress. To address this, we conducted a systematic review and meta-analysis to assess the response of soil microbial biomass and respiration to drought stress. The research found that applying drought stress had a negative impact on microbial respiration in agriculture, forest and grassland ecosystems, resulting in decreased microbial activity (overall negative effect size). However, during the resilience phase, there was an increase in respiration, indicating a recovery and an overall positive effect. Conversely, microbial biomass showed a negative effect size during both the resistance and resilience phases, suggesting a decrease in the microbial biomass. In agricultural systems, soil pH was primarily correlated with soil respiration resistance and resilience, potentially influenced by frequent land preparation and fertiliser applications.

The microbial communities are essential components of soil ecosystem that play vital roles in maintaining soil fertility, nutrient cycling and plant productivity. Environmental stresses such as drought can severely affect soil microbial biomass and respiration, with negative impacts on soil fertility and ecosystem functioning. This meta-analysis has shown that soil microbial respiration can recover with the cessation of drought in different environmental conditions. This recovery may be due to increased moisture availability and increase in readily available organic carbon which can stimulate microbial activity and restore respiration rates to pre-drought levels. However, the recovery of microbial biomass was often more limited or slower than that of respiration. This might be due to microbial biomass requiring more time to rebuild after periods of stress, particularly if the initial stress has caused some microorganisms to die off. Resistance and resilience of microbial biomass and respiration in response to drought can vary depending on the intrinsic soil properties of a particular land-use type. Soils with higher organic matter content and nutrient availability may have more resilient microbial communities as these soils provide more resources for microbial growth and activity. Similarly, soils with a more favourable texture, such as loam or clay, may also have greater resistance and resilience to stress as these soils can retain moisture more effectively than sandy soils. Overall, the response of soil microbial

communities to drought is a complex process that is influenced by many factors. Therefore, understanding the underlying mechanisms that drive these responses, such as intrinsic soil properties and microbial community dynamics, can help to identify the land management practices that promote soil health and resilience.

Chapter 2: Unveiling Soil Health: Initial Assessment After Implementing Ecosystem-Based vs Conventional Management Practices

This chapter investigated the impact of various agricultural management practices on soil properties and microbial dynamics across different sites. While most paired sites showed no significant differences in water-holding capacity, exceptions highlighted the significance of practices like minimum tillage, lime application, and cover crop management, crucial for sustaining agricultural productivity during water stress periods. Soil pH and electrical conductivity varied across management practices, influenced by factors like organic matter input, tillage methods, and soil amendments. Total carbon and total nitrogen contents also varied significantly among paired sites, highlighting the influence of specific practices on soil fertility. Hot water extractable organic carbon (HWEOC) and hot water extractable nitrogen (HWEON) content were notably affected by cover crop management and tillage practices, emphasising the role of organic matter input and soil disturbance in shaping soil organic dynamics. Total mineral nitrogen content varied between management approaches, influenced by factors such as cover crop management, tillage practices, and planting methods. Microbial dynamics, including basal respiration, functional diversity, microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and metabolic quotient (qCO_2), exhibited significant variations across management practices, reflecting the complex interactions between soil management techniques and microbial activity. Overall, the study underscores the importance of altered soil management strategies considering site-specific factors to optimise soil health, fertility, and microbial dynamics for sustainable agricultural practices. The findings highlighted the critical role of specific strategies such as minimum tillage, cover crop management, lime application, and organic matter input in influencing soil biogeochemical properties. These results underscore the intricate interplay between management practices, soil properties, and microbial dynamics, emphasising the need for tailored approaches to soil management based on site-specific conditions and objectives.

Chapter 3: Assessing Soil Biological Resistance and Resilience to Drought Stress

This chapter investigated the impact of various agricultural management practices on soil microbial functional resistance and resilience to drought stress in several Soil CRC long-term trials. The collective findings from investigation into soil responses to stress conditions and diverse management practices underscored the pivotal role of strategic soil management in promoting resilience and nutrient dynamics within ecosystems. The Bednar method emerged as an effective strategy for mitigating the adverse effects of environmental stressors, maintaining stable microbial processes, and enhancing MBC and MBN concentrations, particularly under moderate drought stress. Similarly, cover crop management demonstrated promise in bolstering soil resilience and microbial functioning, evident through increased resistance and resilience indices, and improved nutrient retention under moisture stress conditions. Moreover, lime treatment showed significant potential in enhancing soil health and resilience to drought stress, offering insights into its capacity to mitigate nitrogen loss and facilitate nutrient cycling. These findings collectively emphasise the importance of sustainable soil management practices in fostering resilient ecosystems amidst climate change challenges and highlight the need for further research to refine and optimise these strategies for long-term environmental conservation and agricultural sustainability.

Chapter 4: Impact of Soil Compaction and its Alleviation on Soil Microbiome Resilience and Soil Health in Agricultural Systems

This chapter investigated the effects of soil compaction stress and tillage intensity on soil respiration, microbial biomass, and nutrient dynamics. Results from the soil incubation experiment revealed no significant differences in cumulative CO_2 emissions between compaction treatments and controls under both minimum and excessive tillage management during the resistance phase, indicating that initial soil response to compaction is not heavily influenced by tillage intensity. Following stress removal, minimal tillage exhibited no significant differences until Day 25, whereas severe stress under minimal tillage resulted in lower CO_2 emissions by Day 28. Moderate stress under minimum tillage had the highest respiration resistance index, whereas severe stress under excessive tillage had the lowest. Resilience indices did not significantly differ among treatments in either tillage regime. Substrate-induced respiration showed no significant differences among compaction treatments by the end of the resilience phase, suggesting microbial adaptation over time. MBC and MBN content varied, with moderate compaction under minimum tillage enhancing MBC, while excessive tillage generally resulted in lower MBC. Microbial qCO_2 was higher under excessive tillage due to disrupted soil structure. HWEOC and HWEON content were more affected by compaction in minimum tillage, with better recovery during the resilience phase. Total mineral nitrogen content did not differ significantly between compaction treatments and controls during the resistance phase but was higher in moderate stress treatments under minimum tillage. The results highlighted the complex interplay between compaction stress and tillage management on soil CO_2 emissions, microbial activity, and nutrient dynamics. Despite initial similarities in cumulative CO_2 emissions across stress treatments and tillage practices, the long-term impacts of severe compaction stress under minimum tillage were evident, demonstrating reduced microbial activity and altered soil structure. Moderate compaction under minimum tillage appeared to enhance soil resilience, supporting higher microbial biomass and more efficient nutrient cycling. In contrast, excessive tillage consistently disrupted soil structure and microbial habitats, leading to lower resistance and resilience indices, particularly under severe stress conditions. The study also revealed that MBC and MBN, as well as substrate-induced respiration, adapted over time, reflecting microbial community recovery.

Chapter 5: Evaluating the Effects of Drought and Compaction Stress on Soil Resistance, Resilience, and Plant Growth: A Pot Experiment

This chapter investigated the impact of various soil management practices on microbial and nutrient dynamics, focusing on MBC, MBN, respiration, qCO_2, enzyme activities, nitrogen content and plant growth under different drought stress conditions. Soils from diverse management histories, including cover cropping, minimum tillage, conservation tillage, lime treatments, Bednar treatment along with corresponding conventional management approaches were studied. The results indicated that ecosystem-based practices, such as cover cropping and minimum tillage, generally enhanced microbial resilience, improved nutrient availability, and supported better plant growth compared to conventional and excessively tilled soils. Specifically, cover cropping increased microbial biomass and enzyme activity, particularly under moderate stress conditions, while minimum tillage improved nutrient retention. Additionally, shoot nitrogen content was consistently higher in cover cropping soils, while conventional soils showed higher shoot carbon content under stress. Lime treatment stabilised soil pH and microbial activity, although its effects varied with stress severity. Contrary to initial assumptions, Bednar treatments did not negatively impact plant productivity, although they showed variable effects on microbial dynamics and enzyme activities.

This study showed that cover cropping, and minimum tillage improved microbial resilience and enzyme stability, enhancing soil health and nutrient cycling. Conversely, conventional management and excessive tillage were associated with reduced microbial activity and enzyme functions under drought stress, highlighting the advantages of sustainable practices in maintaining soil functionality. Cover cropping and minimum tillage generally led to better nitrogen retention and higher plant biomass and nutrient uptake under stress whereas conventional and excessively tilled soils showed less resilience and lower nutrient availability.

The knowledge generated in this project would be critical for farmers, advisors and farming groups that work towards adoption of ecosystem-based management practices for the re-engineering of stressed soils for higher crop productivity and farm profitability. In the longer term, the knowledge generated in this project would have broader application across Australian grower group networks and agricultural industries.

OBJECTIVES

- To better understand and monitor the effect of ecosystem processes in existing Soil CRC field sites, and on the improvement of soil carbon stocks and soil functional resilience to environmental stresses.
- To quantify the changes in soil microbial functional resistance and resilience to key environmental stresses as induced by the ecosystem-based management practices in long-term field sites within the Soil CRC.
- To better understand the limitations in the adoption of farming practices that may result in more resilient soils and make recommendations on when these systems would be most likely to deliver positive outcomes for farmers.

RESULTS

Many farming systems are interested in using ecosystem-based management practices to enhance soil health and resilience. There is also an increasing interest in better understanding of the functionalities of SOC and their role in improvement of soil resilience and productivity. Ecosystem-based management can increase soil carbon storage, nutrient bioavailability, and microbial biodiversity. This project investigated the impact of various soil management practices on microbial and nutrient dynamics, focusing on soil microbial biomass, respiration, qCO_2, enzyme activities, nitrogen content, and plant growth under different drought stress conditions. Soils from diverse management histories, including cover cropping, minimum tillage, conservation tillage, lime treatments and Bednar treatment, along with corresponding conventional management approaches, were studied. The results indicated that ecosystem-based practices, such as cover cropping and minimum tillage, generally enhanced microbial resilience, improved nutrient availability, and supported better plant growth compared to conventional and excessively tilled soils. Specifically, cover cropping increased microbial biomass and enzyme activity, particularly under moderate stress conditions, while minimum tillage improved nutrient retention. Additionally, shoot nitrogen content was consistently higher in cover cropping soils, while conventional soils showed higher shoot carbon content under stress.

Disturbances to the environment caused by natural events and human activities can impact the resistance and resilience of soil microbial functional activities. The microbial communities are essential components of soil ecosystem that play vital roles in maintaining soil fertility, nutrient cycling, and plant productivity. Environmental stresses such as drought can severely affect soil microbial activities, with negative impacts on soil fertility and ecosystem functioning. This project investigated the impact of various agricultural management practices on soil microbial functional resistance and resilience to drought stress in several Soil CRC long-term trials. The collective findings from investigation into soil responses to stress conditions and diverse management practices underscored the pivotal role of strategic soil management in promoting resilience and nutrient dynamics within ecosystems. The Bednar method emerged as an effective strategy for mitigating the adverse effects of environmental stressors, maintaining stable microbial processes, and enhancing MBC and MBN concentrations, particularly under moderate drought stress. Similarly, cover crop management demonstrated promise in bolstering soil resilience and microbial functioning, evident through increased resistance and resilience indices, and improved nutrient retention under moisture stress conditions. Moreover, lime treatment showed significant potential in enhancing soil health and resilience to drought stress, offering insights into its capacity to mitigate nitrogen loss and facilitate nutrient cycling.

Soil microbial functional resistance and resilience to drought can vary depending on intrinsic soil properties. Soils with higher organic matter content and nutrient availability may have more resilient microbial communities as these soils provide more resources for microbial growth and activity. Similarly, soils with a more favourable texture, such as loam or clay, may also have greater resistance and resilience to stress as these soils can retain moisture more effectively than sandy soils. In agricultural systems, soil pH is primarily correlated with soil respiration resistance and resilience, potentially influenced by frequent land preparation and fertiliser applications. This project investigated the impact of various agricultural management practices on soil properties and microbial dynamics across different sites. The results highlighted the significance of practices like minimum tillage, lime application, and cover crop management, crucial for sustaining agricultural productivity during water stress periods. Total carbon and total nitrogen contents varied significantly among paired sites, highlighting the influence of specific practices on soil fertility. HWEOC and HWEON content were notably affected by cover crop management and tillage practices, emphasising the role of organic matter input and soil disturbance in shaping soil organic dynamics. This project showed that cover cropping and minimum tillage improved microbial resilience and enzyme stability, enhancing soil health and nutrient cycling. Conversely, conventional management and excessive tillage were associated with reduced microbial activity and enzyme functions under drought stress, highlighting the advantages of sustainable practices in maintaining soil functionality. Cover cropping and minimum tillage generally led to better nitrogen retention and higher plant biomass and nutrient uptake under stress condition, whereas conventional and excessively tilled soils showed less resilience and lower nutrient availability.

NEXT STEPS	TIMING
1. Deliver decision support tools for better quantification of the benefits of ecosystem-based land management practices on soil carbon stocks and resilience in different farming systems.	1–2 years
2. Contribute to Soil CRC goal for education and training of end-users (through providing face-to-face workshops and online trainings).	2–3 years
3. Provide farmers with a better understanding of the underlying ecosystem processes that are responsible for improvement in soil carbon stocks, function and resilience, and assist them in determining when management practices would be most likely to improve soil performance and productivity.	2–3 years

CHAPTER 1: A META-ANALYSIS OF THE ROLE OF EDAPHIC VARIABLES AND MANAGEMENT PRACTICES IN REGULATING SOIL MICROBIAL RESILIENCE TO DROUGHT

1.1 Abstract

Disturbances to the environment caused by natural events and human activities can impact the resistance (ability to withstand environmental and anthropogenic pressure) and resilience (ability to recover functional and structural integrity after disturbance) of soil microbial functional activities. A paucity of information exists on the impact of water stress on soil and how soil biological systems respond to and demonstrate resilience to stress. To address this, we conducted a systematic review and meta-analysis to assess the response of soil microbial biomass and respiration to drought stress. We selected laboratory studies focused either on microbial biomass or respiration of soils under drought stress (compared to control treatment without stress) for this review and analysis. Most of the research found that applying drought stress had a negative impact on microbial respiration in agriculture, forest, and grassland ecosystems, resulting in decreased microbial activity (overall negative effect size). However, during the resilience phase, there was an increase in respiration, indicating a recovery and an overall positive effect. Conversely, microbial biomass showed a negative effect size during both the resistance and resilience phases, suggesting a decrease in the microbial biomass. In agricultural systems, soil pH was primarily correlated with soil respiration resistance and resilience, potentially influenced by frequent land preparation and fertiliser applications, while in forest ecosystems factors such as soil organic matter, clay content, and pH significantly impacted microbial biomass and respiration resistance and resilience. In grassland ecosystems, soil organic matter was strongly associated with biomass resilience to drought. The impact of drought stress on soil microbiome showed different patterns in natural ecosystems and agricultural lands, and the magnitude of microbial functional responses regulated by soil intrinsic properties.

1.2 Introduction

Soil is a heterogeneous material consisting of a complex mixture of minerals, organic matter, and a network of water- and air-filled pore spaces which host a wide range of organisms in micro to macro scales (Joos and De Tender 2022). Different environmental stresses (e.g., drought, heat, fire, compaction) can significantly influence soil's physicochemical and biological properties and alter soil functions. The terms 'soil resistance' and 'soil resilience', as the core components of soil ecosystem stability, have been introduced to describe the influence of environmental and anthropogenic disturbances on soil and are used to address the sustainability of the soil resources (Fialho et al. 2013; Griffiths and Philippot 2013; Silva et al., 2013) . Therefore, soil resistance and resilience can be used to assess soil health, performance, and improvement towards sustainable state (Quinlan et al. 2015) in the context of changing climate with more frequent and prolonged periods of drought worldwide (Dai 2013; Vicente-Serrano et al. 2020). Figure 1.1 summaries the soil properties and mechanisms that are responsible for regulating the resistance and resilience of soil system to drought stress.

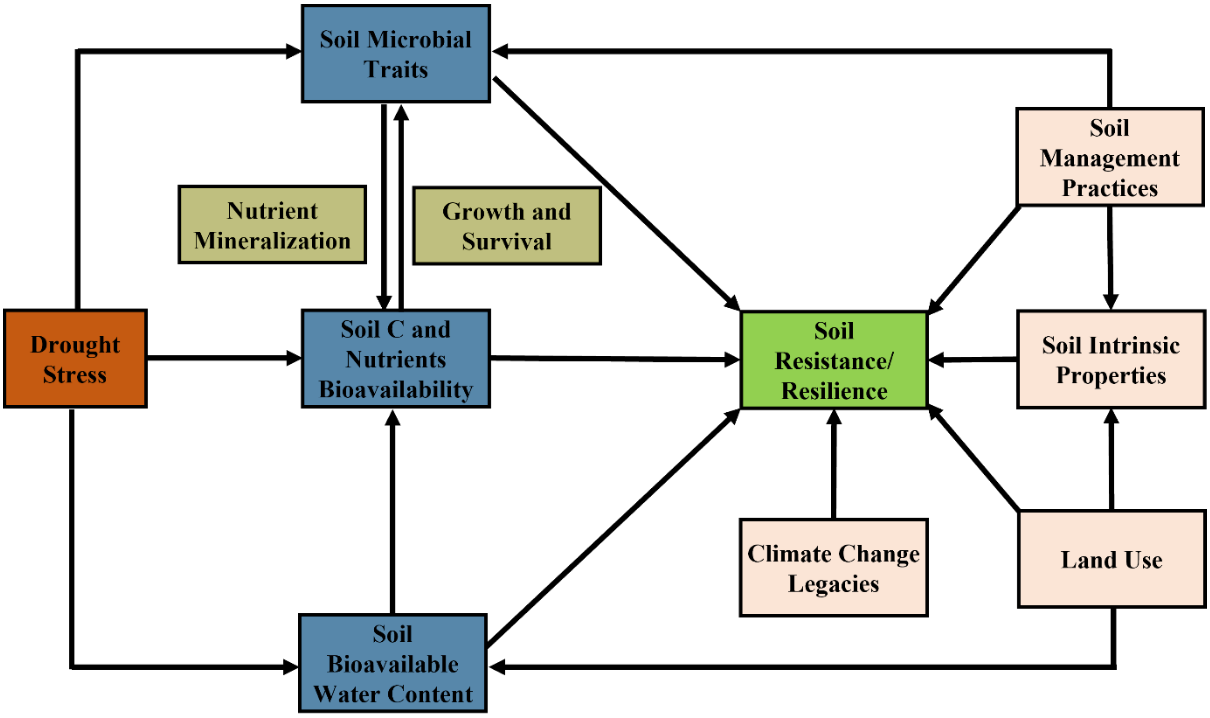

Figure 1.1. The properties and mechanisms that are responsible for regulating the resistance and resilience of soil system to environmental stress.

Soil resistance measures a soil's ability to withstand (i.e., maintain its functions) environmental and anthropogenic pressure (de Vries et al. 2012; Isbell et al. 2015). The magnitude to which soil functions are impaired after stress is dependent on the soil systems' resistance to disturbance (Smith et al. 2022; Wada and Toyota 2007). Therefore, a smaller change is usually observed in the response variable to stress in soils with higher resistance, whereas relatively bigger changes occur in poorly resistant soils (Seybold et al. 1999). Soil resilience has been defined as the capacity of a soil to recover its functional (e.g., microbial activity, decomposition of organic matter, nutrient cycling) and structural (e.g., soil aggregate stability, porosity) integrity to original status (similar level of function) after experiencing a specific disturbance (Griffiths et al. 2008; Li et al. 2019; Quinlan et al. 2015; Schwalm et al. 2017). Ludwig et al. (2018) suggest that soil resilience is a collective output of past and present soil management, as well as the prospect of possible soil responses to future disturbances. Both resistance and resilience concepts are considered as the components of soil functional stability (Wada and Toyota 2007). The resistance and resilience of soil functional properties to environmental stresses can be assessed by comparing the response variables of the target function relative to that of undisturbed soil (Orwin and Wardle 2004). It is also suggested that resistance and resilience have opposite patterns and ecosystems with high resistance tend to have low resilience to environmental stresses (Stuart-Haëntjens et al. 2018; Yao et al. 2022).

In recent decades, intensive agricultural practices (e.g., use of heavy machinery, large-scale farming with monocropping, excessive usage of chemical fertilisers) have contributed to several negative environmental impacts, including widespread degradation of fertile soils (Akamani 2021; Kibblewhite et al. 2008). Soil degradation gradually diminishes soil health which in turn influences crop productivity. Currently, many farming systems are interested in using ecosystem-based management practices (sustainable management practices) to enhance soil health and resilience. Ecosystem-based management is considered as an integrated management of resources (i.e., land, water, living beings) that enhances conservation and sustainable use. Further, it is intended to restore and enhance the resilience of the ecosystem (Abdourahamane Illiassou and Oeba 2020; Delacámara et al. 2020). An ecosystem-based approach to management would facilitate maintaining the fertility of agricultural lands, balance the nutrient requirement of crops, and boost the functional capacity of the ecosystem services (Agula et al. 2018). Better understanding of the basis of soil resistance and resilience would help to improve management practices for effective enhancement of soil health and productivity, especially in underperforming soils.

In addition to human intervention on soil systems stability, the climatic conditions prevailing in a particular geographical location can have a significant influence on biodiversity and nutrient cycling (Ye et al. 2019). Shifting of microbial communities and reduction in microbial biomass (i.e., bacterial and fungal biomass) under warming (Frey et al. 2008) and low rainfall conditions have been reported to decrease soil bacterial abundance and microbial community composition (Dacal et al. 2022). However, Hawkes et al. (2017) and de Moraes Sa et al. (2014) suggest that soil microbial attributes cannot be predicted based only on current environmental conditions, and resilience can also be affected by the past climate (precipitation, temperature, radiation, relative humidity, evapotranspiration) conditions. Further, current changing climate (i.e., drought and high temperature) also influence on the pattern of microbial functional redundancy (Berard et al. 2015) Therefore, frequent exposure to climatic disturbances in the past may have influence on the response of soil microbial community to the current and future climatic extremes. For example, drought history in a particular area would strongly influence soil microbial respiration, potential enzyme activities, microbial community composition, and microbial carbon use efficiency in response to drying/wetting cycles (Leizeaga et al. 2021; Mikha et al. 2005). Further, frequent drying/wetting cycles can break down soil aggregates and alter water penetration pathways through the soil profile to make new ecological niches for soil microorganisms (Kaisermann et al. 2017).

Soil microbes respond differently to long-term drought history and their responses are varied between and within bacterial and fungal communities (Barnard et al. 2013; Preece et al. 2019). Certain soil fungi are generally more tolerant to drought than bacteria since they are common in extreme environments, and their reproduction is mainly through budding which is a more stress-tolerant mechanism (Preece et al. 2019; Wan et al. 2023). Further, the chitinous cell wall present in the fungi will increase the resistance to environmental perturbations and the characteristic nature of hyphal growth enabling fungi to penetrate small areas of dry soil and survive the stress (Ene et al. 2015; Sun et al. 2020). Long-term drought can cause acclimatisation of soil microbial communities and create a drought-tolerant fungal-dominated microbial community (Dacal et al. 2022; Leizeaga et al. 2021). In contrast, sustained nutrient enrichment in agricultural systems may decrease the resistance of microbial communities to drought due to the reduction in the abundance of fungi relative to bacteria (Bardgett and Caruso 2020). Therefore, a higher fungi:bacteria ratio can be considered an indication of drought legacy influence on soil microbiome and formation of a drought induced ecological memory. For example, soil microbiome present in temperate ecosystems adapts to the annual variability in soil moisture, hence taxonomic and functional diversity might not significantly disturb with the limits of a particular region’s seasonal variability (Bouskill et al. 2013; Waring and Hawkes 2018). However, regions with consistent soil moisture history (i.e., no history in drought) may develop a microbiome sensitive to moisture due to changes in soil microbial biomass and composition (Wang and Allison 2021).

The past climatic condition prevailed in a geographical area can have influence on soil microbiome both positively and negatively. Soil microbes play a vital role in soil carbon and nutrient cycling as they are involved in the decomposition of organic matter by releasing extra-cellular enzymes (Hammerl et al., 2019). The changes in microbial biomass and respiration are inevitable with historical climatic events which ultimately influence the availability of soil carbon and nutrients. Therefore, the resistance and resilience capacity of soil microbiome can be considered as a function of climate legacies, soil management practices, intrinsic soil properties, and soil microbial community composition and traits. Therefore, the main objective of this study (using a systematic review and meta-analysis) was to assess how edaphic variables and management practices would affect soil microbial functional characteristics (i.e., microbial biomass and respiration) and performance (i.e., resistance and resilience) under drought stress. This may then enable future management to consider factors that promote soil resistance and resilience under drought conditions to maximize productivity both during and after drought stress.

1.3 Methods

1.3.1 Study Selection

The research for this meta-analysis was retrieved from Web of Science databases for the period between 2000 and 2023 using the search terms "drought" AND "microbial*" AND "resilience" AND "resistance" AND "soil". We first screened the titles, abstracts and methods sections of peer-reviewed publications returned from searches for appropriateness considering drought's effects on microbial biomass and respiration. The eligibility criteria for inclusion of the studies in the meta-analysis were considered during full text screening. The studies needed to:

- include information on microbial respiration and microbial biomass data
- have clearly defined treatment and control groups
- report standard error or were able to convert the data to standard error, and
- be a primary study (Grados et al. 2022; Zhang et al. 2021).

Based on the minimum requirement of a reliable comparison of drought effect on microbial biomass and respiration during resistance and resilience phases, 17 studies were then selected (laboratory experiments). The data reported in field experiments were not included in the analysis, as this study focused on the nature of microbial change only affected by drought stress. Field data could be affected by many other factors other than the drought, as control conditions cannot be implemented under field conditions (Table S1).

1.3.2 Data Acquisition

Relevant data on soil texture, pH, water (H_2O), and soil organic carbon content (SOC) were extracted from the figures and tables of the selected studies (Table S2). PlotDigitizer was used to extract relevant data points when data was provided in graphical format. Each study's measurements were standardised to common units. Microbial biomass was reported in g kg^{-1} soil, and microbial respiration was reported in µg CO_2-C g^{-1} soil h^{-1}. When soil organic matter (SOM) content was provided, the following formula was used to calculate the SOC content:

SOC% = SOM% / 1.724 (Rashti et al. 2015)

The resistance (RS) and resilience (RL) indexes were calculated where necessary (i.e., to calculate the indexes using the mean values provided in the manuscript) using the following formula (Orwin and Wardle 2004):

$$\text{Resistance (RS)} = 1 - [(2 \times |D_{rs}|) / (C_{rs} + |D_{rs}|)]$$

$$\text{Resilience (RL)} = [(2 \times |D_{rs}|) / (|D_{rs}| + |D_{rl}|)] - 1$$

Where D_{rs} is the difference between soil response (i.e., microbial biomass or respiration) value in the control condition (C_{rs}) and the stressed condition at the end of the RS phase. D_{rl} is the difference between soil response (i.e., microbial biomass or respiration) value in the control condition and the stressed condition at the end of the RL phase. The values of RS and RL indices range between −1 and +1. The value of +1 indicates the maximum RS (no impact of stress) or RL (complete recovery from the stress), and lower values indicate less RS or RL of soil microbial biomass and respiration to drought stress. Negative values of the RS index indicate a change greater than 100% in the response variable compared with that in the control condition. If the absolute value of D_{rl} becomes higher than the absolute value of D_{rs}, then the RL index will have a negative value. Mathematically, the RL index of control condition will be infinitely close to +1, while it has been considered as +1 in the RL equation.

1.3.3 Statistical Analysis

The meta-analysis was conducted using the OrchaRd 2.0 function in R to synthesise data from multiple studies that we extracted the data (i.e., sample size, means, standard deviation). First, standardised mean difference (SMD) of the parameters (i.e., microbial biomass and respiration) between the control (without stress) and the treatment (drought stress) was used to calculate the effect size (Nakagawa et al. 2021). Subsequently, the meta-analysis was conducted using the 'mod_results' function, which facilitated the combination of effect sizes across studies using random-effects model. Heterogeneity among studies was assessed using statistical measures such as the Q statistic and I^2. I^2 values of 25%, 50%, and 75% are considered low, moderate, and high heterogeneity respectively (Dougherty 2023). If the 95% confidence interval (CI) did not contain zero, the mean difference within the group was considered significant (null hypothesis). In addition to the overall analysis, the effect of potential moderators, soil intrinsic properties (i.e., SOC %, Clay % and pH) and type of land use (i.e., agriculture, forest, grassland) on microbial biomass and respiration during drought and upon releasing the drought was analysed. In order to analyse the impact of different moderators, a meta-regression model was conducted including one moderator at a time as a fixed effect, considering it as either categorical or continuous. Each moderator was examined in a separate model and did not assess interactions between the moderators (Nakagawa et al. 2021). The Q_M statistics was used to assess significance of each moderator on the response of microbial attributes to drought. The Orchard plot summarised the meta-analysis by indicating whether the overall or subgroup effect was based on many or few studies, and whether studies differed significantly. Publication bias was assessed using the funnel plot asymmetry (Figure S1). A one-way ANOVA was performed to compare the mean resistance and resilience indexes for microbial biomass and respiration among different land uses (i.e., agriculture, forest, grassland) followed by Tukey's HSD. Regression analysis followed by a Principal Component Analysis of the soil intrinsic properties affecting resistance and resilience index was conducted using the SPSS statistical package.

1.4 Results and Discussion

1.4.1 Resistance and Resilience of Soil Microbial Biomass to Drought Stress

Soil microbial biomass carbon (MBC) as an indicator of heterotrophic respiration (decomposition of soil organic matter by microorganisms) is a crucial component of soil ecosystem and is responsible for many important functions such as nutrient cycling and soil structure formation. The meta-analysis of the effect of drought stress on soil microbial biomass indicated that the observed SMD between control and drought treatment on the resistance of soil microbial biomass was negative (–1.58, 95% CI [–2.19, –0.97]) and is statistically significant ($p < 0.0001$, Figure 1.2a). According to the Q-test, the true outcomes appear to be heterogeneous ($Q = 87.14$, $p < 0.0001$, $I^2 = 59\%$), and the linear regression test indicated no funnel plot asymmetry ($p = 0.04$, Figure S1a). We found that the test of the moderator variable (land use) was not significant ($p = 0.51$, Figure 1.2b) and the estimated mean effects were negative for all three land use types, suggesting that mean biomass in control is higher than the drought treatment. The mean effect sized was lower in forest ecosystem (–2.28) compared to agriculture (–1.42) and grassland (–1.42) ecosystems. Further, from the soil intrinsic properties, SOC was found to have significant impact on the microbial biomass response to drought in the resistance phase ($p = 0.01$). The overall effect size was negative under both levels of SOC with –2.16 (95% CI [–2.89, –1.42]) at > 2% SOC and –0.91 (95% CI [–1.67, –0.15]) at 1–2% SOC (Figure S2a). The overall effect sizes for the different levels of clay content (Figure S2b) and pH (Figure S2c) were also negative, however, they were statistically not significant.

Drought stress can have a significant effect on microbial communities (e.g., cell desiccation and lysis, or denaturation of microbial enzymes), which in turn can affect soil processes and ecosystem functioning (Berard et al. 2011; Göransson et al. 2013). Previous studies revealed both positive (due to release of labile carbon, nitrogen, and phosphorus via soil aggregate breakdown) and negative (due to stress-sensitive microorganisms) responses of microbial biomass to drying/wetting cycles (Guillot et al. 2019; Huang et al. 2020). We also observed few positive effects of microbial biomass to drought in the agricultural ecosystem, compared to forest and grassland ecosystems which indicates more resistance to drought and is further associated with alkaline pH ($pH > 7.5$), low SOC ($SOC < 2\%$) and low clay content ($clay < 5\%$) (Figure S2a, S2b and S2c). Microorganisms are known for their ability to adapt to various environmental conditions. For instance, certain microbial taxa may possess traits that enable them to tolerate challenging conditions (i.e., low organic carbon, low clay content, high pH soils) and thrive under drought stress. Homyak et al. (2017) reported possible mechanisms for increasing microbial biomass under drought conditions, such as changes in the composition of microbial communities or their transition into dormant states and some fungi capable of withstanding drought may continue to generate biomass. Drought-tolerant microorganisms may therefore demonstrate metabolic changes, osmotic regulatory mechanisms, or alternative nutrient acquisition strategies that allow them to use available resources more efficiently and maintain growth and biomass production under drought treatments.

The analysis for resilience of microbial biomass to drought stress showed that the overall SMD between control and drought treatments was not significantly different from zero ($p = 0.93$, Figure 1.2c). However, we observed both positive and negative effects of drought on microbial biomass resilience. According to the Q-test, the true outcomes appear to be heterogeneous ($Q = 66.95$, $p < 0.0001$, $I^2 = 70\%$), and there was no funnel plot asymmetry according to the linear regression test ($p = 0.48$, Figure S1b). Additionally, the type of land use has no effect on microbial biomass resistance to drought ($p = 0.96$, Figure 1.2d). The overall effect size for agriculture and forest ecosystem were negative (–0.06, 95% CI [–1.02, 0.91] and –0.11, 95% CI [–1.79, 1.57] respectively). Further, we observed positive effect size for the microbial biomass resilience to drought under 1–2% SOC (Figure S2d), clay content < 5% and > 15% (Figure S2e), and 7–8 pH level (Figure S2f). However, they were statistically not significant.

Resistance index of soil microbial biomass to drought stress for all three assessed ecosystems (agriculture, forest, grassland) ranged from –0.32 to 1 with 65% showed values higher than 0.5 (Figure S3a). Resilience index of soil microbial biomass ranged from –1 to 0.96 with 42% showed values higher than 0.5 (Figure S3b). Overall, the resistance indices of soil microbial biomass to drought stress showed the order of agriculture (0.63) > forest (0.56) > grassland (0.53) while resilience was forest (0.44) > grassland (0.25) > agriculture (0.22) (Table 1.1). We further observed that grassland ecosystem separated from the agriculture and forest ecosystems for the resistance and resilience index of the microbial biomass (Figure 1.4a and 4b). In both grassland and forest ecosystems, microbial biomass resistance and resilience are strongly associated with SOC content, while forest ecosystems additionally exhibit a strong association with clay content and pH (Table 1.3).

Overall, these observations indicate a generally low resistance of soil microbial biomass to drought stress (significantly negative mean difference values) and its low resilience (non-significant mean difference values) to recover from drought stress compared with the pre-disturbance condition. Maestre et al. (2015) reported that microbial communities maintain their structure and functioning even under severe drought conditions and develop more resistance and resilience communities after the drought period is over. They suggested that the response of soil microbial communities was dependent on the severity of drought stress, with more severe droughts leading to a slower recovery of microbial biomass. Barnard et al. (2013) indicated that the resistance and resilience of soil microbial biomass to drought stress would vary depending on soil type and land use. We did not observe any significant differences in resistance and resilience of microbial biomass to drought stress within three land use types. However, more negative effect size in forest system for resistance in microbial biomass indicated that forest systems tend to be less resistant to drought compared to agriculture and grassland ecosystems. Therefore, the resistance and resilience of microbial biomass under drought stress can be attributed to several other factors that need to be focused on in future studies.

Intensive land management practices, such as agricultural use, can shift the distribution of soil microbial biomass to bacterial, rather than fungal, dominated species (De Vries et al. 2013). Soil fungi are generally more resistant than soil bacteria to drought stress due to their thicker cell walls, sporulation activity, and higher capacity to access water and nutrients under stress through fungal hyphae (Barnard et al. 2013; Schimel 2018). In contrast, soil bacteria are more resilient to drought stress due to their rapid growth and reproduction capacity (de Vries et al. 2012). Therefore, the variation of the resistance and resilience indexes under different land uses can be attributed to the fungi:bacteria ratio present under different land uses.

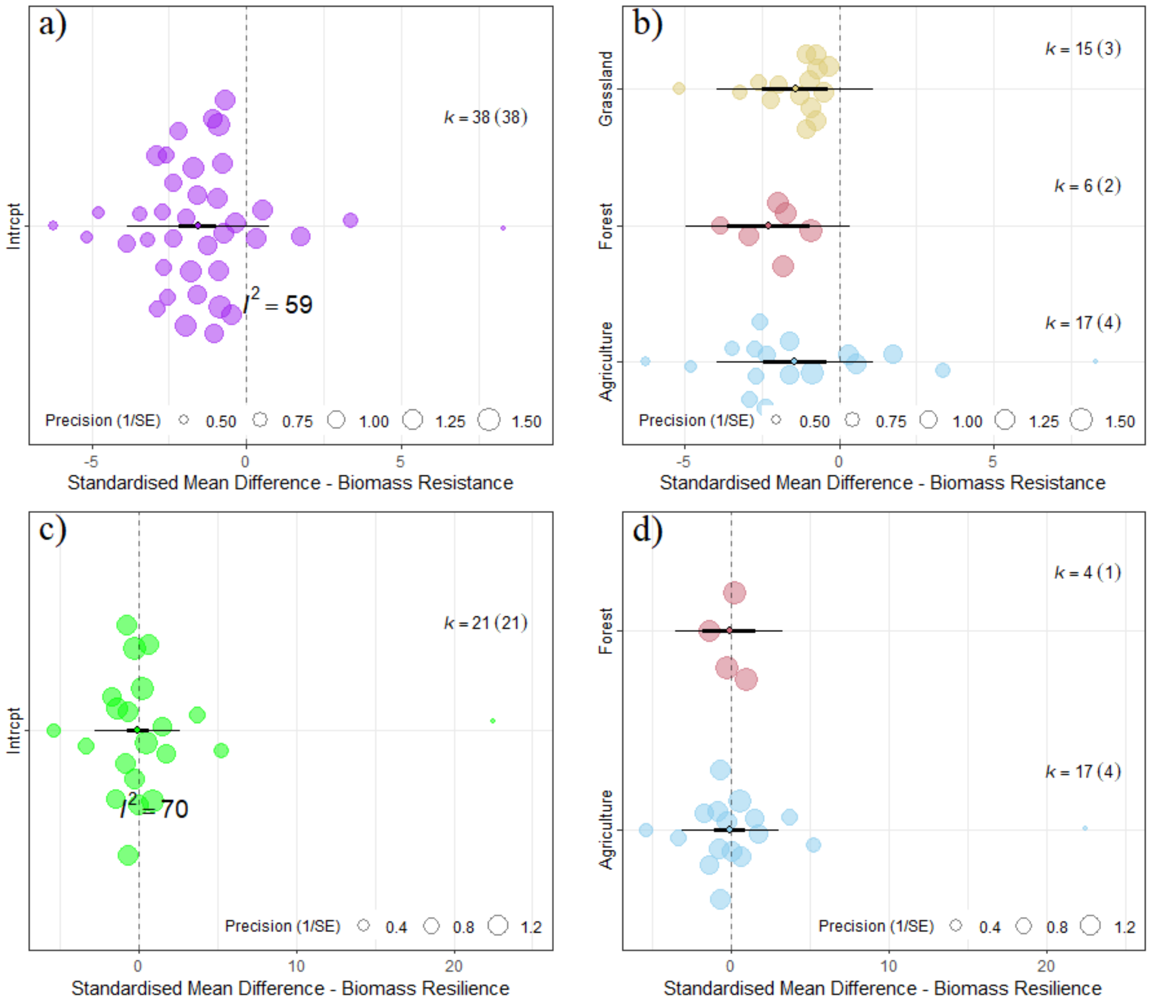

Figure 1.2. Orchard plots of the meta-analytic model showing the overall response of microbial biomass to drought between the control and treatment during resistance (a) and resilience (c) phases and meta-regression model showing the effect of land use type on resistance (b) and resilience (d) of microbial biomass to drought. Mean effect sizes with 95% confidence intervals (bold lines) are shown in the middle of the dot charts, where individual dots are individual (i.e., different data points in the studies) effect sizes. The size of dots shows the precision (inverse of standard error) of the effect size estimate. Negative effect sizes larger values in control, whilst positive values mark larger values in treatment in the respective parameter.

1.4.2 Resistance and Resilience of Soil Microbial Respiration to Drought Stress

The results of the meta-analysis revealed that there is an overall negative effect size across the included studies for the microbial respiration during drought. Specifically, the SMD between control and drought treatment was –0.87 (95% CI [–1.68, –0.07]) which was statistically significant (p = 0.03, Figure 1.3a). This negative effect size suggests that drought is associated with a decrease in microbial respiration. According to the Q-test, the true outcomes appear to be heterogeneous ($Q = 53.73$, $p = 0.005$, $I^2 = 60\%$), and the linear regression test indicated potential funnel plot asymmetry ($p = 0.0002$; Figure S1c). The multivariate meta-analysis model revealed that the type of land use has no effect on response of microbial respiration to drought. Overall, the mean effect of all the land uses were negative in an order of grassland ecosystem (–1.31, 95% CI [–2.90, 0.29]), forest ecosystem (–1.28, 95% CI [–2.61, 0.06]), and agriculture ecosystem (–0.35, 95% CI [–1.50, 0.81]) (Figure 1.3b). Moreover, the overall effect sizes for different levels of SOC% (Figure S4a), clay content (Figure S4b) and pH (Figure S4c) were negative which is statistically not significant. However, the negativity decreased with increases in SOC%, clay% and with neutral and acidic pH indicating that higher SOC and clay content, and neutral and acidic soil favours microbial respiration during the drought and increase their resistant to drought.

Soil microbial respiration during the recovery phase (i.e., following the breaking of drought; the resilience phase) showed overall positive effect (0.30, 95% CI [0.02, 0.57]) which was statistically significant ($p = 0.04$, Figure 1.3c). The Q-test indicated that the results were not heterogeneous ($Q = 27.37$, $p = 0.50$, $I^2 = 2\%$). The linear regression test also indicated potential funnel plot asymmetry ($p < 0.0001$, Figure S1d). The results obtained from the multivariate meta-analysis model indicated that the impact of land use on microbial respiration response to drought was not significant ($p = 0.26$). When considering all types of land use, including grassland ecosystems, forest ecosystems, and agriculture ecosystems, the overall mean effects were found to be positive. Specifically, the effect sizes for the forest ecosystem were estimated at 0.05 (95% CI [–0.58, 0.67]), for the agriculture ecosystem at 0.24 (95% CI [–0.07, 0.56]), and for the grassland ecosystem at 0.74 (95% CI [0.12, 1.37]) (Figure 1.3d). The overall effect size of microbial respiration between treatment and control under different levels of SOC, clay and pH were positive during the resilience phase and the positivity increased with higher SOC contents. However, they were not statistically significant (Figure S4d, S4e, and S4f respectively).

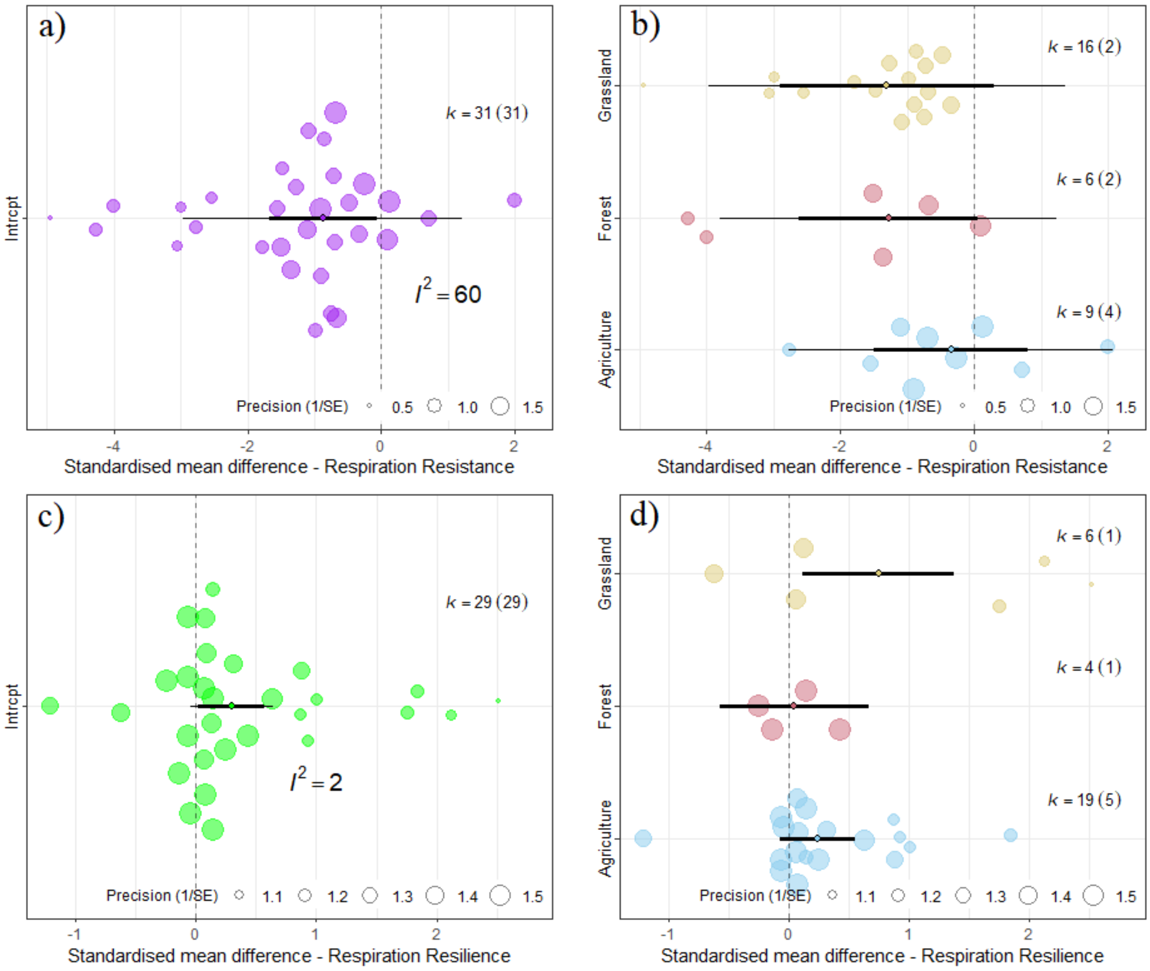

Figure 1.3. Orchard plots of the meta-analytic model showing the overall response of microbial respiration to drought between the control and treatment during resistance (a) and resilience (c) phases and meta-regression model showing the effect of land use type on resistance (b) and resilience (d) of microbial respiration to drought. Mean effect sizes with 95% confidence intervals (bold lines) are shown in the middle of the dot charts, where individual dots are individual (i.e., different data points in the studies) effect sizes. The size of dots shows the precision (inverse of standard error) of the effect size estimate. Negative effect sizes larger values in control, whilst positive values mark larger values in treatment in the respective parameter.

Resistance index of soil microbial respiration for all three ecosystems ranged from –0.65 to 0.97 with 82% showed values lower than 0.5 (Figure S5a). Resilience index of soil microbial biomass also ranged from –0.71 to 0.99 with 52% showing values lower than 0.5 (Figure S5b). The resistance and resilience indices of soil microbial respiration to drought stress showed the order of forest (0.43) > grassland (0.20) > agriculture (0.12), and grassland (0.61) > forest (0.54) > agriculture (0.44), respectively (Table 1.1). The results did not indicate a clear separation of the three land use types (agriculture, forest, and grassland) in terms of resistance and resilience index of the respiration under drought stress as observed in microbial biomass (Figure 1.4c and 1.4d). However, the resistance and resilience of microbial respiration in agricultural ecosystems are primarily regulated by soil pH, whereas in forest ecosystems it is strongly associated with SOC content, clay content, and soil pH (Table 1.3).

Table 1.1. Resistance and resilience indexes for the microbial biomass and microbial respiration in different land use types.

Land Use	Microbial Biomass		Microbial Respiration	
	RS	RL	RS	RL
Agriculture	0.63 ± 0.05	0.23 ± 0.14	0.14 ± 0.12	0.44 ± 0.12
Forest	0.56 ± 0.10	0.44 ± 0.14	0.43 ± 0.10	0.54 ± 0.10
Grassland	0.53 ± 0.08	0.21 ± 0.18	0.20 ± 0.04	0.61 ± 0.04
P value	(0.56)	(0.76)	(0.15)	(0.55)

The table displays the individual column p-values calculated to assess the statistical significance of each variable. RS=Resistance, RL=Resilience.

Soil microbial respiration is regulated by abundant microbial species with high functional redundancy (Shen et al. 2022). In response to drought, microbial communities would exhibit both resistance and resilience, which can have varying impacts on their respiration, as soil microbial biomass and SOC are considered the main contributors to soil respiration resilience (Yu et al. 2021). Microbial response to drought also varies depending on factors such as soil type and land use history, microbial community composition and duration and severity of drought stress. Generally, drought stress decreases soil microbial respiration when soil moisture content falls below the optimum level, while rewetting increases soil microbial respiration due to an increase in dissolved organic carbon and microbial biomass (Yu et al. 2021; Zhang et al. 2015). When soil microorganisms are under stress, they allocate more carbon and nutrients to survival pathways (e.g., synthesising protective substances to stabilise proteins and osmolytes to maintain hydration) than to growth pathways. This would result in higher synthesis of molecules such as polyols or amino acids, which are energetically expensive and consequently decrease soil microbial respiration (Guillot et al. 2019). Some studies have shown that some microbial taxa (gram-positives such as Actinobacteria and Firmicutes) exhibit increased respiration activity during drought, while others (Gram-negatives such as

Proteobacteria and Bacteroidetes) would show decreased activity (Graham et al. 2016; Lipson et al. 2015). The impact of drought on microbial respiration can vary depending on the specific microbial communities and environmental conditions involved (Barnard et al. 2013). Therefore, improved understanding of the mechanisms underlying microbial responses to drought, such as the capacity to survive drought induced osmotic shock, or to assimilate dissolved SOC during rewetting of soil aggregates, is crucial for predicting the long-term effects of ecosystem-based management on ecosystem functioning (Homyak et al. 2018; Slessarev and Schimel 2020; Sun et al. 2017). For example, it has been suggested that microbial communities can adapt to drought stress by altering their carbon utilisation patterns (i.e., higher carbon use efficiency) or shifting to more drought-tolerant taxa. These adaptive responses may increase resistance and resilience, thereby enabling microbial communities to maintain their respiration activity and prevent significant disruptions to ecosystem functioning (de Nijs et al. 2019; Evans and Wallenstein 2014).

1.4.3 Role of soil intrinsic properties and management practices in shaping resistance and resilience of soil microbiome to drought stress

Adoption of ecosystem-based management practices enables the development of a self-recovering soil ecosystem against disturbances (Orwin and Wardle 2004). These include management practices such as organic farming systems (minimal use of external resources), conservation agriculture (minimum soil disturbance, diversified crop rotations, and maintenance of organic soil cover) (Chabert and Sarthou 2020; Nyanga et al. 2020), no-till and low-till farming, mix cropping, crop rotation, cover crops (i.e., crops grown to protect soil surface and improve soil quality), minimum use of off-farm inputs, reliance on biological processes for pest management, and application of organic amendments (Akamani 2021; Horrigan et al. 2002; Jian et al. 2020) (Table 1.2). The stability of a soil ecosystem is a vital factor for its sustainable productivity that is governed by various soil and environmental processes. It has been reported that the structural stability of soil can be increased by utilising a no-till cropping system, due to the potential for development of uniform aggregate strength with more resistant to breakdown after rewetting (Skaalsveen et al. 2019), as well as increasing the number and continuity of soil macropores (Dang et al. 2020). Other than the reduction in soil disturbance, no-till systems can also increase SOC content due to higher retention of crop residues in the soil surface (de Moraes Sa et al. 2014). Additionally, it has been found that the addition of biochar can lead to an increase in the resistance of both bacterial and fungal networks to drought, and biochar-amended soils usually characterised by a faster recovery of soil microbial properties to basal values (Liang et al. 2014).

Table 1.2. The effects of ecosystem-based management practices on soil properties

Management strategy	Region	Management outcome	Reference
No-tillage/Reduced tillage	USA	No-tillage enhanced soil structural stability and soil carbon than conventional tillage.	(Nouri et al. 2021)
	Guadeloupe	Reduced tillage (replacing mouldboard ploughing with disc harrowing) resulted in diminishing SOC losses by 4-fold.	(Chopin and Sierra 2019)
	Spain	Long-term (20 years) no-tillage management stimulated soil biological activity and formation of water-stable macroaggregates.	(Pareja-Sánchez et al. 2017)
	USA	no-till promote soil resilience in terms of moisture retention, effectiveness of drought mitigation, and crop yields.	(Eeswaran et al. 2021)
Organic amendments	Germany	High organic carbon soil amendment (i.e., wheat straw) effectively buffered an excess of nitrogen at a field scale.	(Reichel et al. 2018)
	Australia	Biochar and biochar-compost soil managements improved soil organic carbon, nutrient contents, and water holding capacity.	(Agegnehu et al. 2016; Lan et al. 2019)
	Canada	Beef feedlot manure applied to the plots where topsoil has been removed had a legacy effect on enhancing soil carbon fraction values and soil aggregation.	(Larney et al. 2016)
	Guadeloupe	The use of organic amendments (i.e., manure, sugar scum, sewage sludge, vinasse, and compost) reduced SOC losses by 1.5-fold.	(Chopin and Sierra 2019)
	Spain	In an incubation experiment, organic amendment increased soil water holding capacity, organic carbon and total nitrogen	(Hueso et al. 2011)

		contents, and soil enzyme activities (i.e., β-glucosidase and phosphomonoesterase).	
	China	Long-term organic matter inputs (corn straw and farmyard manure) increased SOC, decreased soil bulk density and increased soil porosity.	(Song et al. 2015)
Cover cropping	France	Cover cropping enhanced soil resilience to nitrogen fertilization by increasing functional redundancy and complementarity within soil bacterial communities and across soil horizons.	(Alahmad et al. 2019)
	Hesston, Kansas	Cover cropping (i.e., sunn hemp and late-maturing soybean) reduced the near-surface soil's susceptibility to compaction and improved the physical properties of soil and SOC contents.	(Blanco-Canqui et al. 2011)
	Italy	The introduction of different cover crops (non-legume cover crop, low nitrogen supply legume cover crop, and high nitrogen supply legume cover crop) increased SOC content at various rates (0.08, 0.32 and 0.34 Mg ha^{-1} $year^{-1}$ respectively) as compared with the control. All cover crops significantly increased soil total nitrogen content compared to the control.	(Mazzoncini et al. 2011)
	Brazil	Cropping systems with summer legume cover crops had higher potential for improving SOM in no-till cropping systems by increasing either the stocks or lability of soil organic C.	(Bayer et al. 2009)

The focus of any ecosystem-based management practice should ensure an increase in soil organic matter, which is one of the main factors regulating soil resilience to disturbance. Organic amendments and cover crops coupled with no-/reduced-tillage practices can enhance soil's physical, chemical and biological properties and increase soil resilience to different environmental stresses. However, the rate and magnitude of recovery attributed to the management practices can be varied with the type of cover crop/ organic amendment / intensity of the tillage (no/reduced), microbial resource availability and intrinsic properties of soil, and climatic conditions (de Vries and Shade 2013; Kaisermann et al. 2013; Shen et al. 2022; Wallenstein and Hall 2012). It has been reported that soils with high organic matter content have higher resistance and resilience of soil functions (e.g., soil respiration, and nitrogen cycling) to disturbances than soils with low organic matter content (Dacal et al. 2022). Organic matter content in soil determines the aggregate stability, porosity and compaction, available water capacity, hydrology and macropore connectivity, and erodibility (Blanco-Canqui and Lal 2010).

Improvements in soil quality and productivity can be achieved through long-term monitoring of the resistance and resilience of soil microbiomes in response to the implementation of different management practices. This meta-analysis indicated that resistance of soil microbial biomass and respiration was negatively influenced by the experimental drought stress. The recovery of the soil microbiome after environmental disturbance can be attributed to many factors including the edaphic properties of soil. In this study, we observed that soil pH was a vital factor for determination of the resistance and resilience of soil microbial respiration to drought in agricultural soils (Figure 1.4c and 1.4d, Table 1.3). It has also been reported that soil microbial composition and functions can be regulated by soil pH in different soils (Yang et al. 2020), and the bacterial growth would be stimulated by an increase in soil pH values (Silva-Sánchez et al. 2019), resulting in a lower respiration resilience to drying/ wetting cycles (Hu et al. 2021). Therefore, soil acidity/alkalinity management is one of the main factors to be considered in ecosystem-based management practices. Since topsoil has usually been disturbed in agricultural fields, surface application of organic amendments may not be able to effectively improve soil microbial resistance and resilience to drought stress.

Table 1.3. Association between resistance and resilience index with soil intrinsic properties

Soil Properties	**Microbial Biomass**					
	Resistance			**Resilience**		
	Agriculture	**Forest**	**Grassland**	**Agriculture**	**Forest**	**Grassland**
SOC	-0.17 (p=0.52)	-0.97** (p=0.001)	-0.47** (p=0.05)	0.05 (p=0.86)	0.85 (p=0.15)	0.75** (p=0.01)
Clay	-0.09 (p=0.72)	-0.87 (p=0.13)	-0.18 (p=0.52)	0.05 (p=0.86)	-0.79 (p=0.21)	0.09 (p=0.79)
pH	0.1 (p=0.69)	0.97** (p=0.001)	0.06 (p=0.86)	-0.07 (p=0.79)	-0.96** (p=0.04)	-0.22 (p=0.54)
Soil Properties	**Microbial Respiration**					
	Resistance			**Resilience**		

	Agriculture	Forest	Grassland	Agriculture	Forest	Grassland*
SOC	-0.21	0.94**	-0.59**	-0.037	0.47	-
	(p=0.42)	(p=0.006)	(p=0.001)	(p=0.86)	(p=0.53)	-
Clay	-0.13	0.58	-0.09	-0.3	-0.68	-
	(p=0.61)	(p=0.42)	(p=0.72)	(p=0.09)	(p=0.32)	-
pH	0.6**	-0.94**	0.28	-0.5**	-0.58	-
	(p=0.01)	(p=0.006)	(p=0.16)	(p=0.01)	(p=0.42)	-

*Could not compute due to limited data availability.

**Correlation is significant at 0.01 level (The table displays the individual cell p-values calculated to assess the statistical significance of the correlation between resistance and resilience index with soil intrinsic properties).

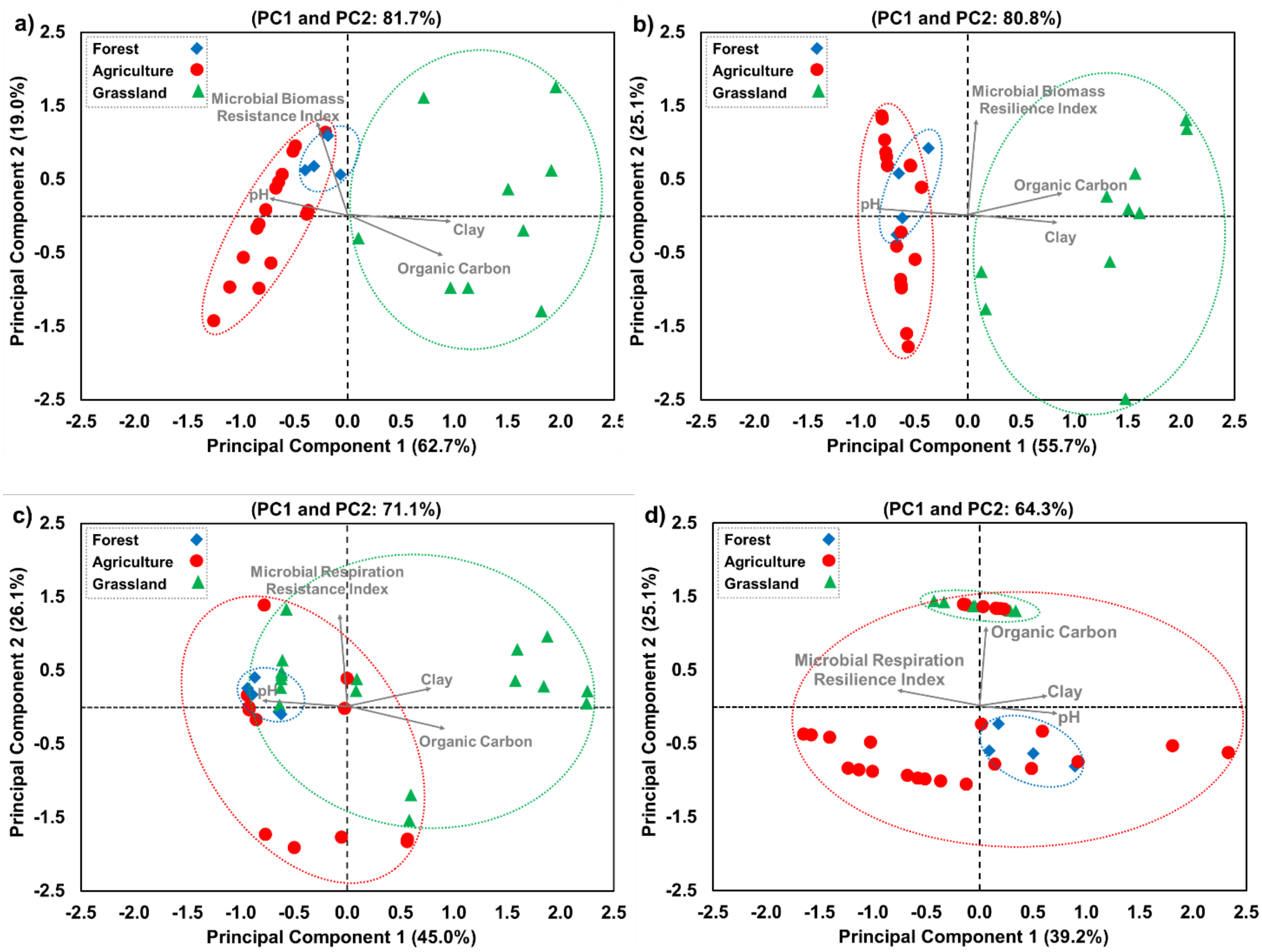

Figure 1.4. Principle Component Analysis (PCA) showing the separation of land use for resistance and resilience indexes of microbial biomass (a and b) and microbial respiration (c and d) under drought stress.

The magnitude of improvement in soil resilience, due to adoption of ecosystem-based management practices, is a cumulative outcome of land management practices, soil intrinsic properties, and climatic factors. Intrinsic properties refer to various soil properties that are inherent to the soil in a particular geographical area such as soil texture, organic matter content, biological characteristics, and structure (Corstanje et al. 2015; de Moraes Sa et al. 2014). The ability of soils to recover from environmental stresses mainly depends on these intrinsic properties. For instance, soil texture is one of the major factors with strong influence on resistance and resilience of soil physical and biological properties to stressors (Corstanje et al. 2015; Seybold et al. 1999). It has been reported that microbial communities in sandy soils are more resilient to drought stress than clay soils, due to the higher frequency of drying/ wetting events in sandy soils (Arthur et al. 2012; Perez-Guzman et al. 2020). The textural differences would affect soil resilience processes such as the leaching of carbon and nutrients, and translocation of fine soil particles within the soil profile (Blanco-Canqui and Lal 2010). The mechanism of higher resilience in organic carbon-rich (i.e., labile carbon, fungal hyphae, root-derived substances) soils can also be attributed to binding and bonding mechanisms of soil carbon with soil clay particles (Arthur et al. 2012).

Since soil is an entity consisting of various properties, processes, and functions, one property/process/function influences another. Therefore, soil resilience is a cumulative function of all the processes that take place in the soil ecosystem. Soil abiotic properties (i.e., soil organic matter content and texture) can influence modulating the environmental stress on soil microbial population by modifying the scale of environmental impact and/or shaping the intrinsic attributes of soil microbial communities such as species richness and functional diversity (Bardgett and Caruso 2020). For example, clay soils would promote a more diverse microbial community with fungal dominance than sandy soils (Bach et al. 2010). A soil microbiome with diverse communities is likely to contain traits of various responsive capacities to disturbance and the capability to recover rapidly after disturbance. This nature in soil microbial community can promote soil resilience (Shade et al. 2012). Six et al. (2006) reported that clay minerals can protect soil microbial community by promoting microbial growth, adsorbing metabolites inhibitory to microbial growth, and providing protection against desiccation and predation. Higher clay and organic matter contents would enhance soil water holding capacity (WHC), which is important in regulating moisture availability during drought periods (Bach et al. 2010; Bardgett and Caruso 2020). Soil moisture is essential for the growth of microorganisms, particularly with fungal biomass and hyphal length (Bach et al. 2010; Frey et al. 1999). The quantity and quality of soil organic matter is determined by the nature of the native vegetation present in the area. Therefore, the nature of the litter and exudates released into the rhizo/mycorrhizosphere, can increase distinct microbial communities in soil (Prescott and Grayston 2013). This can then lead to modification of soil functions including nutrient cycling, carbon dynamics (Carnovale et al. 2019), organic matter transformation processes, and stabilization of soil structure (Gispert et al. 2013; Lozano et al. 2016). Therefore, the inherent abiotic properties of soil and their influence on soil microbiome can frame soil resistance and resilience to environmental stress and ecosystem-bases management practices.

1.5 Conclusions

The microbial communities are essential components of soil ecosystem that play vital roles in maintaining soil fertility, nutrient cycling, and plant productivity. Environmental stresses such as drought can severely affect soil microbial biomass and respiration, with negative impacts on soil fertility and ecosystem functioning. This meta-analysis has shown that soil microbial respiration can recover with the cessation of drought in different environmental conditions. This recovery may be due to increased moisture availability and increase in readily available organic carbon which can stimulate microbial activity and restore respiration rates to pre-drought levels. However, the recovery of microbial biomass was often more limited or slower than that of respiration. This might be since microbial biomass would require more time to rebuild after periods of stress, particularly if the initial stress has caused some microorganisms to die off. Resistance and resilience of microbial biomass and respiration in response to drought can vary depending on the intrinsic soil properties of a particular land use type. Soils with higher organic matter content and nutrient availability may have more resilient microbial communities as these soils provide more resources for microbial growth and activity. Similarly, soils with a more favourable texture, such as loam or clay, may also have greater resistance and resilience to stress, as these soils can retain moisture more effectively than sandy soils. Overall, the response of soil microbial communities to drought is a complex process that is influenced by many factors. Therefore, understanding the underlying mechanisms that drive these responses, such as intrinsic soil properties and microbial community dynamics, can help to identify the land management practices that promote soil health and resilience.

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CHAPTER 2: UNVEILING SOIL HEALTH: INITIAL ASSESSMENT AFTER IMPLEMENTING ECOSYSTEM-BASED VERSUS CONVENTIONAL MANAGEMENT PRACTICES

2.1 Abstract

The study investigated the impact of various agricultural management practices on soil properties and microbial dynamics across different sites. While most paired sites showed no significant differences in water holding capacity (WHC), exceptions highlighted the significance of practices like minimum tillage, lime application, and cover crop management, crucial for sustaining agricultural productivity during water stress periods. Soil pH and electrical conductivity (EC) varied across management practices, influenced by factors like organic matter input, tillage methods, and soil amendments. Total carbon and total nitrogen content also varied significantly among paired sites, highlighting the influence of specific practices on soil fertility. Hot water extractable organic carbon (HWEOC) and nitrogen (HWEON) content were notably affected by cover crop management and tillage practices, emphasising the role of organic matter input and soil disturbance in shaping soil organic dynamics. Total mineral nitrogen content varied between management approaches, influenced by factors such as cover crop management, tillage practices, and planting methods. Microbial dynamics, including basal respiration, functional diversity, biomass carbon (MBC), biomass nitrogen (MBN), and metabolic quotient (qCO2), exhibited significant variations across management practices, reflecting the complex interactions between soil management techniques and microbial activity. Overall, the study underscores the importance of altered soil management strategies considering site-specific factors to optimise soil health, fertility, and microbial dynamics for sustainable agricultural practices. Continued research in this area is crucial for addressing the challenges of modern agriculture and ensuring food security in the face of evolving environmental conditions.

2.2 Introduction

Soil, a natural and finite resource, serves as a crucial mediator between the atmosphere and lithosphere, and mismanagement of this invaluable asset can lead to profound detrimental impacts on the environment (Blanco and Lal 2008; Lal 2016). The intensification of agricultural practices in recent decades, characterised by the heavy use of machinery, monocropping on large scales, and excessive application of chemical fertilisers, has been identified as a significant contributor to environmental degradation, particularly in terms of widespread soil deterioration (Akamani 2021; Kibblewhite et al. 2008). Such degradation inevitably compromises soil health, which, in turn, directly impacts crop productivity. Soil health, a composite measure influenced by various physical, chemical, and biological properties, is essential for sustaining biological productivity within ecosystems and facilitating vital ecosystem services (Kibblewhite et al. 2008; Lal 2016; Rinot et al. 2019; Srivastava et al. 2020). Key functions such as carbon transformation, nutrient cycling, soil structure maintenance, and pest and disease regulation are integral to soil health (Kibblewhite et al. 2008). Plant growth and production heavily reliant on the provision of adequate nutrients and a conducive microbial environment within the soil (Srivastava et al. 2020). Thus, different soil management practices ultimately exert either positive or negative influences on soil health and, consequently, on plant growth and productivity.

Currently, there is a growing interest in adopting ecosystem-based management practices within farming systems to bolster soil health and resilience. Ecosystem-based management, characterised by an integrated approach to resource management aimed at conservation and sustainable use, holds promise for restoring and enhancing ecosystem resilience (Abdourahamane Illiassou and Oeba 2020; Delacámara et al. 2020). Such practices are anticipated to help maintain agricultural land fertility, balance nutrient requirements for crops, and augment the functional capacity of ecosystem services (Agula et al. 2018). Stability within soil systems is a critical factor influenced by both soil and ecosystem processes, and the adoption of ecosystem-based management practices can foster the development of self-recovering soil systems resilient to disturbances (Orwin and Wardle 2004). Therefore, the adoption of ecosystem-based management practices offers a promising pathway towards sustaining agricultural productivity while safeguarding soil health and ecosystem resilience against environmental pressures.

Several ecosystem-based management practices are currently in use to promote sustainability, including organic farming systems with minimal external resource inputs and conservation agriculture, which emphasises minimal soil disturbance, diversified crop rotations, and the maintenance of organic soil cover (Chabert and Sarthou 2020; Nyanga et al. 2020). A core focus of these practices is the enhancement of soil organic matter, a vital determinant of soil resilience, as it governs structural stability, porosity, hydrology, and erodibility (Blanco-Canqui and Lal 2010). To better understand the benefits of ecosystem-based land management practices on soil stability and sustainability, it is essential to assess the effectiveness of different management approaches through long-term monitoring of soil resistance and resilience, including the responses of soil microbiomes to various management interventions.

This project aims to understanding the influence of soil management history on the resistance and resilience of soil microbiome and soil nutrients to environmental stress. In this chapter, we present the results of the baseline assessment conducted on soil samples collected from diverse agricultural sites with varying management histories. The soil samples were analysed for key microbiological parameters including microbial respiration, substrate induced respiration, MBC, MBN, qCO_2, soil chemical properties, total carbon, total nitrogen, HWEOC and HWEON and mineral nitrogen (NH_4^+ and NO_3^-). The findings from the baseline assessment were used to select soil samples for the subsequent experiments to determine the relationship between soil management history and the resistance and resilience of soil microbiome and nutrient dynamics to environmental stress.

2.3 Methodology

2.3.1 Soil Sample Collection

Soil samples were gathered from a range of agricultural sites with differing management backgrounds, detailed in Table 2.1. Within each site, paired locations were chosen, comprising conventionally managed areas alongside those employing ecosystem-based management practices, to facilitate comparative soil sampling.

Table 2.1. Sampling locations and Management Practices

Location	Site 1 (Conventional)	Site 2 (Ecosystem-based)
CWFS	No cover crop	Cover crop trial
Facey Group	No soil disturbance (Bednar)	Bednar treatment
HCPSL Pair 1	No legume cover crop during the fallow period, significant tillage practiced, and furrow planted. (RUSSO SHP Farmer – RSF)	A multi-species legume cover crop during the fallow period, minimum tillage practiced, with mound planted cane. (RUSSO SHP Demo – RSD)
HCPSL Pair 2	A cowpea and lablab during the fallow period, conventional tilled and furrow planted. (CAREY SHP Farmer – CSF)	A large multi-species legume cover crop during the fallow period, minimum tillage practices, with mound planted cane. (CAREY SHP Demo – CSD)
HCPSL Pair 3	Plant cane. The block was planted to a very large mixed fallow legume crop, minimum tilled and mound planted to cane. (DiBella 0135A 3-1 – D_3-1)	Old ratoon which was planted to a mixed cowpea, soybean and lablab cover crop during the fallow period, minimum tilled and furrow planted to cane. (DiBella 0135A 3-2 – D_3-2)
HCPSL Pair 4	This block has been excessively tilled, no stubble conservation practices (aligns with a traditional sugarcane farming system (Cauchi Fallow – CF)	This block has been under controlled traffic, minimum tillage, a large mixed fallow cover crop during the fallow period and all cane stubbles retained. (DiBella 0135A 1-1 – D_1-1)
PIRSA Pair 1	Soil has been spaded to 30 cm	Soil has been spaded with organic matter.
PIRSA Pair 2	No lime added	Lime treated.
Soils for Life Trust	No cover crop	Cover crop trial

CWFS Central West Farming Systems Inc., Condobolin, NSW
Facey Group Inc.,Wickepin WA
HCPSL Herbert Cane Productivity Services Ltd., Ingham, QLD
PIRSA Department of Primary Industries and Regions in South Australia
Soils for Life Trust, Canberra, ACT.

2.3.2 Soil Sample Preparation for the Analysis

Soil samples obtained from Soils for Life (SFL), Central West Farming Systems (CWFS), Facey Group (FGp), Herbert Cane Productivity Services Ltd. (HCPSL), and the Department of Primary Industries and Regions in South Australia (PIRSA) underwent sieving using a 2.0 mm stainless steel sieve to eliminate impurities. These sieved samples were stored at 4 °C until analysis. Subsequently, the cooled samples were incubated at 25 °C for two weeks to revive microbial activity.

2.3.3 Soil Sample Analysis

Soil carbon content (total organic carbon, labile carbon, and soil organic compounds), soil nitrogen content (total nitrogen, labile nitrogen, mineral nitrogen), soil biological properties (microbial respiration, microbial biomass, microbial functional diversity, microbial qCO_2), and soil physical properties (WHC and moisture content) were all measured at the end of the pre-incubation period (Figure 2.1).

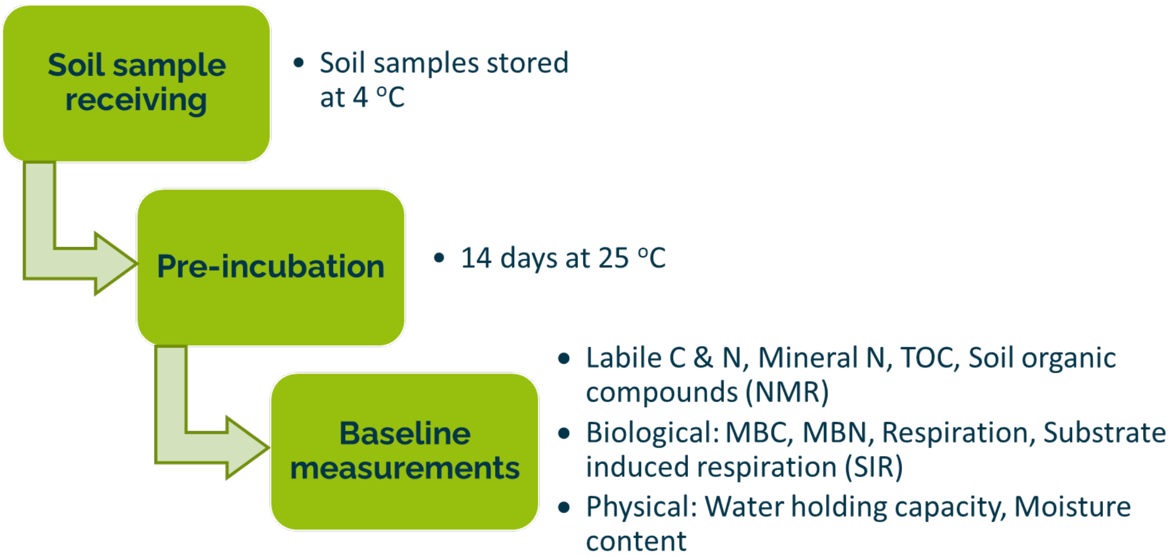

Figure 2.1. The procedure of soil sample preparation for the analysis and parameters measured.

2.3.3.1 Soil Physical and Chemical Properties

Soil WHC was determined using soil saturation from the bottom of the container. Briefly, soil samples were placed on a perforated cups and placed the cup filled with soil in a petri dish. Then distilled water was added to the petri dish and saturated the soil from the bottom. Once the soil in the cup was saturated, excess moisture was allowed to drain out. The gravimetric water content of all the samples was measured by placing the moist soil in the 105 °C oven until it achieved a constant weight (overnight). The total carbon and total nitrogen contents of soil samples were measured by dry-combustion using a LECO CNS-2000 analyser (LECO Corporation, MI, USA). The concentration of HWEOC and HWEON in the samples was determined following the method outlined by Chen et al. (2000). In summary, 4.0 g of fresh soil was combined with 40 mL of water in a capped falcon-tube and incubated at 70°C for 18 hours. The tubes underwent agitation on an end-over-end shaker for 15 minutes and were then filtered through a Whatman 42 filter paper (Whatman Ltd., Maidstone, UK). The filtrates were analysed for concentrations of extractable organic carbon and total nitrogen using a SHIMADZU TOC-VCPH (Shimazu, Kyoto, Japan) TOCN analyser. Soil mineral nitrogen was extracted with 2M KCl at a 1:4 ratio of soil to extractant using an end-over-end shaker, followed by filtration through a Whatman 42 filter paper (Rayment and Lyons 2011) after one hour of shaking. Concentrations of NH_4^+-N and NO_3^--N were measured using a SEAL AA3 Continuous Segmented Flow Analyzer (SEAL Analytical Limited. USA). All results were expressed on an oven-dry basis.

2.3.3.2 Biological Properties

We determined the microbial respiration and functional profiles (substrate-induced respiration) using the MicroResp™ technique. This approach involves the measurement of short-term changes in soil respiration in response to a range of substrates that vary in structural complexity (Campbell et al. 2003). Differences in substrate utilisation (i.e., carbon, amino acid, and complex carbohydrates) can therefore be used specifically as a tool to indicate relative differences in community composition or functional diversity (Burton et al. 2010; Campbell et al. 2003; Knox et al. 2014). This technique has been successfully applied to detect post-disturbance changes in soil functional capacity (Gartzia-Bengoetxea et al. 2016) and assessment of microbial functional recovery in environmental plantings (Amarasinghe et al. 2021). The substrate-induced respiration by soil microbes was calculated by subtracting the basal respiration rates (water blanks) from the substrate-induced respiration values (µg C–CO_2 g-1 h-1) for substrates.

2.3.3.2.1 Preparation of Deep-well Plates

A layout of the deep-well plate demonstrating the positioning of the nutrient substrate to be added and the soil sample was prepared, and soil samples added to the wells with the help of a filling device according to this pre-prepared layout (Figure 2.2 and Figure 2.3). The moisture content of soil samples placed in the deep-well plates was adjusted to 30% of its maximum WHC by adding filter-sterilised distilled water accordingly. Prior to carrying out the MicroResp™ method, the deep-well plates with moisture adjusted soils were incubated at 25°C for three to five days in a sealed container containing a dish of self-indicating soda lime and lined with wet paper towels/a beaker of water.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Con	Con	Con	Con	Con	Con	EBM	EBM	EBM	EBM	EBM	EBM
B	Con	Con	Con	Con	Con	Con	EBM	EBM	EBM	EBM	EBM	EBM
C	Con	Con	Con	Con	Con	Con	EBM	EBM	EBM	EBM	EBM	EBM
D	Con	Con	Con	Con	Con	Con	EBM	EBM	EBM	EBM	EBM	EBM
E	Con	Con	Con	Con	Con	Con	EBM	EBM	EBM	EBM	EBM	EBM
F	Con	Con	Con	Con	Con	Con	EBM	EBM	EBM	EBM	EBM	EBM
G	Con	Con	Con	Con	Con	Con	EBM	EBM	EBM	EBM	EBM	EBM
H	Con	Con	Con	Con	Con	Con	EBM	EBM	EBM	EBM	EBM	EBM

Figure 2.2. Layout of the 96-well deep-well plates for the respiration measurement. Con = Soil under conventional management practices, EBM = Soil under ecosystem-based management practices.

01	1	2	3	4	5	6	7	8	9	10	11	12
A	Basal	Ala	Ara	Arg	Asp	Fru	Gal	Gluc	Gly	His	Hydr	Leu
B	Basal	Ala	Ara	Arg	Asp	Fru	Gal	Gluc	Gly	His	Hydr	Leu
C	Basal	Ala	Ara	Arg	Asp	Fru	Gal	Gluc	Gly	His	Hydr	Leu
D	Basal	Ala	Ara	Arg	Asp	Fru	Gal	Gluc	Gly	His	Hydr	Leu
E	Basal	Ala	Ara	Arg	Asp	Fru	Gal	Gluc	Gly	His	Hydr	Leu
F	Basal	Ala	Ara	Arg	Asp	Fru	Gal	Gluc	Gly	His	Hydr	Leu
G	Basal	Ala	Ara	Arg	Asp	Fru	Gal	Gluc	Gly	His	Hydr	Leu
H	Basal	Ala	Ara	Arg	Asp	Fru	Gal	Gluc	Gly	His	Hydr	Leu

02	1	2	3	4	5	6	7	8	9	10	11	12
A	Lys	Mal	Meth	Phe	Raf	Ser	Suc	Thre	Treh	Try	Val	Xyl
B	Lys	Mal	Meth	Phe	Raf	Ser	Suc	Thre	Treh	Try	Val	Xyl
C	Lys	Mal	Meth	Phe	Raf	Ser	Suc	Thre	Treh	Try	Val	Xyl
D	Lys	Mal	Meth	Phe	Raf	Ser	Suc	Thre	Treh	Try	Val	Xyl
E	Lys	Mal	Meth	Phe	Raf	Ser	Suc	Thre	Treh	Try	Val	Xyl
F	Lys	Mal	Meth	Phe	Raf	Ser	Suc	Thre	Treh	Try	Val	Xyl
G	Lys	Mal	Meth	Phe	Raf	Ser	Suc	Thre	Treh	Try	Val	Xyl
H	Lys	Mal	Meth	Phe	Raf	Ser	Suc	Thre	Treh	Try	Val	Xyl

04	1	2	3	4	5	6	7	8	9	10	11	12
A	Basal	Ala	Ara	Arg	Asp	Fru	Gal	Gluc	Gly	His	Hydr	Leu
B	Basal	Ala	Ara	Arg	Asp	Fru	Gal	Gluc	Gly	His	Hydr	Leu
C	Basal	Ala	Ara	Arg	Asp	Fru	Gal	Gluc	Gly	His	Hydr	Leu
D	Basal	Ala	Ara	Arg	Asp	Fru	Gal	Gluc	Gly	His	Hydr	Leu
E	Basal	Ala	Ara	Arg	Asp	Fru	Gal	Gluc	Gly	His	Hydr	Leu
F	Basal	Ala	Ara	Arg	Asp	Fru	Gal	Gluc	Gly	His	Hydr	Leu
G	Basal	Ala	Ara	Arg	Asp	Fru	Gal	Gluc	Gly	His	Hydr	Leu
H	Basal	Ala	Ara	Arg	Asp	Fru	Gal	Gluc	Gly	His	Hydr	Leu

05	1	2	3	4	5	6	7	8	9	10	11	12
A	Lys	Mal	Meth	Phe	Raf	Ser	Suc	Thre	Treh	Try	Val	Xyl
B	Lys	Mal	Meth	Phe	Raf	Ser	Suc	Thre	Treh	Try	Val	Xyl
C	Lys	Mal	Meth	Phe	Raf	Ser	Suc	Thre	Treh	Try	Val	Xyl
D	Lys	Mal	Meth	Phe	Raf	Ser	Suc	Thre	Treh	Try	Val	Xyl
E	Lys	Mal	Meth	Phe	Raf	Ser	Suc	Thre	Treh	Try	Val	Xyl
F	Lys	Mal	Meth	Phe	Raf	Ser	Suc	Thre	Treh	Try	Val	Xyl
G	Lys	Mal	Meth	Phe	Raf	Ser	Suc	Thre	Treh	Try	Val	Xyl
H	Lys	Mal	Meth	Phe	Raf	Ser	Suc	Thre	Treh	Try	Val	Xyl

Figure 2.3. Layout of the 96-well deep-well plates for the substrate-induced respiration measurement. Green shading = Soil under conventional management practices, orange shading = Soil under ecosystem-based management practices. Basal = water control, Ala = Alanine, Ara = Arabinose, Arg = Arginine, Asp = Aspartic acid, Fru = Fructose, Gal = Galactose, Gluc = Glucose, Gly = Glycine, His = Histidine, Hydr = Hydroxy-L-proline, Leu = Leucine, Lys = Lysine, Mal = Maltose, Meth = Methionine, Phe = Phenylalanine, Raf = Raffinose, Ser = Serine, Suc = Sucrose, Thre = Threonine, Treh = Trehalose, Try = Tryptophan, Val = Valine, Xyl = Xylose.

2.3.3.2.2 Preparation of Substrates

Carbon and amino acids substrate solutions were prepared based on their solubility in water (Table 2.2). Once prepared the required volume, the solution was filter sterilised and stored in sterile tubes at 4 °C.

Table 2.2. Nutrient substrates and their solubility

No	Chemical	Solubility
1	Alanine	H_2O soluble 50 mg/mL, clear, colourless
2	Arabinose	H_2O soluble 100 mg/mL, clear, colourless
3	Arginine	H_2O soluble 50 mg/mL, clear, colourless
4	Aspartic acid	H_2O soluble 50 mg/mL (with heat), clear, colourless
5	Fructose	H_2O soluble 50 mg/mL, clear, colourless
6	Galactose	H_2O soluble 100 mg/mL, clear, colourless
7	Glucose	H_2O soluble 130mg/mL, clear, colourless
8	Glycine	H_2O soluble 100 mg/mL, clear, colourless
9	Histidine	H_2O soluble 50 mg/mL, clear, colourless
10	Hydroxy-L-proline	H_2O soluble 50 mg/mL, clear, colourless
11	Leucine	H_2O soluble 20 mg/mL, clear, colourless
12	Lysine	H_2O soluble 50 mg/mL, clear, colourless
13	Maltose	H_2O soluble 50 mg/mL, clear, colourless
14	Methionine	H_2O soluble 50 mg/mL, clear, colourless
15	Phenylalanine	H_2O soluble 25 mg/mL, clear, colourless
16	Raffinose	H_2O soluble 50 mg/mL, clear, colourless
17	Serine	H_2O soluble 50 mg/mL, clear, colourless
18	Sucrose	H_2O soluble 342 mg/mL, clear, colourless
19	Threonine	H_2O soluble 50 mg/mL, clear, colourless
20	Trehalose	H_2O soluble 50 mg/mL, clear, colourless
21	Tryptophan	H_2O soluble 13.4 mg/mL (with heat), clear, colourless
22	Valine	H_2O soluble 50 mg/mL, clear, colourless
23	Xylose	H_2O soluble 150 mg/mL, clear, colourless

2.3.3.2.3 Detection of Substrate Utilisation

Thirty microliters (30 µl) from each prepared substrates were added to the pre-incubated soil (3–5 days) in the deep-well plates. In order to calculate carbon utilisation, the detection plate colour was measured as absorbance at 570 nm immediately before and after the six-hour incubation using a microplate reader (Burton et al. 2010; Campbell et al. 2003). Microbial respiration was observed for nine days. Detection plates were replaced before saturation and initial absorbance was measured at each time of using a new detection plate.

2.3.3.3 Microbial Biomass

Soil MBC and MBN contents were measured using the fumigation-extraction method with an EC conversion factor of 2.64 (Vance et al. 1987) and an EN conversion factor of 2.22 (Jenkinson 1988) as described by Liu et al. (2018). In brief, soil samples were pre-incubated at 40% WHC at 25 °C. Four subsamples of 5 g of each soil were fumigated with ethanol-free chloroform for 24 hours at 25 °C in a sealed desiccator in the dark. The non-fumigated three subsamples of 5 g of each soil were stored at 4 °C during this period. The following day, after removing the beaker of chloroform, the desiccator was evacuated four times to remove the chloroform from the soils. Both fumigated and non-fumigated subsamples were extracted with freshly prepared 0.5 M K_2SO_4 at a 1:4 ratio and filtered. The filtrate was analysed using a Shimadzu TOC-VCSH/CSN total organic carbon and nitrogen analyser (Shimadzu Scientific Instruments, Japan) to calculate the concentrations of dissolved organic carbon and total nitrogen in fumigated and unfumigated samples.

2.3.3.4 Microbial Metabolic Quotient

Microbial qCO2 was computed by dividing the measured CO_2 production during respiration by the MBC content of the soil sample. This quotient serves as an indicator of the metabolic efficiency of the microbial community in the soil, reflecting the amount of CO_2 produced per unit of microbial biomass.

2.3.4 Data Analysis

Differences in soil properties (chemical, physical and biological) between management practices of each pair sites were analysed using RStudio. Differences between patterns of microbial metabolic function profiles were tested with permutational analysis of variance

(PERMANOVA) and visualised using multidimensional scaling (MDS) of bootstrap averages. Monte Carlo P–values were used to detect significant differences among management practices (Amarasinghe et al. 2021). PCA analysis was performed to select the most influenced substrates for the variability of the microbial respiration rates between management practices. MDS, PERMANOVA, and PCA were run in PRIMER v7 with PERMANOVA+.

2.4 Results and Discussion

2.4.1 Physical and Chemical Properties of Soil

2.4.1.1 Water Holding Capacity

Most of the paired sites showed no significant differences in WHC. However, an exception occurred at the HCPSL site between two management practices: conventional tillage and furrow planting with legume cover during the fallow period (CAREY SHP Farmer) and minimum tillage practices, with mound planting with legume cover during the fallow period (CAREY SHP Demo) (HCPSL Pair 2). In this instance, 'CAREY SHP Farmer' displayed a significantly lower WHC compared to 'CAREY SHP Demo' ($p < 0.05$) (Table 2.3). Additionally, soil treated with lime in the PIRSA and soil managed with cover crop in SFL exhibited higher WHC compared to conventionally managed soil (without lime treatment and control respectively) (Table 2.3). Overall, these findings imply that certain management practices, such as minimum tillage, lime application, and cover crop management can play a significant role in maintaining or improving soil WHC. This is crucial for agricultural productivity, as soils with higher WHC can better sustain crops during periods of water stress and contribute to overall soil health and resilience.

The variations in WHC observed between different management practices likely stem from a combination of factors. Conventional tillage practices may disrupt soil structure, reducing WHC, while minimum tillage methods preserve soil integrity and organic matter content, enhancing WHC. Planting methods involving legume cover crops during fallow periods contribute organic matter to the soil, improving structure and water retention compared to conventional methods. Lime application modifies soil pH, promotes aggregation, and increases WHC. Cover crop management further enhances WHC by increasing organic matter input, improving soil structure, and reducing erosion. These practices collectively promote soil health, increase organic matter content, and stimulate microbial activity, all of which contribute to improved WHC and overall soil resilience crucial for sustaining agricultural productivity during water stress periods.

Table 2.3: Water Holding Capacity (WHC) of soil across varied management histories.

Sites	Management practice	WHC (%)	P value
CWFS	CWFS_Conventional	48.97 ± 0.77[a]	p = 0.08
	CWFS_EBM	47.26 ± 0.27[a]	
Facey Group	Facey_Conventional	30.77 ± 0.87[a]	p = 0.46
	Facey_EBM	29.14 ± 1.86[a]	
HCPSL Pair 1	RSF_Conventional	35.78 ± 0.25[a]	p = 0.22
	RSD_EBM	40.64 ± 1.68[a]	
HCPSL Pair 2	CSF_Conventional	23.66 ± 1.27[b]	p = 0.04
	CSD_EBM	29.51 ± 1.40[a]	
HCPSL Pair 3	D 3-2_Furrow	43.81 ± 1.30[a]	p = 0.09
	D 3-1_Mound	47.17 ± 0.81[a]	
HCPSL Pair 4	CF_Conventional	40.87 ± 0.03[a]	p = 0.02
	D 1-1_EBM	43.36 ± 0.34[a]	
PIRSA Pair 1	Spading	15.95 ± 1.80[a]	P = 0.92
	Spading+OM	16.30 ± 2.64[a]	
PIRSA Pair 2	No lime	41.10 ± 0.77[b]	P = 0.01
	Lime added	44.80 ± 0.66[a]	
Soils for Life	SFL_Conventional	44.21 ± 1.09[b]	P = 0.00
	SFL_EBM	54.54 ± 0.76[a]	

Significant differences in WHC between two management practices at a 95% confidence level, indicated by different letters in each comparison.

2.4.1.2 Soil pH and Electrical Conductivity

Soil pH dynamics were observed across various management practices in different agricultural settings (Table 2.4). In CWFS and HCPSL pair 2, soils under cover crop management (CWFS_EBM and CSD_EBM) exhibited higher pH levels compared to their respective control sites (CWFS_Conventional and CSD_Conventional). Conversely, SFL displayed decreased pH under cover crop management (SFL_EBM) compared to its control (SFL_Conventional). In HCPSL pair 4, minimum tillage practices (D 1-1_EBM) led to an increase in soil pH relative to excessive tillage practices (CF_Conventional). Additionally, in HCPSL pair 3, the mound planting method resulted in elevated pH compared to the furrow planting method. Furthermore, both lime treatment and spading combined with organic matter treatment significantly raised soil pH levels compared to their control counterparts in PIRSA.

Soil electrical conductivity (EC) exhibited notable variations under different management practices across various agricultural contexts (Table 2.4). In CWFS and HCPSL pair 2, soils under cover crop management and cover crop combined with minimum tillage demonstrated a significant decrease in EC compared to their respective controls. Conversely, mound planting in HCPSL pair 3 resulted in increased EC compared to furrow planting, while minimum tillage practices in HCPSL pair 4 led to higher EC levels compared to excessive tillage methods. Additionally, the Bednar treatment in the FGp samples and lime treatment in PIRSA samples significantly elevated EC compared to their control sites. These findings highlight the diverse impacts of management practices on soil EC, emphasising the need for tailored approaches to soil management to optimise soil salinity levels based on specific agricultural contexts.

The observed variations in soil pH and EC across different management practices can be attributed to a combination of factors including organic matter input, nutrient cycling, soil structure, and root activity. Cover crop management practices influence soil pH and EC through organic matter decomposition and nutrient release, leading to either pH increases or decreases depending on the decomposition products. Minimum tillage practices generally result in higher soil pH and lower EC compared to excessive tillage due to improved soil structure and organic matter retention. Differences in planting methods such as mound planting versus furrow planting can affect soil pH and EC through alterations in soil moisture levels and root distribution. Additionally, soil amendments like lime and organic matter treatments can significantly impact soil pH and EC by altering nutrient availability and microbial activity. Overall, these findings highlight the multifaceted nature of soil management practices and their intricate effects on soil properties and nutrient dynamics.

Table 2.4. pH and EC of soil across varied management histories.

Sites	Management practice	pH	EC
CWFS	CWFS_Conventional	3.93 ± 0.03	134.67 ± 1.76
	CWFS_EBM	4.43 ± 0.03	68.67 ± 0.88
		$p < 0.001$	$p < 0.001$
Facey Group	Facey_Conventional	5.14 ± 0.00	82.00 ± 1.15
	Facey_EBM	5.71 ± 0.05	97.00 ± 4.51
		$p < 0.001$	$p = 0.032$
HCPSL Pair 1	RSF_Conventional	4.03 ± 0.08	76.00 ± 7.55
	RSD_EBM	4.06 ± 0.02	64.33 ± 5.90
		$p = 0.799$	$p = 0.290$
HCPSL Pair 2	CSF_Conventional	3.90 ± 0.02	63.5 ± 1.5
	CSD_EBM	4.35 ± 0.01	51.67 ± 1.2
		$p < 0.001$	$p = 0.008$
HCPSL Pair 3	D 3-2_Furrow	4.06 ± 0.01	48.00 ± 0.58
	D 3-1_Mound	4.28 ± 0.02	60.67 ± 1.45
		$p < 0.001$	$p = 0.001$
HCPSL Pair 4	CF_Conventional	3.78 ± 0.01	53.00 ± 1.53
	D 1-1_EBM	4.09 ± 0.02	65.33 ± 2.96
		$p < 0.001$	$p = 0.021$
PIRSA Pair 1	Spading	5.10 ± 0.01	192.00 ± 5.69
	Spading+OM	5.32 ± 0.03	187.00 ± 2.08
		$p = 0.001$	$p = 0.455$
PIRSA Pair 2	No lime	4.08 ± 0.03	112.33 ± 1.45
	Lime added	5.61 ± 0.01	118.33 ± 1.20
		$p < 0.001$	$p = 0.034$
Soils for Life	SFL_Conventional	5.10 ± 0.08	94.33 ± 9.84
	SFL_EBM	4.83 ± 0.05	105.33 ± 2.19
		$p = 0.037$	$p = 0.336$

Significant differences in pH and EC between two management practices at a 95% confidence level, indicated by different letters in each comparison.

2.4.1.3 Total Carbon and Total Nitrogen

Significant differences were found in the total carbon and total nitrogen content between paired sites within the FGp and SFL ($p < 0.05$) (Table 2.5). Specifically, soils from the cover crop trial (SFL_EBM) in the SFL group and the Bednar treatment-applied soil in the FGp (Facey_EBM) exhibited notably higher total carbon and total nitrogen content compared to their respective controls (SFL_Conventional and Facey_Conventional, respectively). However, in the CWFS, no significant difference was observed between the paired sites. In the HCPSL, notable differences in total nitrogen content were observed only in Pair 4, where higher total nitrogen content was noted under minimum tillage practices (D 1-1_EBM) compared to conventional tillage practices (CF_Conventional). Moreover, total carbon content was also higher under minimum tillage practices in Pair 4 compared to CF_Conventional. In PIRSA Pair 2, soil treated with lime showed no difference in total carbon content compared to its control, but lower total nitrogen content was observed. Conversely, contradictory total carbon and total nitrogen content were observed in PIRSA Pair 1, where spading plus organic matter management exhibited lower total carbon and total nitrogen compared to spading-only management practices.

The results demonstrate significant variations in total carbon and total nitrogen content among paired sites within the FGp and SFL samples, indicating the influence of specific management practices on soil fertility. Soil treatments such as cover crop trials and specific applications led to notably higher total carbon and total nitrogen content compared to controls in both groups. While no significant differences were found in the CWFS, notable increases in total nitrogen content were observed under minimum tillage practices in the HCPSL, suggesting potential benefits for nitrogen retention and carbon sequestration. Lime treatment in PIRSA Pair 2 did not affect total carbon content but resulted in lower total nitrogen content, while contradictory results were observed in PIRSA Pair 1, emphasising the complexity of interactions between management practices and soil carbon and nitrogen dynamics. Overall, these findings underscore the importance of considering specific agricultural contexts when assessing soil fertility and highlight the potential for targeted management practices to enhance soil health and productivity.

Table 2.5. Total Carbon and Total Nitrogen content of soil across varied management histories.

Sites	Management practice	Total Carbon (%)	Total Nitrogen (%)
CWFS	CWFS_Conventional	1.18 ± 0.03	0.11 ± 0.00
	CWFS_EBM	1.17 ± 0.02	0.11 ± 0.00
		p = 0.834	p = 0.125
Facey Group	Facey_Conventional	1.06 ± 0.06	0.09 ± 0.01
	Facey_EBM	1.22 ± 0.02	0.10 ± 0.00
		p = 0.049	p = 0.043
HCPSL Pair 1	RSF_Conventional	1.54 ± 0.01	0.12 ± 0.00
	RSD_EBM	1.21 ± 0.00	0.11 ± 0.01
		p < 0.001	p = 0.585
HCPSL Pair 2	CSF_Conventional	0.90 ± 0.01	0.10 ± 0.02
	CSD_EBM	0.83 ± 0.01	0.08 ± 0.01
		p = 0.002	p = 0.317
HCPSL Pair 3	D 3-2_Furrow	1.71 ± 0.00	0.13
	D 3-1_Mound	1.57 ± 0.01	0.12
		p < 0.001	p = 0.539
HCPSL Pair 4	CF_Conventional	1.38 ± 0.01	0.11
	D 1-1_EBM	1.63 ± 0.03	0.13
		p < 0.001	p = 0.021
PIRSA Pair 1	Spading	0.82 ± 0.05	0.08
	Spading+OM	0.49 ± 0.01	0.05
		p < 0.001	p < 0.001
PIRSA Pair 2	No lime	1.27 ± 0.03	0.13
	Lime added	1.27 ± 0.07	0.11
		p = 0.981	p = 0.042
Soils for Life	SFL_Conventional	1.54 ± 0.07	0.14 ± 0.01
	SFL_EBM	2.60 ± 0.01	0.19 ± 0.00
		p < 0.001	p < 0.001

Significant differences in total carbon and total nitrogen between two management practices at a 95% confidence level, indicated by different letters in each comparison.

2.4.1.4 Hot Water Extractable Organic Carbon and Nitrogen

The content of HWEOC was notably elevated in soils managed with cover crops in the CWFS (CWFS_EBM) and SFL (SFL_EBM) compared to their respective control sites (CWFS_Conventional and SFL_Conventional) (Table 2.6). Additionally, the Bednar treatment in the FGp (Facey_EBM) and minimum tillage practice in HCPSL (D_1-1_EBM) exhibited higher HWEOC contents compared to their control sites (Table 2.6). Interestingly, the combination of spading and organic matter treatment in PIRSA resulted in a lower HWEOC content compared to solely spading management (Table 2.6). However, there was no significant difference observed in HWEOC between soils treated with lime and those without lime in PIRSA (Table 2.6). The HWEON exhibited a significant decrease under cover crop management in CWFS compared to its control (Table 2.6). Conversely, SFL displayed a contrasting pattern, with significantly higher HWEON content under cover crop management compared to conventionally managed soil (Table 2.6). Similarly, in HCPSL, minimum tillage management resulted in higher HWEON content compared to excessive tillage practices (Table 2.6). In PIRSA, both spading only and spading plus organic matter amendment management practices followed a trend similar to that observed for HWEOC, with spading only management showing higher HWEON content compared to the spading plus organic amendment approach (Table 2.6). Additionally, there was no notable difference in HWEON content between lime-treated soil and soil with no lime added in PIRSA (Table 2.6).

The elevated content of HWEOC in soils managed with cover crops in the CWFS (CWFS_EBM) and SFL (SFL_EBM), as well as in treatments like the Bednar treatment in the FGp (Facey_EBM) and minimum tillage practice in HCPSL, can be attributed to increased organic matter input and reduced soil disturbance, fostering organic carbon accumulation. Conversely, the lower HWEOC content in soils managed with a combination of spading and organic matter treatment in PIRSA suggests potential interactions hindering organic carbon preservation. Regarding HWEON, the contrasting patterns observed between cover crop management in CWFS and SFL likely reflect differences in the type and nitrogen content of cover crops utilised, while minimum tillage practices in HCPSL promote nitrogen retention. Additionally, the insignificant effect of lime treatment in the PIRSA samples on both HWEOC and HWEON suggests that lime primarily influences soil pH and nutrient availability rather than directly impacting organic carbon and nitrogen dynamics. These results underscore the intricate interplay between management practices and soil organic matter dynamics, emphasising the need for holistic approaches in soil management strategies.

Table 2.6. Hot water extractable organic carbon and nitrogen content of soil across varied management histories.

Sites	Management practice	HWEOC (mg/kg)	HWEON (mg / kg)
CWFS	CWFS_Conventional	161.93 ± 1.14	68.59 ± 0.74
	CWFS_EBM	177.26 ± 1.76	30.50 ± 0.84
		$p < 0.001$	$p < 0.001$
Facey Group	Facey_Conventional	427.25 ± 19.06	60.19 ± 2.66
	Facey_EBM	574.33 ± 29.62	57.79 ± 1.99
		$p = 0.005$	$p = 0.497$
HCPSL Pair 1	RSF_Conventional	179.57 ± 12.3	23.6 ± 3.18
	RSD_EBM	164.22 ± 2.39	18.02 ± 0.44
		$p = 0.266$	$p = 0.133$
HCPSL Pair 2	CSF_Conventional	162.83 ± 10.98	23.25 ± 2.79
	CSD_EBM	155.45 ± 5.03	20.99 ± 1.08
		$p = 0.564$	$p = 0.480$
HCPSL Pair 3	D 3-2_Furrow	234.66 ± 24.85	27.28 ± 2.58
	D 3-1_Mound	244.12 ± 12.92	32.88 ± 0.61
		$p = 0.747$	$p = 0.079$
HCPSL Pair 4	CF_Conventional	207.77 ± 8.00	26.69 ± 1.16
	D 1-1_EBM	362.72 ± 16.61	52.1 ± 2.84
		$p < 0.001$	$p < 0.001$
PIRSA Pair 1	Spading	381.01 ± 4.96	111.66 ± 1.04
	Spading+OM	258.01 ± 2.93	103.21 ± 0.81
		$p < 0.001$	$p < 0.001$
PIRSA Pair 2	No lime	406.85 ± 7.32	69.8 ± 1.72
	Lime added	381.28 ± 9.49	64.97 ± 1.79
		$p = 0.076$	$p = 0.100$
Soils for Life	SFL_Conventional	544.98 ± 18.73	97.8 ± 1.75
	SFL_EBM	672.82 ± 8.74	112.37 ± 1.23
		$p < 0.001$	$p < 0.001$

Significant differences in HWEOC and HWEON between two management practices at a 95% confidence level, indicated by different letters in each comparison.

2.4.1.5 Mineral Nitrogen Content

There were notable differences in the overall mineral nitrogen content between the two management approaches at each site. For instance, in CWFS and SFL, implementing cover crop management (CWFS_EBM and SFL_EBM) led to a lower total mineral Nnitrogen content compared to their respective control methods (CWFS_Conventional and SFL_Conventional) (Table 2.7). Similarly, within the FGp, using the Bednar treatment (Facey_EBM) resulted in lower total mineral nitrogen content compared to the control (Facey_Conventional) (Table 2.7). Additionally, in HCPSL, combining cover crop management with minimum tillage (RSD_EBM and CSD_EBM) demonstrated significantly lower total mineral nitrogen content than their respective controls (RSD_Conventional and CSD_Conventional) (Table 2.7). However, in HCPSL Pair 4, where minimum tillage and cover crop practices were applied (D_1-1_EBM), there was notably higher total mineral nitrogen content compared to excessive tillage practices (CF_Conventional) (Table 2.7). Moreover, in HCPSL Pair 3, cover crop management coupled with furrow planting exhibited higher total mineral nitrogen content compared to mound planting (Table 2.7). We did not observe a significant difference in soil mineral nitrogen content between PIRSA Pair 1, where spading combined with organic matter management was compared to spading-only management practices. However, in PIRSA Pair 2, soil treated with lime showed an increased total mineral nitrogen content compared to the treatment without lime (Table 2.7).

The differences observed in total mineral nitrogen content between management strategies at each site can be attributed to several factors. The implementation of cover crop management in CWFS, SFL, some sites in HCPSL as well as the Bednar treatment in the FGp, led to lower total mineral nitrogen content compared to conventional control treatments, possibly due to competition for nitrogen resources and variations in nitrogen cycling dynamics. Conversely, in HCPSL, cover crop management combined with minimum tillage demonstrated significantly higher total mineral nitrogen content than conventional controls, likely as a result of improved soil structure, increased microbial activity, and enhanced nitrogen retention associated with minimum tillage practices. Additionally, in HCPSL Pair 3, the use of furrow planting resulted in higher total mineral nitrogen content compared to mound planting, potentially due to differences in soil moisture retention and microbial activity influenced by planting methods. The lack of a significant difference in soil mineral nitrogen content between spading with and without organic matter in PIRSA Pair 1 could be due to the similar nitrogen release rates from the organic matter and the existing soil organic matter. In contrast, the increased total mineral nitrogen content in the lime-treated soil of PIRSA Pair 2 can be attributed to lime's ability to enhance soil pH, thereby improving nitrogen mineralisation and availability. These variations underscore the complex interplay of cover crop management, tillage practices, organic matter amendment, lime treatments, planting methods, and site-specific factors in shaping soil nitrogen dynamics.

Table 2.7. Mineral nitrogen content (NH_4^+ and NO_3^-) of soil across varied management histories.

Sites	Management practice	NH4+ (mg / Kg)	NO3- (mg / Kg)	Total MN (mg / Kg)
CWFS	CWFS_Conventional	6.09 ± 0.12	51.55 ± 0.67	57.64 ± 0.69
	CWFS_EBM	7.45 ± 1.50	12.80 ± 0.11	20.24 ± 1.53
		p = 0.403	p < 0.001	p < 0.001
Facey Group	Facey_Conventional	1.00 ± 0.02	8.00 ± 0.06	9.00 ± 0.06
	Facey_EBM	0.95 ± 0.02	7.30 ± 0.09	8.25 ± 0.11
		p = 0.147	p < 0.001	p < 0.001
HCPSL Pair 1	RSF_Conventional	18.23 ± 0.27	6.09 ± 0.06	24.31 ± 0.27
	RSD_EBM	14.70 ± 0.05	3.85 ± 0.01	18.55 ± 0.06
		p < 0.001	p < 0.001	p < 0.001
HCPSL Pair 2	CSF_Conventional	13.63 ± 0.47	7.58 ± 0.11	21.21 ± 0.46
	CSD_EBM	11.74 ± 0.05	5.94 ± 0.08	17.68 ± 0.12
		p = 0.007	p < 0.001	p < 0.001
HCPSL Pair 3	D 3-2_Furrow	16.48 ± 0.04	6.82 ± 0.02	23.30 ± 0.05
	D 3-1_Mound	13.06 ± 0.03	13.31 ± 0.05	26.37 ± 0.08
		p < 0.001	p < 0.001	p < 0.001
HCPSL Pair 4	CF_Conventional	18.60 ± 0.2	5.50 ± 0.06	24.1 ± 0.20
	D 1-1_EBM	28.24 ± 0.09	11.4 ± 0.05	39.64 ± 0.13
		p < 0.001	p < 0.001	p < 0.001
PIRSA Pair 1	Spading	129.68 ± 6.89	250.23 ± 9.54	379.92 ± 5.12
	Spading+OM	113.62 ± 3.97	247.72 ± 6.45	361.33 ± 10.35
		p = 0.09	p = 0.833	p = 0.158
PIRSA Pair 2	No lime	133.09 ± 6.14	83.95 ± 5.13	217.04 ± 10.94
	Lime added	146.70 ± 7.23	115.59 ± 3.41	262.29 ± 10.34
		p = 0.201	p = 0.002	p = 0.024
Soils for Life	SFL_Conventional	115.39 ± 1.14	2.17 ± 0.01	117.57 ± 1.14
	SFL_EBM	34.83 ± 0.13	14.68 ± 0.21	49.51 ± 0.29
		p < 0.001	p < 0.001	p < 0.001

Significant differences in mineral nitrogen content (NH_4^+, NO_3^- and Total) between two management practices at a 95% confidence level, indicated by different letters in each comparison.

2.4.2 Biological Properties of the Soil

2.4.2.1 Microbial Basal Respiration

Microbial basal respiration exhibited significant variations across different management practices. Specifically, it was notably higher under cover crop management (SFL_EBM) compared to its control (SFL_Conventional) in SFL and under minimum tillage practice (D 1-1_EBM) compared to excessive tillage practice (CF_Conventional) in HCPSL (Table 2.8). Additionally, the addition of lime treatment increased microbial respiration compared to soil without lime in PIRSA (Table 2.8). However, contrasting results were observed in other pairs of HCPSL and CWFS, where cover crop management (RSD_EBM and CSD_EBM, CWFS_EBM) did not show a significant difference compared to their respective controls (RSD_Conventional, CSD_Conventional and CWFS_Conventional respectively) (Table 2.8). Similarly, no significant difference in microbial respiration was observed between furrow planting (D 3-2_Furrow) and mound planting (D 3-1_Mound) methods in HCPSL (Table 2.8). The observed variations in microbial basal respiration across different management practices reflect the complex interactions between soil management techniques and microbial activity. In SFL, the implementation of cover crop management (SFL_EBM) resulted in significantly higher microbial basal respiration compared to its control (SFL_Conventional), indicating that cover crops may enhance soil microbial activity by providing additional organic matter and habitat for microorganisms. Similarly, in HCPSL, the adoption of minimum tillage practices (D 1-1_EBM) led to notably higher microbial respiration compared to excessive tillage practices (CF_Conventional), suggesting that reduced soil disturbance promotes microbial activity by preserving soil structure and organic matter content. Conversely, the addition of lime treatment in PIRSA increased microbial respiration compared to soil without lime, indicating that lime application may enhance soil microbial activity by adjusting soil pH or providing additional nutrients favourable for microbial growth. However, contrasting results observed in other pairs of HCPSL and CWFS, suggesting that the impact of cover crops on microbial activity may vary depending on site-specific conditions or management practices. Similarly, no significant difference in microbial respiration between furrow planting (D 3-2_Furrow) and mound planting (D 3-1_Mound) methods in HCPSL, indicating that planting techniques may not have a substantial effect on microbial activity under the conditions tested. Overall, these results highlight the importance of considering multiple factors, including soil management practices, site-specific conditions, and plant–soil interactions, when evaluating the impact of management strategies on soil microbial activity.

Table 2.8. Microbial Basal Respiration of soil across varied management histories.

Sites	Management practice	Microbial Respiration (CO_2-C µg g^{-1} h^{-1} dry soil)	P value
CWFS	CWFS_Conventional	0.22 ± 0.02	p = 0.836
	CWFS_EBM	0.23 ± 0.04	
Facey Group	Facey_Conventional	0.31 ± 0.04	p = 0.045
	Facey_EBM	0.61 ± 0.1	
HCPSL Pair 1	RSF_Conventional	0.29 ± 0.01	p = 0.335
	RSD_EBM	0.24 ± 0.05	
HCPSL Pair 2	CSF_Conventional	0.25 ± 0.04	p = 0.378
	CSD_EBM	0.32 ± 0.05	
HCPSL Pair 3	D 3-2_Furrow	0.43 ± 0.06	p = 0.318
	D 3-1_Mound	0.36 ± 0.03	
HCPSL Pair 4	CF_Conventional	0.43 ± 0.02	p = 0.007
	D 1-1_EBM	0.57 ± 0.03	
PIRSA Pair 1	Spading	0.27 ± 0.01	p < 0.001
	Spading+OM	0.05 ± 0.02	
PIRSA Pair 2	No lime	0.35 ± 0.03	p = 0.050
	Lime added	0.48 ± 0.04	
Soils for Life	SFL_Conventional	1.24 ± 0.08	p = 0.025
	SFL_EBM	1.70 ± 0.13	

Significant differences in microbial respiration between two management practices at a 95% confidence level, indicated by different letters in each comparison.

2.4.2.2 Microbial Functional Diversity

Metabolic function profiles, such as substrate-induced respiration, of soil microbial communities were compared under different cover crop management practices and their corresponding controls at HCPSL (CSF_EBM vs CSF_Conventional, Figure 2.5B) and SFL (SFL_EBM vs SFL_Conventional, Figure 2.8), revealing significant differences. Additionally, comparisons between excessive tillage (CF_Conventional) and minimum tillage practices (D 1-1_EBM) (Figure 2.6A), as well as mound planting (D 3-1_Mound) and furrow planting (D 3-2_Furrow) (Figure 2.6B) at HCPSL, showed significant variations in microbial functional diversity. Similarly, paired sites within PIRSA (Control vs Lime treated and Spading vs Spading+Organic Matter) exhibited notable differences in functional diversity (Figure 2.7A and 2.7B). However, no significant differences were observed in microbial functional diversity between management histories in soils from CWFS (Cover crop management vs conventional management) (Figure 2.4A), the FGp (Bednar treatment vs Control) (Figure 2.4B) and one pair in HCPSL (RSF_EBM vs RSF_Conventional) (Figure 2.5A).

The significant differences in metabolic function profiles observed across various management practices likely due to a combination of factors influencing soil microbial communities. Variances in cover crop management practices, such as organic matter enrichment and nutrient availability, can foster diverse substrates for microbial respiration, enhancing functional diversity compared to conventional methods. Distinctions between tillage practices, like minimum tillage preserving soil structure and organic matter, versus excessive tillage disrupting these conditions, contribute to microbial diversity variations. Planting methods, such as mound versus furrow planting, alter microclimates and nutrient distribution, affecting microbial community dynamics. Soil amendments like lime treatment and organic matter addition can shift microbial community structure by changing pH, nutrient availability, and substrate utilization efficiency.

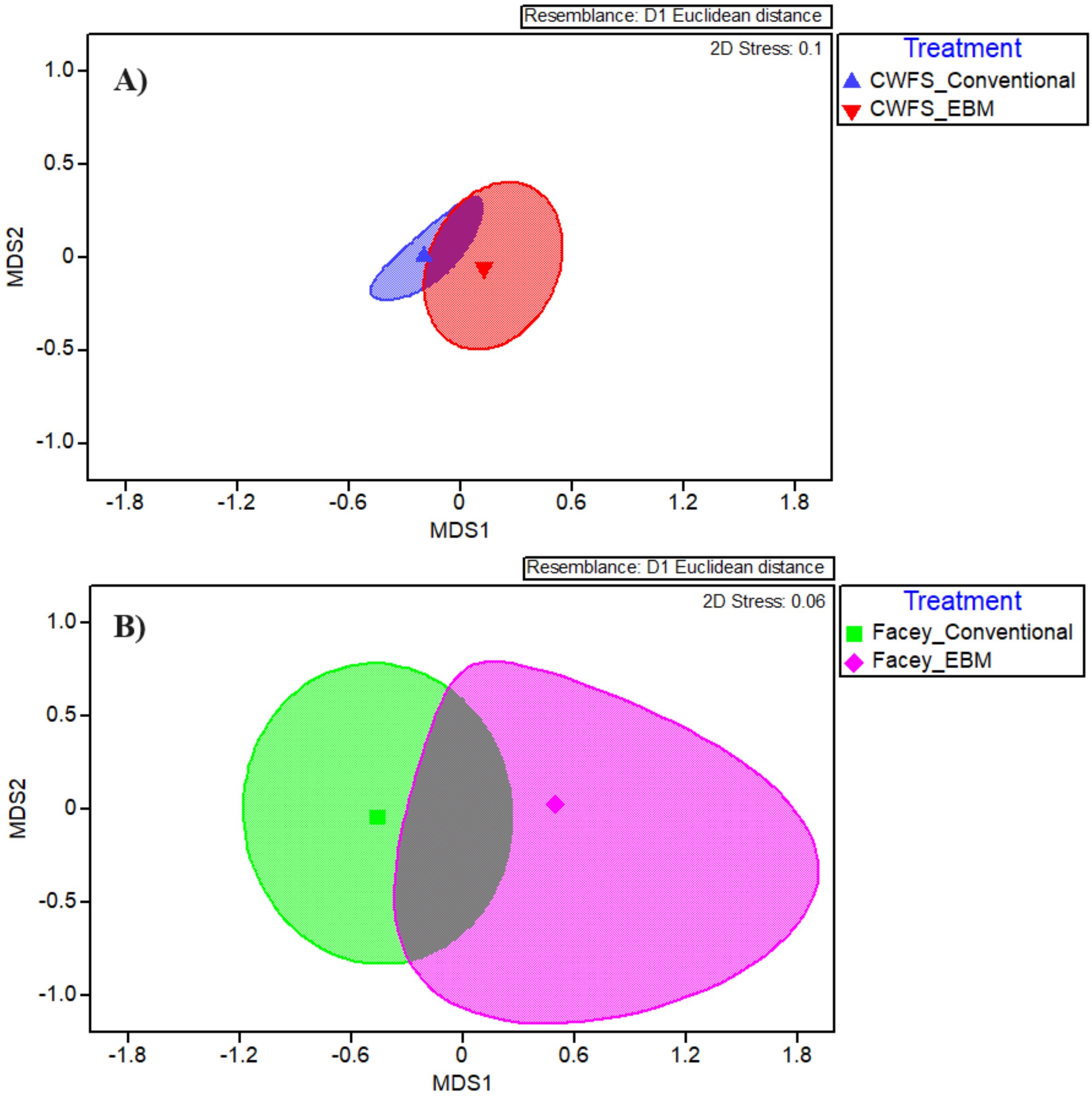

Figure 2.4. Ordinations of bootstrap averages (50 bootstraps) of substrate-induced respiration data under different management history of soils from CWFS (a) and from FGp (b). Two-dimensional metric multi-dimensional scaling ordinations (mMDS) of 50 bootstrap sample averages for each management practice and the empirical approximate 95% confidence region, based on Euclidean distance. The distance between the points corresponds to the functional diversity of the microbes in particular management practice: the shorter the distance, the higher similarity between the management practices.

CWFS *= Central West Farming Systems,* ***CWFS_Conventional*** *= no cover crop,* ***CWFS_EBM*** *= cover crop trial,* ***Facey_Conventional*** *= No Bednar treatment,* ***Facey_EBM*** *= Bednar treatment*

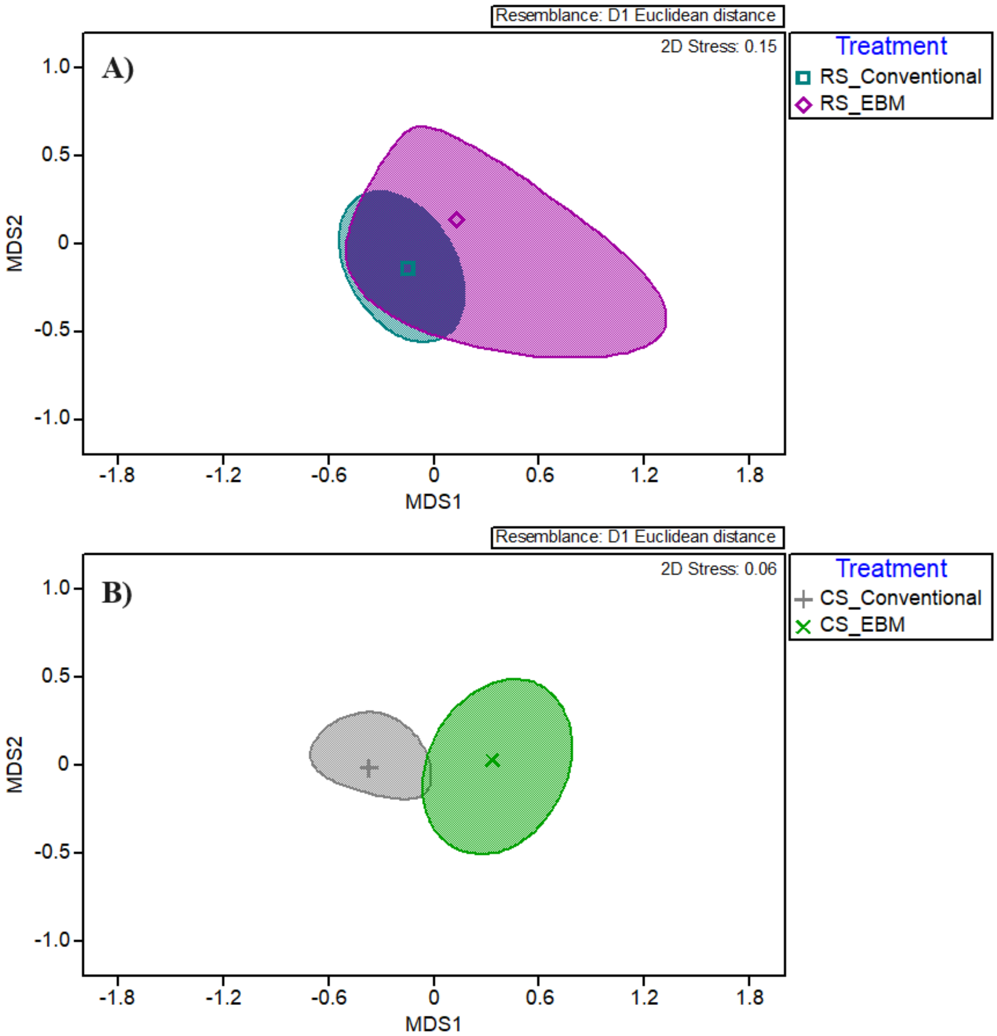

Figure 2.5. Ordinations of bootstrap averages (50 bootstraps) of substrate induced respiration data under conventional and ecosystem-based management (EBM) history of soils from HCPSL, pair 1 (a) and 2 (b). Two-dimensional metric multi-dimensional scaling ordinations (mMDS) of 150 bootstrap sample averages for each management practice and the empirical approximate 95% confidence region, based on Euclidean distance. The distance between the points corresponds to the functional diversity of the microbes in particular management practice: the shorter the distance, the higher similarity between the management practices.

RS_Conventional *= no legume cover crop during the fallow period, significant tillage practiced, and furrow planted,* ***RS_EBM*** *= a multi-species legume cover crop during the fallow period, minimum tillage practiced, with mound planted cane,* ***CS_Conventional*** *= a cowpea and lablab during the fallow period, conventional tilled and furrow planted,* ***CS_EBM*** *= a large multi-species legume cover crop during the fallow period, minimum tillage practices, with mound planted cane*

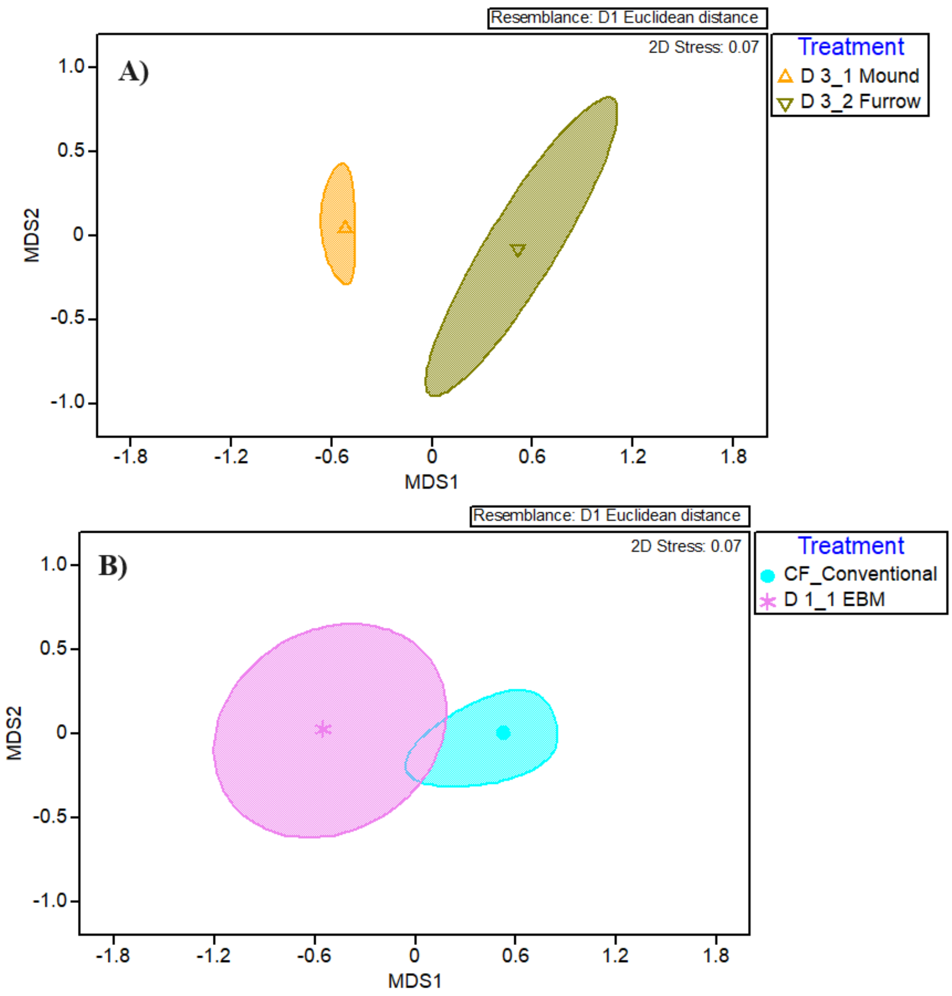

Figure 2.6. Ordinations of bootstrap averages (50 bootstraps) of substrate induced respiration data under different management history of soils from HCPSL pair 3 (a) and 4 (b) Two-dimensional metric multi-dimensional scaling ordinations (mMDS) of 150 bootstrap sample averages for each management practice and the empirical approximate 95% confidence region, based on Euclidean distance. The distance between the points corresponds to the functional diversity of the microbes in particular management practice: the shorter the distance, the higher similarity between the management practices.

D 3_1 Mound *= plant cane. The block was planted to a very large mixed fallow legume crop, minimum tilled and mound planted to cane,* ***D 3_2 Furrow*** *= a old ratoon which was planted to a mixed cowpea, soybean and lablab cover crop during the fallow period, minimum tilled and furrow planted to cane,* ***CF_Conventional*** *= This block has been excessively tilled, no stubble conservation practices (aligns with a traditional sugarcane farming system,* ***D 1-1_EBM*** *= This block has been under controlled traffic, minimum tillage, a large mixed fallow cover crop during the fallow period and all cane stubbles retained,*

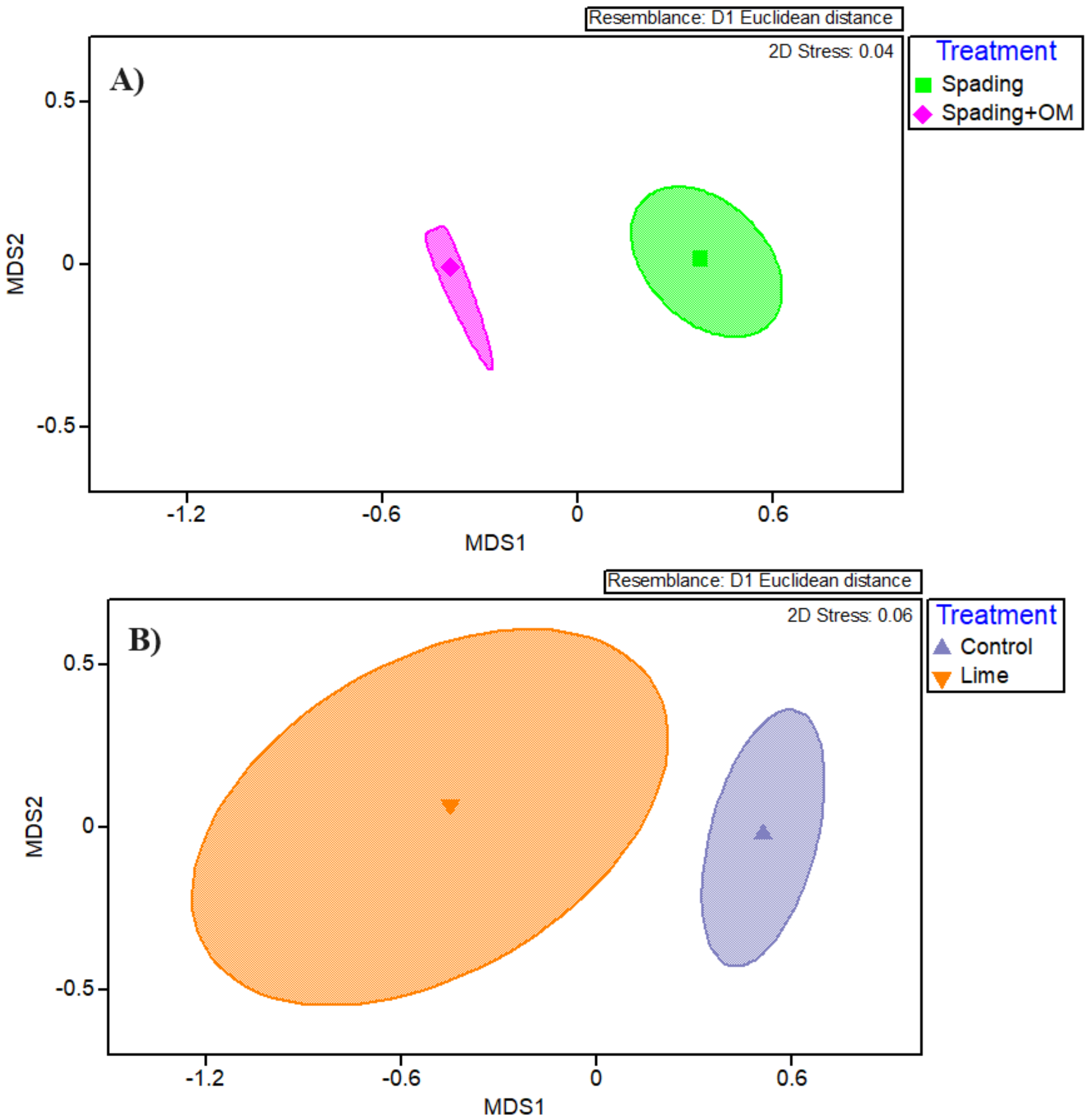

Figure 2.7. Ordinations of bootstrap averages (50 bootstraps) of substrate induced respiration data under different management history of soils from PIRSA pair 1 (A) and 2 (B) Two-dimensional metric multi-dimensional scaling ordinations (mMDS) of 150 bootstrap sample averages for each management practice and the empirical approximate 95% confidence region, based on Euclidean distance. The distance between the points corresponds to the functional diversity of the microbes in particular management practice: the shorter the distance, the higher similarity between the management practices.

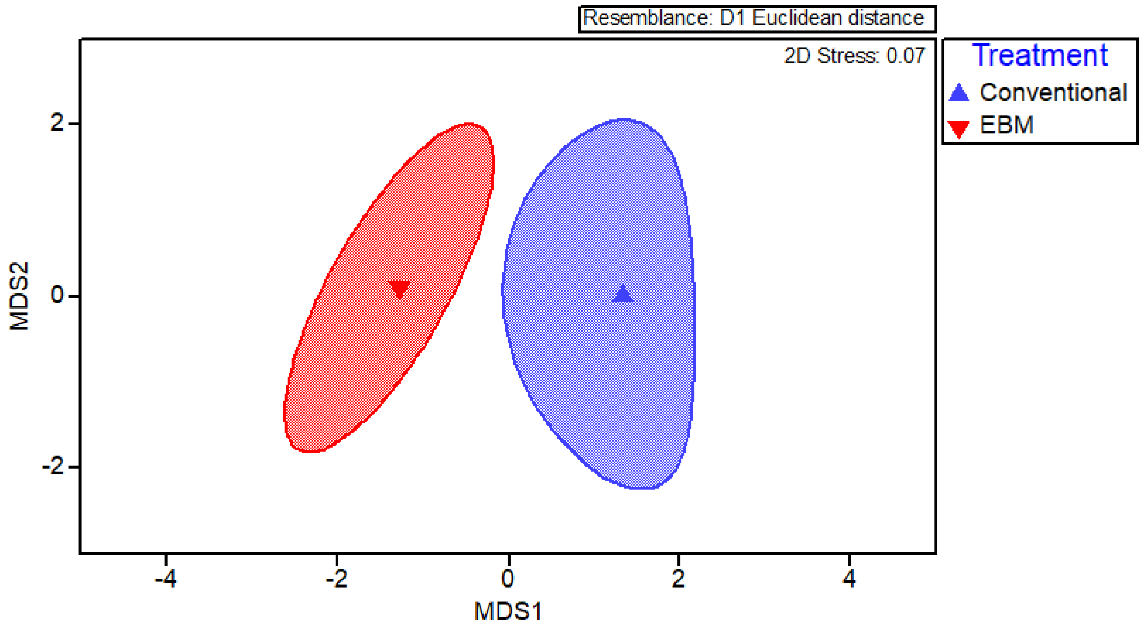

Figure 2.8. Ordinations of bootstrap averages (50 bootstraps) of substrate induced respiration data under different management history of soils from Soils for Life. Two-dimensional metric multi-dimensional scaling ordinations (mMDS) of 150 bootstrap sample averages for each management practice and the empirical approximate 95% confidence region, based on Euclidean distance. The distance between the points corresponds to the functional diversity of the microbes in particular management practice: the shorter the distance, the higher similarity between the management practices. Conventional = no cover crop management practice, EBM = cover crop management practice.

2.4.2.3 Microbial Biomass Carbon and Nitrogen Content

Microbial biomass carbon (MBC) content exhibited notable variations across different management practices within various pairs (Table 2.9). In CWFS (CWFS_EBM), SFL (SFL_EBM), and HCPSL pair 1 (RSD_EBM), MBC content was significantly elevated under cover crop management compared to respective controls (CWFS_Conventional, SFL_Conventional and RSF_Conventional respectively). Moreover, within HCPSL pair 4, adopting minimum tillage practices (D 1-1_EBM) resulted in higher MBC content than excessive tillage methods (CF_Conventional). Notably, in PIRSA pair 1, employing spading only management demonstrated significantly higher MBC content compared to a spading plus organic matter amendment approach. Additionally, Bednar treatment (Facey_EBM) in the FGp exhibited higher MBC content than its control (Facey_Conventional), although this difference lacked statistical significance. Interestingly, in HCPSL pair 3, no significant difference in MBC content was observed between furrow planting and mound planting; however, mound planting exhibited a higher MBC value compared to furrow planting. Among the groups analysed, only the FGp and SFL displayed a notable contrast in MBN content. Specifically, within the FGp , the Bednar treatment showed higher MBN content compared to its control. Similarly, in SFL, implementing cover crop management led to elevated MBN content in comparison to its control.

The variations in MBC content across different management practices within various pairs suggest that factors such as cover crop management, tillage practices, organic matter input, and planting methods influence soil microbial communities. The significant elevation in MBC content under cover crop management implies that the presence of cover crops enhances organic matter input into the soil, promoting microbial growth. Adoption of minimum tillage practices likely preserves soil structure and organic matter, contributing to higher MBC content compared to excessive tillage methods. The higher MBC content under spading only management compared to spading combined with organic matter amendment suggests complex interactions between soil microorganisms and organic inputs. Similarly, the observed higher MBC content in the Bednar treatment group indicates potential positive effects on microbial biomass. Moreover, the significant contrast in MBN content between certain management practices and their controls, particularly in the FGp and SFL, suggests that these practices may enhance nitrogen availability in the soil, possibly through increased nitrogen fixation or retention. Overall, these findings highlight the importance of management practices in shaping soil microbial communities and nutrient cycling processes.

Table 2.9. Microbial Biomass Carbon and Nitrogen content of soil across varied management histories.

Sites	Management practice	MBC (mg / Kg)	MBN (mg / Kg)
CWFS	CWFS_Conventional	50.77 ± 19.75	8.36 ± 3.58
	CWFS_EBM	119.7 ± 11.15	18.07 ± 6.00
		$p = 0.038$	$p = 0.214$
Facey Group	Facey_Conventional	157.95 ± 21.29	30.16 ± 4.14
	Facey_EBM	235.45 ± 14.77	51.07 ± 2.71
		$p = 0.095$	$p = 0.005$
HCPSL Pair 1	RSF_Conventional	104.35 ± 3.66	7.89 ± 1.34
	RSD_EBM	152.36 ± 20.46	9.05 ± 2.78
		$p = 0.042$	$p = 0.719$
HCPSL Pair 2	CSF_Conventional	112.46 ± 38.65	30.09 ± 8.27
	CSD_EBM	73.53 ± 10.03	14.97 ± 5.19
		$p = 0.310$	$p = 0.197$
HCPSL Pair 3	D 3-2_Furrow	156.56 ± 4.64	16.56 ± 1.48
	D 3-1_Mound	175.45 ± 32.04	20.16 ± 4.09
		$p = 0.591$	$p = 0.504$
HCPSL Pair 4	CF_Conventional	140.43 ± 2.85	18.18 ± 4.45
	D 1-1_EBM	266.08 ± 26.45	31.06 ± 4.81
		$p = 0.009$	$p = 0.121$
PIRSA Pair 1	Spading	114.12 ± 4.83	12.4 ± 1.21
	Spading+OM	86 ± 2.46	9.51 ± 4.8
		$p = 0.002$	$p = 0.580$
PIRSA Pair 2	No lime	137.89 ± 5.45	10.6 ± 2.52
	Lime added	121.23 ± 5.89	12.79 ± 1.39
		$p = 0.083$	$p = 0.476$
Soils for Life	SFL_Conventional	202.53 ± 11.37	39.14 ± 1.76
	SFL_EBM	479.9 ± 34.16	84.71 ± 5.65
		$p < 0.001$	$p < 0.001$

Significant differences in MBC and MBN content between two management practices at a 95% confidence level, indicated by different letters in each comparison.

2.4.2.4 Microbial Metabolic Quotient

The microbial qCO_2, indicating the amount of CO_2 produced per unit of microbial biomass, exhibited lower values in soils managed with cover crops compared to those under conventional management practices in CWFS, HCPSL pairs 1 and 2, and SFL (Table 2.10). Notably, in SFL, the qCO_2 was significantly lower under cover crop management than in its control. In HCPSL pair 4, minimum tillage practices showed a lower qCO2 value compared to excessive tillage practices. Additionally, spading combined with organic matter management significantly decreased qCO_2 in soil compared to spading-only treatment in PIRSA pair 1. While lime treatment resulted in a higher qCO_2 compared to soils without lime in PIRSA pair 2, the difference was not statistically significant. Furthermore, there was no significant difference in qCO_2 between furrow planting and mound planting in HCPSL pair 3.

The observed differences in qCO_2 across various management practices can be attributed to several factors. Cover crop management likely enhances soil organic matter and microbial biomass, thereby promoting microbial activity and carbon sequestration, leading to lower qCO2 values compared to conventional practices. Minimum tillage practices preserve soil structure and organic matter, reducing disturbance and enhancing carbon retention, resulting in lower qCO2 values compared to excessive tillage. Spading combined with organic matter management likely improves soil structure and nutrient availability, fostering microbial activity and carbon sequestration, thus lowering qCO2 values compared to spading-only treatments. Lime treatment may alter soil pH and microbial community composition, influencing microbial metabolic activity and resulting in higher qCO2 values. However, the lack of significant difference between furrow and mound planting methods suggests that planting technique may have less impact on qCO2 compared to other management practices. Overall, these findings underscore the critical role of management practices in shaping soil microbial communities and their metabolic activities, with implications for soil carbon dynamics and ecosystem sustainability.

Table 2.10. Microbial qCO_2 across varied management histories.

Sites	Management practice	qCO_2 ($\mu g\ CO_2\text{-}C\ h^{-1}\ mg^{-1}$)	P value
CWFS	CWFS_Conventional	5.02 ± 1.84	$p = 0.223$
	CWFS_EBM	2.36 ± 0.67	
Facey Group	Facey_Conventional	1.85 ± 0.2	$p = 0.602$
	Facey_EBM	1.71 ± 0.13	
HCPSL Pair 1	RSF_Conventional	2.8 ± 0.09	$p = 0.102$
	RSD_EBM	1.86 ± 0.48	
HCPSL Pair 2	CSF_Conventional	4.09 ± 0.84	$p = 0.358$
	CSD_EBM	3.01 ± 0.36	
HCPSL Pair 3	D 3-2_Furrow	2.42 ± 0.23	$p = 0.441$
	D 3-1_Mound	2.09 ± 0.31	
HCPSL Pair 4	CF_Conventional	3.07 ± 0.16	$p = 0.05$
	D 1-1_EBM	1.83 ± 0.44	
PIRSA Pair 1	Spading	2.4 ± 0.12	$p < 0.001$
	Spading+OM	0.61 ± 0.2	
PIRSA Pair 2	No lime	2.96 ± 0.15	$p = 0.06$
	Lime added	3.42 ± 0.1	
Soils for Life	SFL_Conventional	6.18 ± 0.49	$p = 0.007$
	SFL_EBM	3.62 ± 0.44	

Significant differences in microbial qCO_2 between two management practices at a 95% confidence level, indicated by different letters in each comparison.

2.5 Conclusion

In conclusion, the comprehensive analysis of various management practices across different agricultural contexts revealed significant impacts on soil properties and microbial communities. The findings highlight the critical role of specific strategies such as minimum tillage, cover crop management, lime application, and organic matter input in influencing soil WHC, total carbon, total nitrogen, HWEOC, HWEON, total mineral nitrogen, microbial basal respiration, metabolic function profiles, MBC content, MBN content, and qCO2. These results underscore the intricate interplay between management practices, soil properties, and microbial dynamics, emphasising the need for tailored approaches to soil management based on site-specific conditions and objectives. By optimising management practices to enhance soil health, nutrient cycling, and microbial activity, agricultural productivity can be sustained while promoting long-term environmental sustainability and resilience. Continued research and implementation of holistic soil management strategies are essential for addressing the challenges of modern agriculture and ensuring food security in the face of changing climatic conditions and resource constraints.

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CHAPTER 3: ASSESSING SOIL BIOLOGICAL RESISTANCE AND RESILIENCE TO DROUGHT STRESS

3.1 Introduction

Understanding the impact of drought stress on soil ecosystems is importance in a world struggling with changing climate patterns and increasing water scarcity (Chieb and Gachomo 2023). Soil, as a complex and dynamic medium, hosts a variety of microorganisms that play vital roles in nutrient cycling, carbon sequestration, and overall ecosystem health. Assessing the biological resistance and resilience of soil to drought stress is crucial for predicting the long-term sustainability of terrestrial ecosystems and agricultural productivity (Martin et al. 2019).

Soil resistance measures soil's ability to withstand (i.e., maintain its functions) environmental and anthropogenic pressure (de Vries et al. 2012; Isbell et al. 2015). The magnitude to which soil functions are impaired after stress is dependent on the soil systems' resistance to disturbance (Smith et al. 2022; Wada and Toyota 2007). Therefore, assessing soil biological resistance refers to understanding how soil microorganisms initially respond to drought stress, determining which species or functional groups are more resistant, and evaluating the impact of drought on microbial activity, diversity, and abundance.

Soil resilience has been defined as the capacity of a soil to recover its functional (e.g., microbial activity, decomposition of organic matter, and nutrient cycling) and structural (e.g., soil aggregate stability, and porosity) integrity to original status (similar level of function) after experiencing a specific disturbance (Griffiths et al. 2008; Li et al. 2019; Quinlan et al. 2016; Schwalm et al. 2017). Therefore, soil biological resilience evaluates how quickly and effectively microbial communities can rebound and resume their essential functions (Martin et al. 2019).

We investigated the soil biological resistance and resilience under two different soil management practices (i.e., conventional and ecosystem-based management) utilising 96-well deep-well plates. We hypothesise that soil biological resistance and resilience to drought will improve with the best management practices compared to conventional management practices. 96-well deep-well plates allow for simultaneous testing of multiple soil samples, enabling researchers to analyse a broad range of conditions and treatments efficiently. Further, the standardised well format ensures precise measurements and facilitates the replication of experiments, increasing the reliability of results. Deep well plates offer controlled conditions, including soil moisture levels and nutrient availability, allowing researchers to simulate drought stress under controlled laboratory settings.

In this chapter, we present the results of changes in soil biological and chemical properties to drought stress under different management practices from four sites: (i) Facey Group, Inc., in Wickepin, WA (FGp); (ii) Central West Farming Systems, Inc., in Condobolin, NSW (CWFS); (iii) Soils for Life Trust in Canberra, ACT (SFL); and (iv) the Department of Primary Industries and Regions in South Australia (PIRSA).

3.2 Methodology

3.2.1 Sampling Site and Soil Preparation

Soil samples were collected from diverse agricultural sites with varying management histories, including those employing cover crop management (e.g., CWFS and SFL), different tillage practices, stubble conservation, and legume cultivation during fallow periods (e.g., Herbert Cane Productivity Services Ltd. in Ingham, QLD), soil disturbance treatments (e.g., Bednar) at FGp and lime application conducted by PIRSA. Soil samples were passed through a 2.0 mm stainless steel sieve to remove the impurities. Sieved soil samples were kept at 4 °C until they were analysed. The cool storage samples were then incubated at 25 °C for two weeks to restore the microbial activities.

3.2.2 Stress Treatment

To establish different moisture levels for our experiment, we maintained three distinct conditions to achieve control (no stress), moderate, and severe levels of stress in two management practices (Table 3.1). The amount of water needed to maintain the desired moisture stress levels in the soil was determined based on the gravimetric water content of the soil.

Table 3.1. Different levels of water stress treatments

Treatment	Drought Stress
C_T1	Conventional Management_No stress (35% WFPS)
C_T2	Conventional Management_Moderate stress (15% WFPS)
C_T3	Conventional Management_Severe stress (7.5% WFPS)
T_T1	Bednar treated/ Lime application / cover crop management_ No stress (35% WFPS)
T_T2	Bednar treated / Lime application / cover crop management _Moderate stress (15% WFPS)
T_T3	Bednar treated / Lime application / cover crop management _Severe stress (7.5% WFPS)

WFPS = Water fill pore space = (GWC × BD) / [1 - (BD / PD)]

Where GWC is gravimetric water content, BD is bulk density and PD is particle density (2.65gcm^{-3}).

3.2.3 Arranging Soil in 96-well Deep-well Plates

We created a layout for the deep-well plate, clearly indicating the placement of the intended treatments and soil samples. Subsequently, we used a filling device to add soil samples into the wells following the predefined layout (Plate 1).

01	1	2	3	4	5	6	7	8	9	10	11	12
	R	R	R	R	R	R	R	R	R	R	R	R
A	C_T1	C_T1	C_T2	C_T2	C_T3	C_T3	T_T1	T_T1	T_T2	T_T2	T_T3	T_T3
B	C_T1	C_T1	C_T2	C_T2	C_T3	C_T3	T_T1	T_T1	T_T2	T_T2	T_T3	T_T3
C	C_T1	C_T1	C_T2	C_T2	C_T3	C_T3	T_T1	T_T1	T_T2	T_T2	T_T3	T_T3
D	C_T1	C_T1	C_T2	C_T2	C_T3	C_T3	T_T1	T_T1	T_T2	T_T2	T_T3	T_T3
E	C_T1	C_T1	C_T2	C_T2	C_T3	C_T3	T_T1	T_T1	T_T2	T_T2	T_T3	T_T3
F	C_T1	C_T1	C_T2	C_T2	C_T3	C_T3	T_T1	T_T1	T_T2	T_T2	T_T3	T_T3
G	C_T1	C_T1	C_T2	C_T2	C_T3	C_T3	T_T1	T_T1	T_T2	T_T2	T_T3	T_T3
H	C_T1	C_T1	C_T2	C_T2	C_T3	C_T3	T_T1	T_T1	T_T2	T_T2	T_T3	T_T3

Plate 1. Layout of the 96-well deep-well plates for the respiration measurement. Blue shading = Soil under conventional management practices, green shading = Soil under Bednar treatment/cover crop management. R = for respiration measurement (16 replicates per treatment), A-H = rows of 96-well deep-well plate, 1-12 = columns of 96-well deep-well plate, 01 = Plate number for identification purposes. C_T1, C_T2, C_T3, T_T1, T_T2 and T_T3 = as described in Table 3.1.

3.2.4 Assessing Resistance and Resilience

96-well deep-well plates with the applied drought stress treatments were place in a sealed container with a beaker of soda lime and water. Soda lime is primarily used to absorb CO_2 produced by soil microbes during incubation to maintain a controlled environment, preventing interference with measurements, while water in a beaker ensures consistent soil moisture essential for accurate research results.

The containers were incubated at 25 °C for 14 days, after which half of the 96-well deep-well plates were removed and kept at 4 °C (maximum of two weeks) for further analysis, marking the end of the resistance phase. In the remaining plates, drought stress was relieved by adding water to reach a controlled moisture level of 35% WFPS, and the incubation continued for an additional 14 days. After the full 28-day incubation period, soil samples were extracted from the plates for analysis, representing the end of the resilience phase. Daily respiration measurements were recorded using a microplate reader throughout the incubation period (as described in section 3.2.5.1). A separate set of plates was maintained specifically for the respiration measurements.

3.2.5 Analysis of Soil Biological Properties

3.2.5.1 Soil Respiration Measurements

We determined the microbial respiration using the MicroResp^{TM} technique. This approach involves the measurement of short-term changes in soil respiration in response to various external factors (i.e., stress treatment and the management practice in our experiment) (Campbell et al. 2003). This technique has been successfully applied to detect post-disturbance changes in soil functional capacity (Gartzia-Bengoetxea et al. 2016) and assessment of microbial functional recovery in environmental plantings (Amarasinghe et al. 2021). To measure the respiration-produced CO_2 calorimetrically, an indicator-gel medium was needed to capture the emitted CO_2. The colorimetric method depends on the change in the pH of a solution of bicarbonate in quasi-equilibrium with the well headspace in MicroResp. Detection plates were prepared by combining the indicator dye, cresol red (12.5 ppm, wt/wt), potassium chloride (150 mM), and sodium bicarbonate (2.5 mM) in 150 µl of Noble agar (1%) in each well of the detection plate. The agar and indicator solutions were prepared separately and combined 1:2 ratio (agar: indicator) prior to use. These plates were stored in sealed plastic boxes with wet paper towels/ a beaker of water and soda lime to ensure that they do not desiccate or react with atmospheric CO_2 until used. In order to calculate respiration, the detection plate colour was measured as absorbance at 570 nm immediately before and after the incubation using a microplate reader (Burton et al. 2010; Campbell et al. 2003). Microbial respiration was observed for 28 days (i.e., throughout the resistance and resilience phases). Detection plates were replaced before saturation and initial absorbance was measured at each time of using a new detection plate.

3.2.5.2 Substrate-induced Respiration

Carbon and amino acid substrate solutions were prepared based on their solubility in water. Once prepared the required volume, the solution was filter sterilised and stored in sterile tubes at 4 °C. At the beginning of the experiment, and end of the resistance and resilience phase, 30 µl from each prepared substrates were added to the soil in the deep-well plates. Basal respiration also measured by adding 30 µl of sterilised water. Carbon utilisation was determined as the same as the respiration measurement.

3.2.5.3 Microbial Biomass

Soil microbial biomass carbon (MBC) and nitrogen (MBN) contents were measured using the fumigation-extraction method with an EC conversion factor of 2.64 (Vance et al. 1987) and an En conversion factor of 2.22 (Jenkinson 1988) as described by Liu et al. (2018). Briefly, four replicates of 4 g of each treatment were fumigated with ethanol-free chloroform for 24 hours at 25 °C in a sealed desiccator in the dark at the beginning, and end of the resistance and resilience phases. The non-fumigated subsamples of 4 g of each soil were stored at 4 °C during this period. The following day, after removing the beaker of chloroform, the desiccator was evacuated four times to remove the chloroform from the soils. Both fumigated and non-fumigated subsamples were extracted with freshly prepared 0.5 M K_2SO_4 at a 1:4 ratio and filtered. The filtrate was analysed using a Shimadzu TOC-VCSH/CSN total organic carbon and nitrogen analyser (Shimadzu Scientific Instruments, Japan) to calculate the concentrations of dissolved organic carbon and total nitrogen in fumigated and unfumigated samples.

3.2.6 Analysis of Soil Chemical Properties

The concentration of hot water extractable organic carbon (HWEOC) and nitrogen (HWETN) in the samples was determined following the method outlined by Chen et al. (2000). In summary, 4 g of fresh soil was combined with 40 mL of water in a capped falcon-tube and incubated at 70°C for 18 hours. The tubes underwent agitation on an end-over-end shaker for 15 minutes and were then filtered through a Whatman 42 filter paper (Whatman Ltd., Maidstone, UK). The filtrates were analysed for concentrations of extractable organic carbon and total nitrogen using a Shimadzu TOC-VCPH (Shimadzu, Kyoto, Japan) TOCN analyser. Soil mineral nitrogen was extracted with 2M KCl at a 1:4 ratio of soil to extractant using an end-over-end shaker, followed by filtration through a Whatman 42 filter paper (Rayment and Lyons 2011) after one hour of shaking. Concentrations of NH_4^+-N and NO_3^--N were measured using a SEAL AA3 Continuous Segmented Flow Analyzer (SEAL Analytical Limited. USA). All results were expressed on an oven-dry basis.

3.2.7 Calculating Resistance and Resilience

The resistance (RS) and resilience (RL) indexes are calculated using the following formular (Orwin and Wardle 2004).

$$\text{Resistance (RS)} = 1 - [(2 \times |D_{rs}|) / (C_{rs} + |D_{rs}|)]$$

$$\text{Resilience (RL)} = [(2 \times |D_{rs}|) / (|D_{rs}| + |D_{rl}|)] - 1$$

Where, D_{rs} is the absolute difference between soil response (i.e., respiration) value in the control treatment (C_{rs}) and the stressed treatment at the end of the RS phase. D_{rl} is the difference between soil response (i.e., respiration) value in the control treatment and the stressed treatment at the end of the RL phase. The values of RS and RL indices range between –1 and +1. The value of +1 indicates the maximum RS (no impact of stress) or RL (complete recovery from the stress), and lower values indicate less RS or RL of soil microbial biomass and respiration to drought stress. Negative values of the RS index indicate a change greater than 100% in the response variable compared with that in the control condition. If the absolute value of D_{rl} becomes higher than the absolute value of D_{rs}, then the RL index will have a negative value. Mathematically, the RL index of control condition will be infinitely close to +1, while it has been considered as +1 in the RL equation.

3.2.8 Data Analysis

Differences in soil chemical and biological properties between the drought stress treatments under conventional management practices and ecosystem-based management practice (treated with Bednar / cover crop management) were analysed using R (RCoreTeam 2023). Differences between patterns of microbial metabolic function profiles were tested with permutational analysis of variance (PERMANOVA) and visualised using multidimensional scaling (MDS) of bootstrap averages. Monte Carlo P–values were used to detect significant differences among management practices. MDS and PERMANOVA were run in PRIMER v7 with PERMANOVA+ (Amarasinghe et al. 2021; Strickland et al. 2017).

3.3 Results and Discussion

3.3.1 Facey Group

3.3.1.1 Soil Biological and Chemical Properties at the Initial Stage

Soil managed using the Bednar approach demonstrated notably higher levels of MBC and MBN compared to those managed conventionally (Figure 3.1A). This result can be attributed to the Bednar approach's focus on sustainable soil management practices, which create a conducive environment for microbial growth and activity, leading to increased carbon and nitrogen contents in the soil. Conversely, despite the higher MBC and MBN levels, the microbial metabolic quotient (qCO_2) was significantly elevated in soils managed conventionally (Figure 3.1B). This discrepancy may arise due to the differences in resource availability and microbial community dynamics between the two management approaches. For example, the Bednar approach fosters a more balanced microbial community with lower metabolic rates, resulting in a lower qCO_2 despite higher MBC and MBN contents compared to conventional management practice.

From the soil chemical properties, HWEOC concentration was significantly higher under Bednar approach compared to conventional management (Figure 3.1C). Conversely, although the Bednar approach exhibited higher values, the soil nitrogen pool components, including HWEON (Figure 2C), NH4+, NO3-, and total mineral nitrogen (Figure 3.1D), did not display a significant difference compared to conventional management.

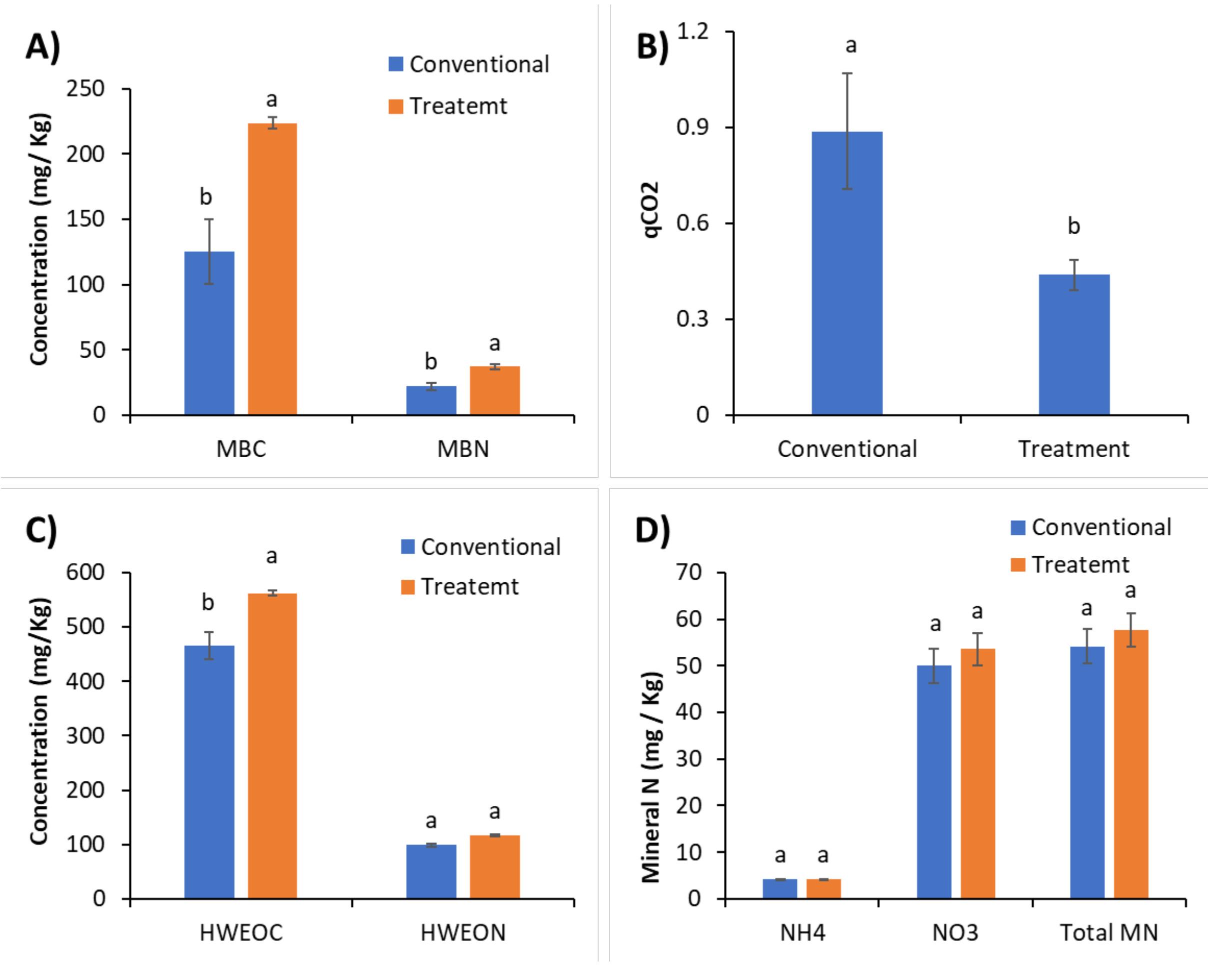


Figure 3.1. Soil biological properties; MBC and MBN (A), and microbial qCO_2 (B), and chemical properties; HWEOC and HWEON concentration (C) and soil mineral nitrogen pool (D) at the initial stage under two management practices. Treatment = Bednar approach. Vertical bars represent standard error. In each soil parameter, the means with the same letter did not differ at the 5% level of significance.

3.3.1.2 The Effect of Moisture Stress on Soil Biological Properties

3.3.1.2.1 Cumulative CO_2 Emission

No significant difference in cumulative CO_2 emission was observed between the control (C_T1) and the moderate stress (C_T2) treatment for the soils obtained from the conventionally managed site until the sixth day (Figure 3.2A). By the end of the first day, the cumulative CO_2 emission in the severe stress (C_T3) treatment was significantly lower than that in the control (C_T1). However, from the second to the sixth day, no significant difference was observed between C_T1 and C_T3. Subsequently, after the sixth day, cumulative CO_2 emission began to decline significantly in both stress treatments (C_T2 and C_T3) when compared to the control treatment (C_T1), with the difference becoming more pronounced at the end of the resistance phase (Day 14) (Figure 3.2A). During the resilience phase, both stress treatments showed significantly lower CO_2 emission compared to control treatment (Figure 3.2A).

Contrastingly, in the soil managed with the Bednar treatment, a different pattern emerged. Under severe stress condition (T_T3), there was a lower cumulative CO_2 emission compared to the control (T_T1). However, under moderate stress condition (T_T2), there was no significant difference in cumulative CO_2 emissions between the control and the stressed soil throughout the resistance phase (Day 1–14) (Figure 3.2B). At the end of the resilience phase (Day 28), soil treated with the Bednar approach exhibited no difference in cumulative CO_2 emissions under moderate (T_T2) and severe stress (T_T3) conditions when compared to the control (T_T1) (Figure 3.2B).

Conventionally managed soil showed a poor resistance and resilience to applied stress treatments compared to Bednar treatment applied soil (Figure 3.3). Further, in soil treated with the Bednar method, there was an observed higher resistance (0.72) index under moderate stress conditions (Figure 3.3). This indicates a robust response to stress and an enhanced capacity for recovery.

The results reveal dynamic patterns in cumulative CO_2 emissions under drought stress conditions for conventionally managed soils and those treated with the Bednar approach. In conventionally managed soils, initial stress-induced reductions in cumulative CO_2 emissions were followed by a convergence with control levels from the second to the sixth day, suggesting microbial adaptation. Subsequently, a significant decline in emissions in both stress treatments indicated a reduction in metabolic activity in response to the applied stress, leading to a slower carbon turnover rate.

Conversely, the Bednar treatment demonstrated a capacity to mitigate stress effects, with lower emissions under severe stress. Furthermore, under moderate stress, the Bednar-treated soil exhibited no significant differences throughout the resistance phase, implying a stabilising influence on microbial processes. The absence of distinctions between moderate and severe stress conditions in the Bednar treatment during the resilience phase suggests enhanced soil resilience and recovery facilitated by this management approach. These findings underscore the complex interaction between soil management practices, stress response, and microbial dynamics in influencing carbon emissions.

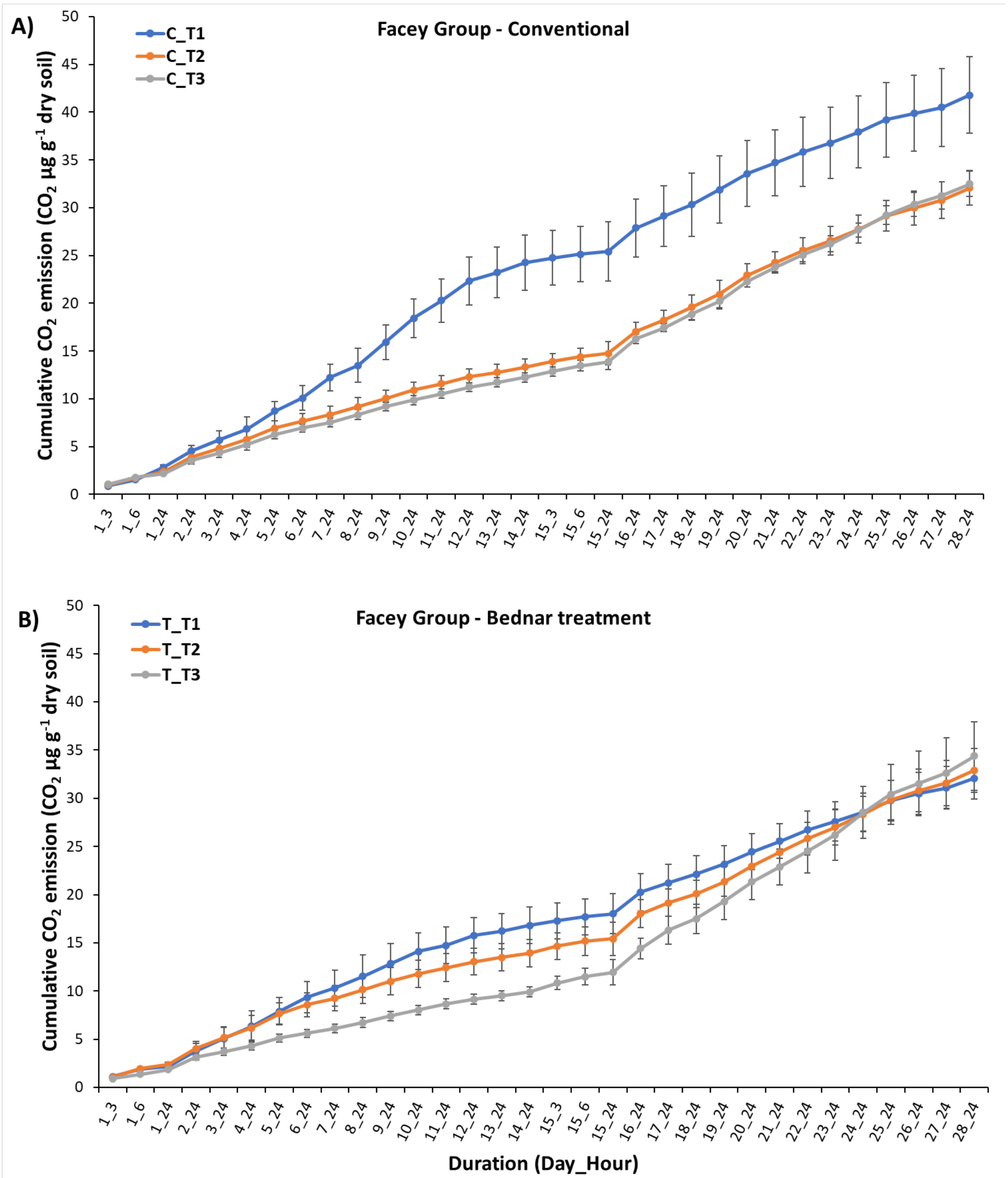


Figure 3.2. Effect of moisture stresses on cumulative CO_2 emissions (CO_2 µg g^{-1} dry soil) in conventionally managed soil (A) and Soil treated with Bednar treatment (B). C = conventionally managed soil, T = Bednar treatment applied soil, T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Vertical bars represent standard error.

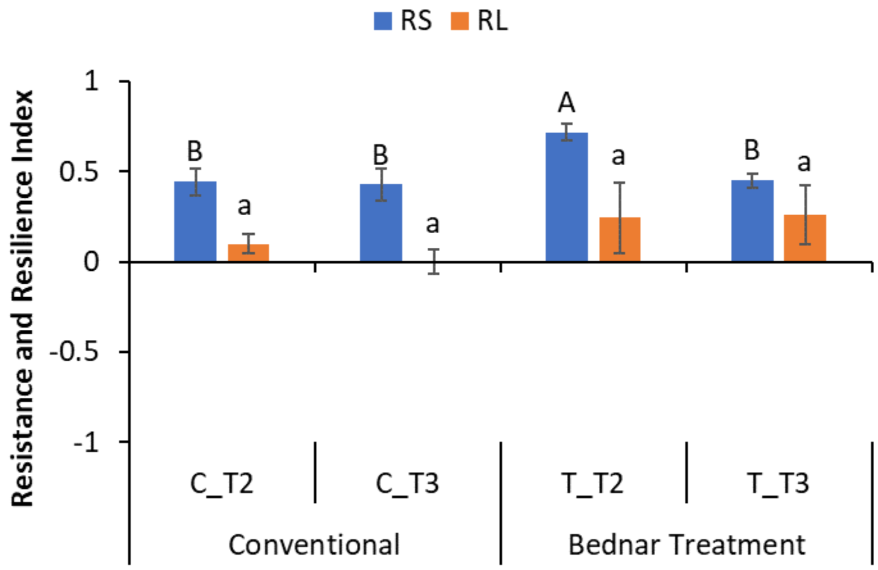


Figure 3.3. Resistance (RS) and Resilience (RL) indices of microbial respiration under moisture stress levels in conventionally managed soil and Bednar treatment applied soil of FGp. RS = Resistance index, RL = Resilience index, T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS), Conventional = Conventionally managed soil. Horizontal bars represent standard error. The means with the same capital letter is not different at the 5% level of significance for the resistance. Means with the same simple letter is not different at the 5% level of significance for the resilience.

3.3.1.2.2 Substrate-induced Respiration

Measurement of substrate-induced respiration using Glucose, Maltose, and Xylose revealed no significant difference among the water stress treatments within each management practice at the end of the resistance and resilience phases. However, within the no-stress treatments (C_T1 and T_T1), there was a significant difference observed between management practices, with higher metabolic rates evident under the Bednar approach at the end of the resistance phase (Figure 3.4). Notably, it was observed that the difference between water stress treatments and the control (no-stress) diminished by the end of the resilience phase compared to the end of the resistance phase in soil treated with the Bednar approach (Table 3.2). This suggests a recovery in microbial functional diversity following stress alleviation. Conversely, the difference increased under conventional soil management (Table 3.2).

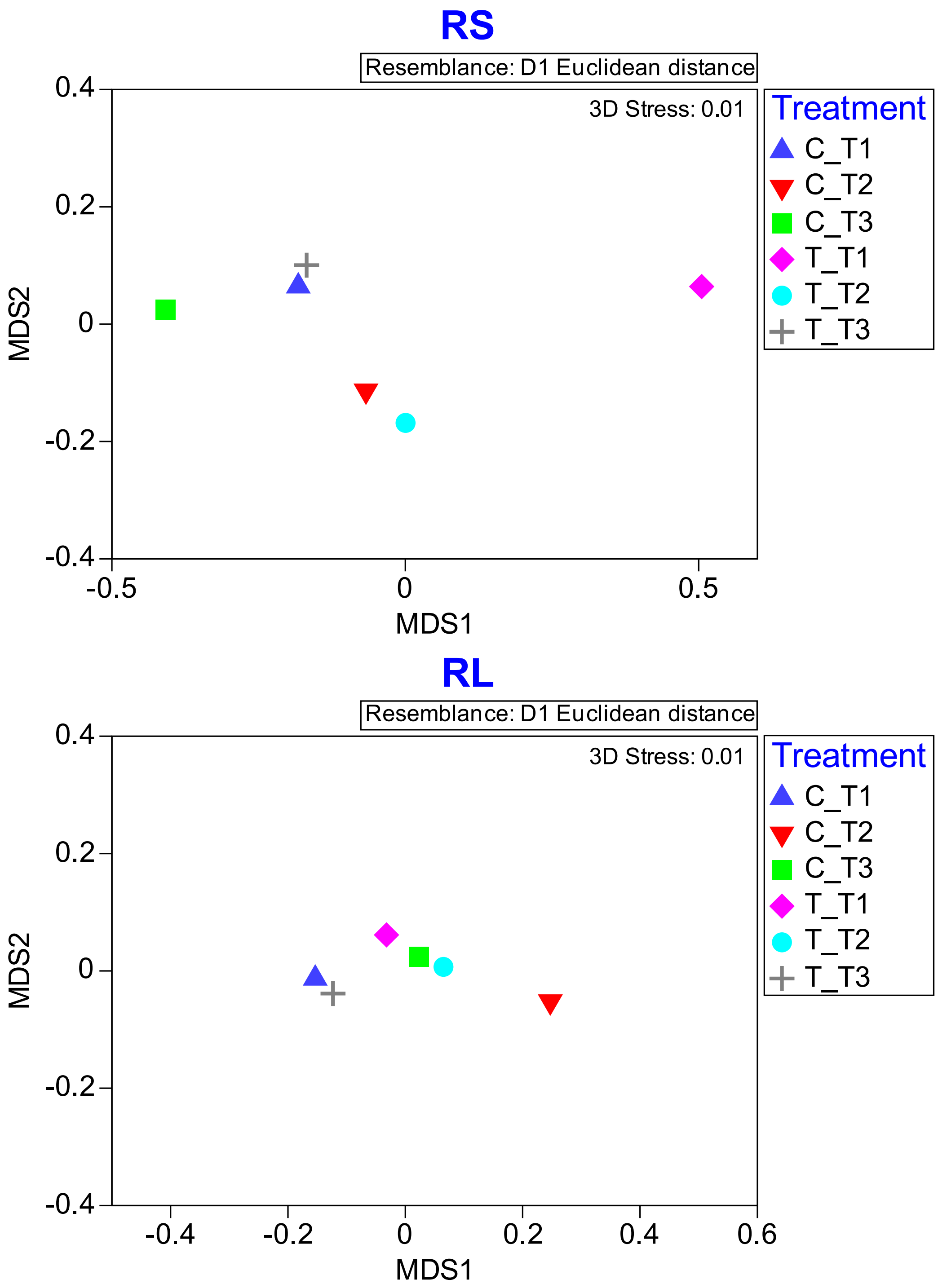


Figure 3.4. Ordinations of bootstrap averages of substrate induced respiration data under water stress treatments for conventional and Bednar treatment history. Two-dimensional metric multi-dimensional scaling ordinations (mMDS) of 50 bootstrap sample averages for each treatment and the empirical approximate 95% confidence region, based on Euclidean distance. The distance between the points corresponds to the functional diversity of the microbes in particular treatment: the shorter the distance, the higher similarity between the treatments. C = conventionally managed soil, T = Bednar treatment applied soil, T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS).

Table 3.2. Pairwise tests from PERMANOVA on Euclidean distance of substrate induced respiration data of microbial population in response to water stress treatments.

Groups	RS		RL	
	Distance	P(MC)	Distance	P(MC)
C_T1, C_T2	0.55	0.37	0.63	0.13
C_T1, C_T3	0.41	0.47	0.48	0.11
T_T1, T_T2	0.90	0.19	0.50	0.80
T_T1, T_T3	0.94	0.09	0.47	0.68

The shorter the distance, the higher similarity between treatments. C = conventionally managed soil, T = Bednar treatment applied soil, T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS).

There were no notable variances observed in the resistance and resilience indexes pertaining to the utilisation rates of individual substrates (such as Glucose, Maltose, and Xylose) between treatments conducted under two different management practices. Nonetheless, the overall impact of the substrate exhibited significance across the treatments. For instance, under moderate stress conditions, the Bednar treatment displayed a higher resistance index, whereas resilience indexes remained consistent across both moderate and severe stress levels (Figure 3.5). In contrast, conventional management showcased similar resistance indexes under both moderate and severe stress, with a higher resilience index observed under severe stress compared to moderate stress (Figure 3.5).

The observed results indicate that despite no notable differences in the resistance and resilience indexes of microbial communities under Bednar and conventional management practices regarding individual substrate utilisation rates, the overall impact of substrates remains significant across treatments. Specifically, under moderate stress conditions, the Bednar treatment exhibits higher resistance, suggesting enhanced functionality maintenance compared to conventional management, while resilience indexes remain consistent regardless of stress severity. Conversely, conventional management demonstrates uniform resistance under both stress levels but displays higher resilience under severe stress, indicating a stronger recovery ability. These findings suggest that while management practices may not significantly alter microbial responses to individual substrates, they do influence the overall stress response dynamics, with differential effects observed under different stress intensities for each management approach.

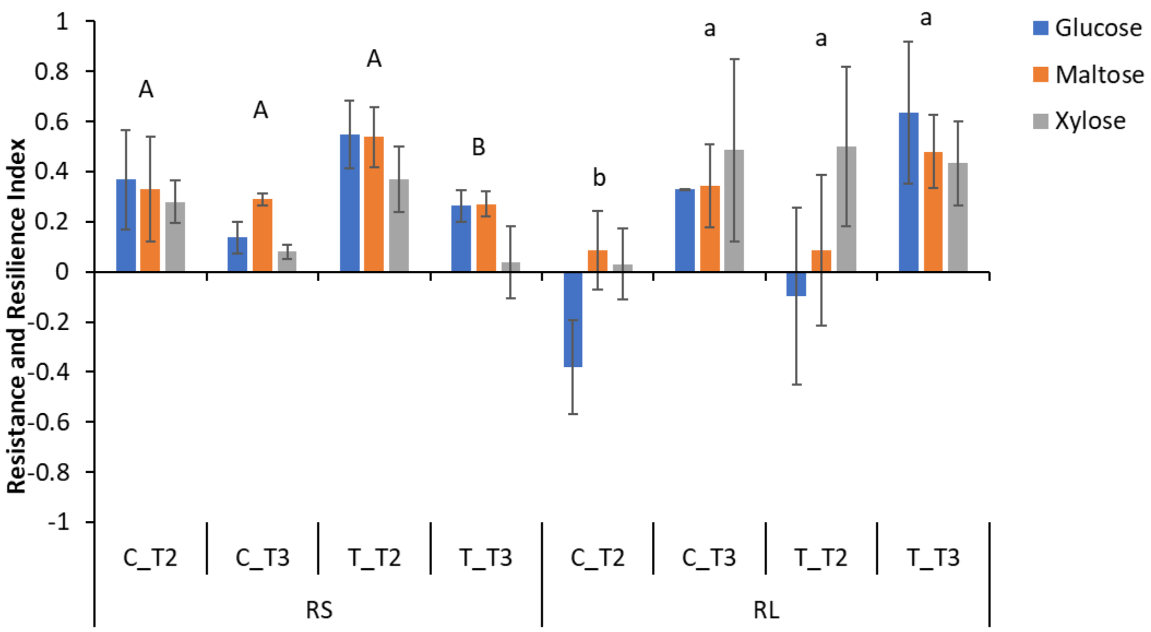


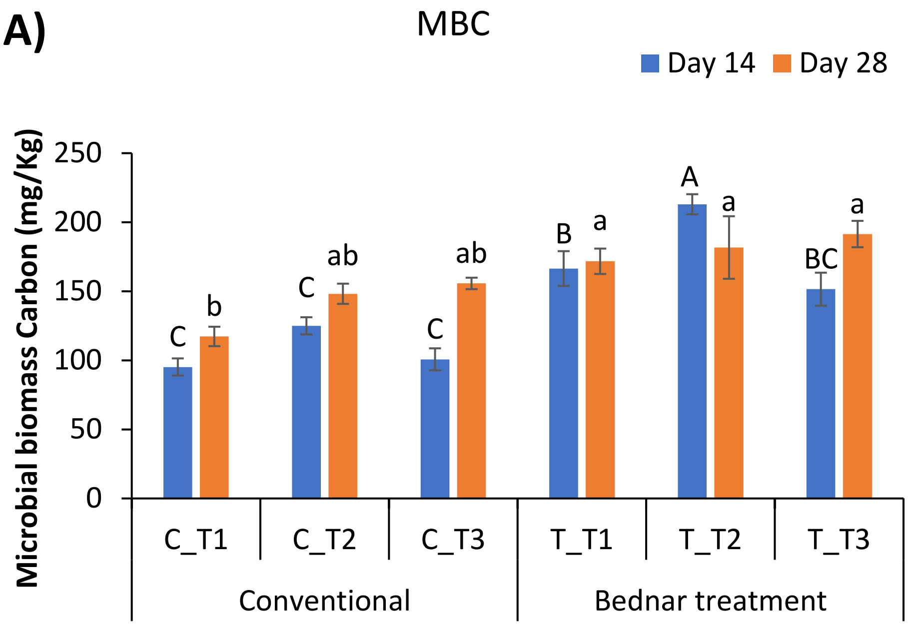
Figure 3.5. Resistance (RS) and Resilience (RL) indices of microbial substrate induced respiration under moisture stress levels in conventionally managed soil and Bednar treatment applied soil of FGp. RS = Resistance index, RL = Resilience index, T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS), Conventional = Conventionally managed soil. Error bars represent standard error. The overall mean of the treatments with the same capital letter is not different at the 5% level of significance for the resistance. The overall mean of the treatments with the same simple letter is not different at the 5% level of significance for the resilience.

3.3.1.2.3 Soil Microbial Biomass Carbon and Nitrogen, and Metabolic Quotient

The soil treated with the Bednar method exhibited significantly higher MBC concentrations under no-stress and moderate stress conditions compared to severe stress and all stress levels under conventional management by the end of the resistance phase (Figure 3.6A). In contrast, conventionally managed soil showed consistent MBC levels across all stress levels at the end of the resistance phase (Figure 3.6A). During the resilience phase, both management practices showed similar MBC levels across all stress levels (Figure 3.6A). The trend in MBN was similar to that of MBC under the three stress levels in soil treated with the Bednar method, with higher MBN levels observed under no-stress and moderate stress by the end of the resistance phase, and consistent MBN levels across all stress levels by the end of the resilience phase (Figure 3.6B). During the resistance phase, conventionally managed soil displayed a higher MBN level at the moderate stress level compared to severe stress, which was lower than the

MBN levels observed under soil treated with the Bednar method. However, by the end of the resilience phase, all stress levels exhibited similar MBN levels (Figure 3.6B).

Resistance and resilience index of the MBC and MBN was not significantly difference for both stress levels under two management systems (Figure 3.7A and 3.7B).



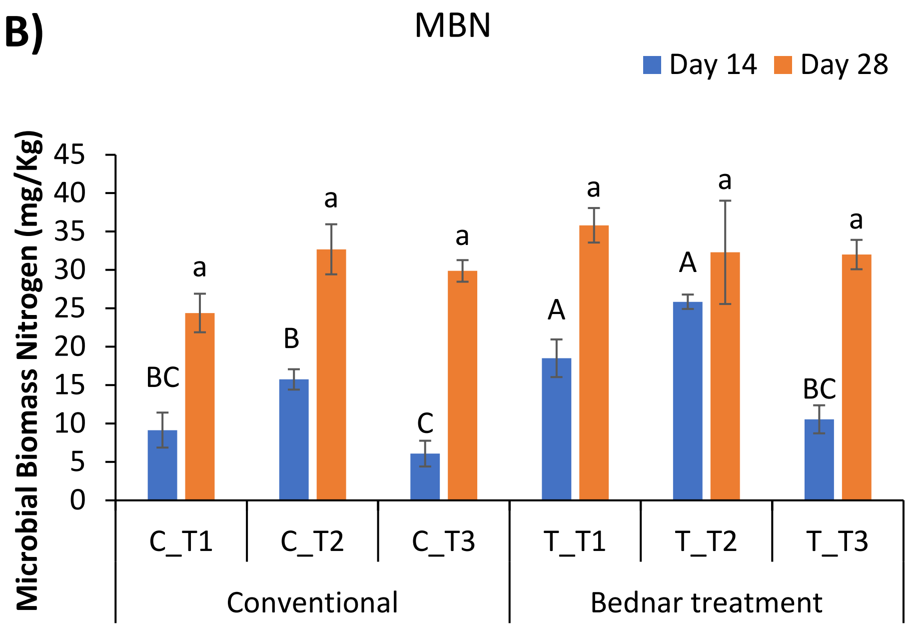


Figure 3.6. Effect of soil management on (A) MBC and (B) MBN concentrations during the incubation period for different water stress treatments in FGp. C = conventionally managed soil, T = Bednar treatment applied soil, T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

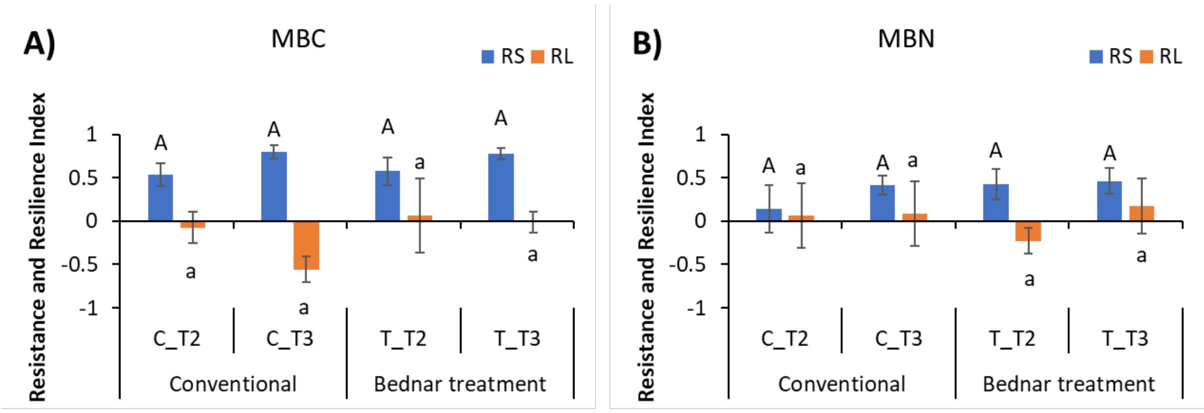


Figure 3.7. The resistance and resilience indices of MBC (A) and MBN (B) between treatments under different management practices in FGp. Error bars represent standard error. C = conventionally managed soil, T = Bednar treatment applied soil. T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). The means with the same capital letter is not different at the 5% level of significance for the resistance. Means with the same simple letter is not different at the 5% level of significance for the resilience.

The microbial qCO_2 exhibited a significant increase in the no-stress level for conventionally managed soil compared to both moderate and severe stress levels, as well as all stress levels in soil treated with the Bednar approach at the end of the resistant phase (Figure 3.8). Notably, the highest qCO_2 levels were observed under the no-stress treatment for conventionally managed soil by the end of the resilience phase. However, by the same phase, there were no significant differences in qCO_2 levels across all stress levels in soil treated with the Bednar approach, nor in moderate and severe stress levels under conventional management (Figure 3.8).

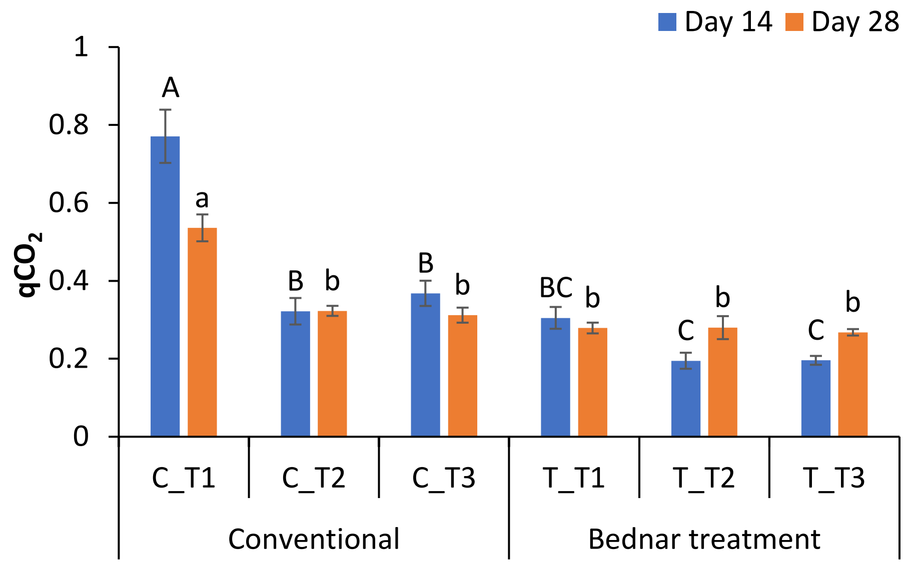


Figure 3.8. Effect of microbial qCO_2 during the incubation period for different water stress treatments in FGp. C = conventionally managed soil, T = Bednar treatment applied soil, T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

Resistance and resilience indexes for the microbial qCO_2 was not significantly different for both stress levels under two management systems. However, the Bednar approach showed comparatively higher RS and RL values for both moderate and severe stress levels compared to the conventional management system (Figure 3.9).

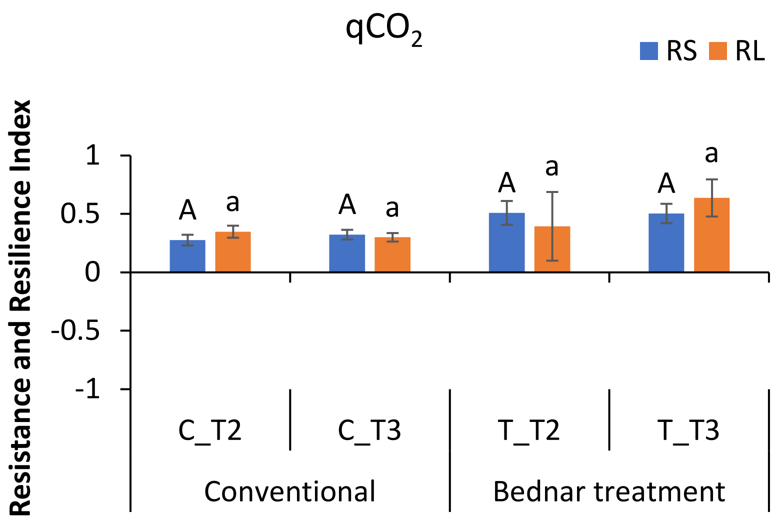


Figure 3.9. The resistance and resilience indices microbial qCO_2 between treatments under different management practices in FGp. Error bars represent standard error. C = conventionally managed soil, T = Bednar treatment applied soil. T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). The means with the same capital letter is not different at the 5% level of significance for the resistance. Means with the same simple letter is not different at the 5% level of significance for the resilience.

The results of the soil biological properties indicate that the Bednar method of soil management fosters higher MBC and MBN concentrations under no-stress and moderate stress conditions compared to conventional management, likely due to improved soil structure, organic matter content, and nutrient availability. However, under severe stress, the differences in MBC and MBN between the two management practices diminish, suggesting that severe stress negatively impacts microbial biomass in both systems. Additionally, the Bednar method maintains more consistent microbial qCO_2 levels across stress levels, indicating greater resilience and efficiency in carbon utilisation compared to conventionally managed soil, which exhibits a significant increase in qCO_2 under no-stress conditions. Moreover, the higher RS and RL values observed in soil treated with the Bednar approach under moderate and severe stress levels highlight its ability to withstand and recover from environmental stressors more effectively, likely due to enhanced soil quality, microbial diversity, and nutrient availability. These findings underscore the potential of the Bednar method for promoting sustainable agriculture and ecosystem resilience.

3.3.1.3 The Effect of Moisture Stress on Soil Chemical Properties

3.3.1.3.1 Hot Water Extractable Organic Carbon and Nitrogen

HWEOC (Figure 3.10A) and HWEON (Figure 3.10B) concentrations were not affected by the stress treatments under both management at the end of the resistance and resilience phases. Further, resistance and resilience indices also not significantly different for the stress treatments under two management practices (Figure 3.11A and 3.11B).

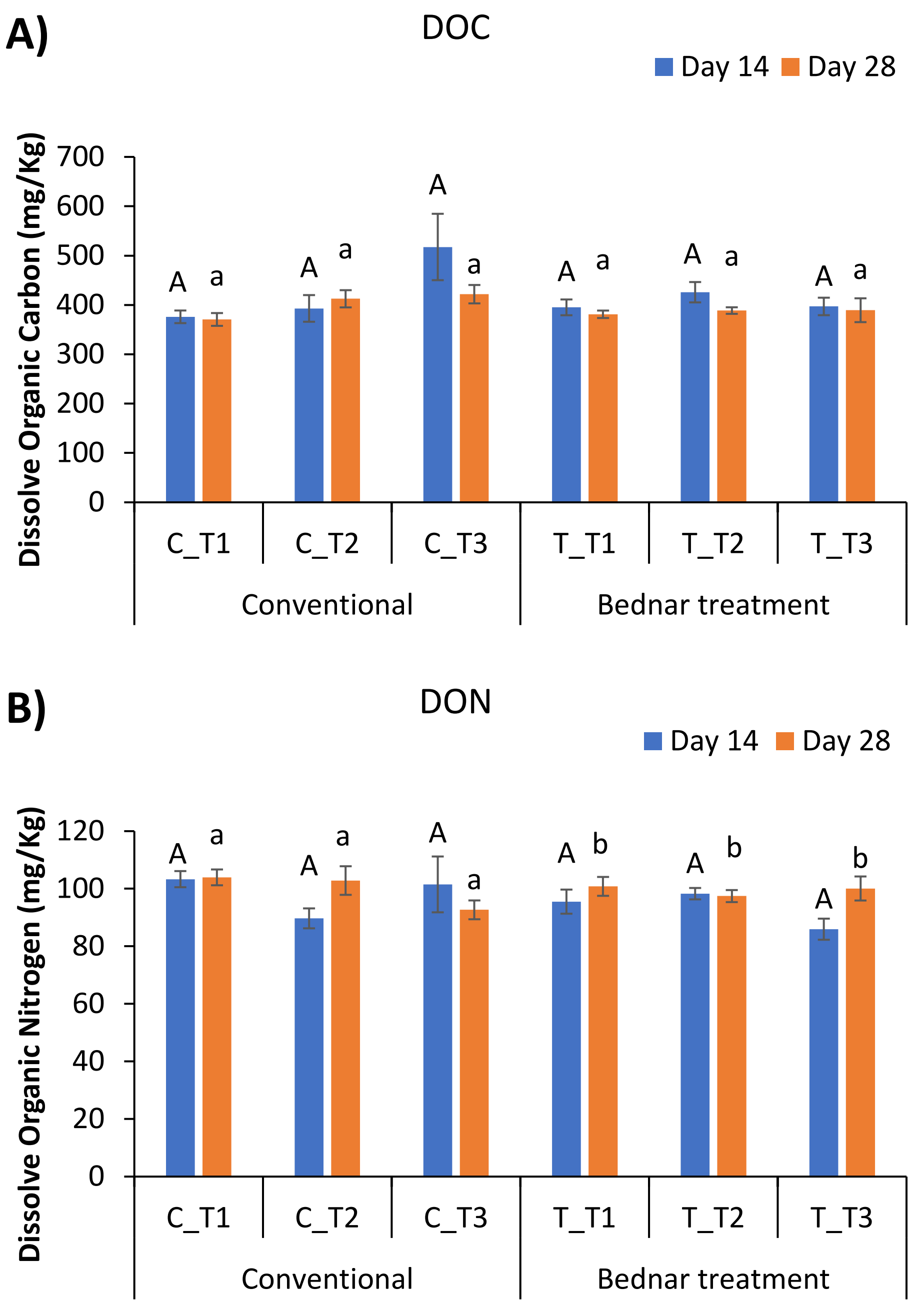


Figure 3.10. Effect of soil management on (A) HWEOC and (B) HWEON concentrations during the incubation period for different water stress treatments in FGp. C = conventionally managed soil, T = Bednar treatment applied soil, T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

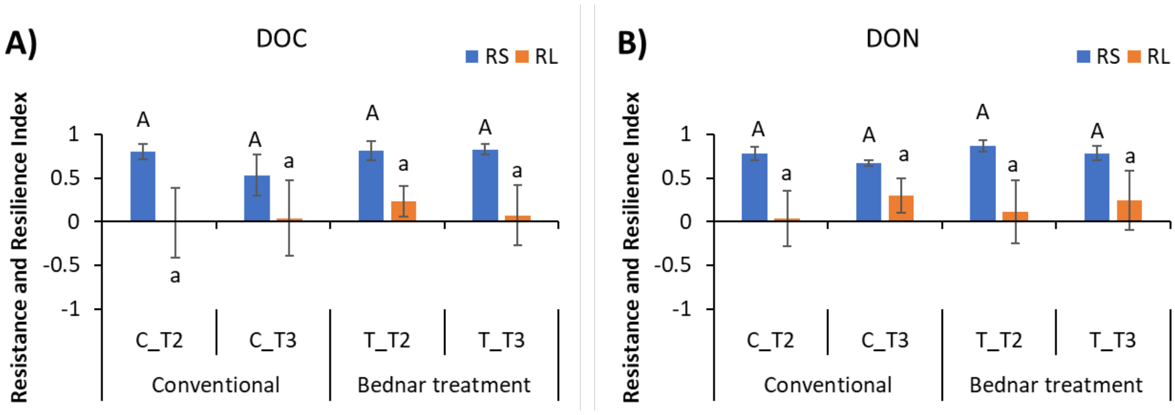


Figure 3.11. The resistance and resilience indices of (A) HWEOC and (B) HWEON between treatments under different management practices in FGp. Error bars represent standard error. C = conventionally managed soil, T = Bednar treatment applied soil. T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). The means with the same capital letter is not different at the 5% level of significance for the resistance. Means with the same simple letter is not different at the 5% level of significance for the resilience.

3.3.1.3.2 Soil Mineral Nitrogen

Moderate and severe stress notably decreased the mineral nitrogen concentration by the end of the resistance phase in conventionally managed soil, in contrast to the no-stress and moderate stress observed in soil treated with the Bednar approach (Figure 3.12). By the end of the resilience phase, the mineral nitrogen concentration was similar between the no-stress and moderate stress conditions under both management systems. However, the severe stress treatment exhibited a markedly lower mineral nitrogen concentration compared to the no-stress treatment under both management systems (Figure 3.12). The resistance and resilience indices were not significantly different for the stress treatments under both management systems. However, higher values were observed under Bednar approach compared to conventional management under moderate stress treatment (Figure 3.13).

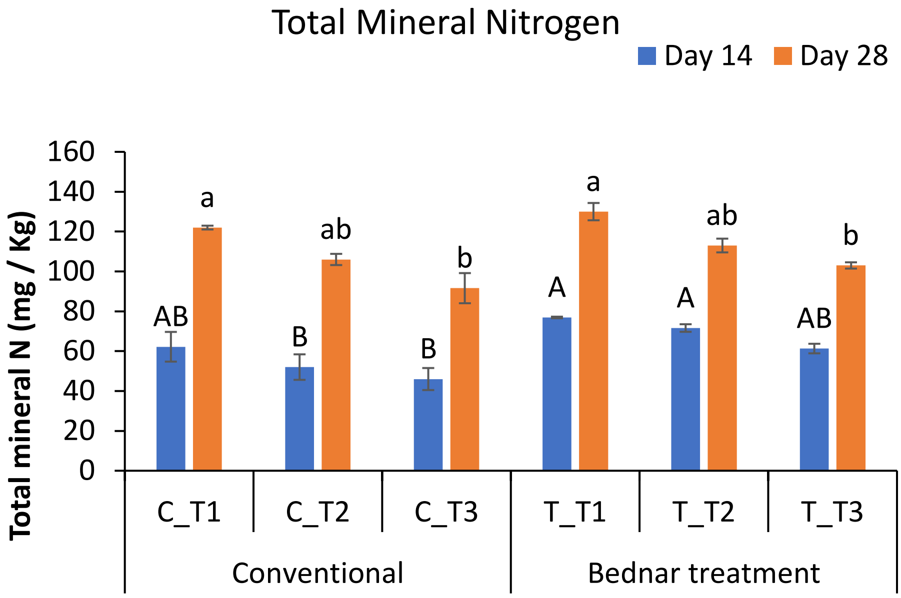


Figure 3.12. Effect of soil management on total mineral nitrogen concentrations during the incubation period for different water stress treatments in FGp. C = conventionally managed soil, T = Bednar treatment applied soil, T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

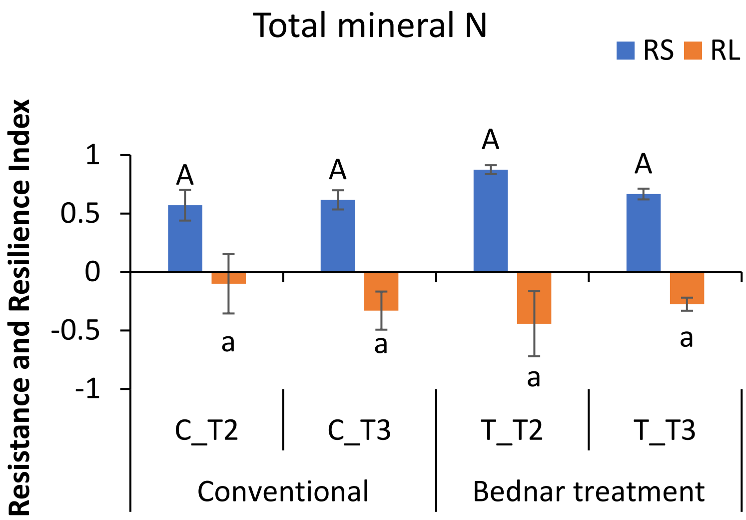


Figure 3.13. The resistance and resilience indices of total mineral nitrogen (NH_4^+ and NO_3^-) between treatments under different management practices in FGp. Error bars represent standard error. C = conventionally managed soil, T = Bednar treatment applied soil. T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). The means with the same capital letter is not different at the 5% level of significance for the resistance. Means with the same simple letter is not different at the 5% level of significance for the resilience.

The results of the soil chemical properties demonstrate that while HWEOC and HWEON concentrations remained largely unaffected by stress treatments, mineral nitrogen concentrations were significantly influenced, particularly during the resistance phase. In conventionally managed soil, both moderate and severe stress treatments led to notable decreases in mineral nitrogen levels, whereas soil treated with the Bednar approach showed resilience under moderate stress conditions, maintaining nitrogen availability. During the resilience phase, mineral nitrogen levels recovered to some extent, but severe stress continued to have a lasting impact, resulting in lower nitrogen availability compared to no-stress conditions. These findings underscore the importance of management practices in modulating soil nutrient dynamics under stress and highlight potential benefits of approaches like the Bednar method in mitigating nutrient loss during challenging conditions.

3.3.2 Central West Farming Systems

3.3.2.1 Soil Biological and Chemical Properties at the Initial Stage

Soil biological properties, such as MBC and MBN, did not exhibit significant differences between conventional management and cover crop management (Figure 3.14A). However, microbial qCO_2 was significantly lower under cover crop management compared to conventional management (Figure 3.14B). This could be attributed to increased organic matter input, fostering diverse microbial communities, improving soil structure, enhancing nutrient cycling, and reducing soil disturbance. This potentially leading to more efficient metabolic processes and lower qCO_2 values under cover crop management.

Although the HWEOC did not display a significant difference between the two management practices, it is noteworthy that cover crop management exhibited a higher value (Figure 3.14C). This observation could potentially be due to the organic matter input from cover crops, which contributes to the HWEOC pool but may not have demonstrated as a statistically significant difference in this study. Conversely, the HWEON (Figure 3.14C), soil nitrate (NO3-) content (Figure 3.14D), and total mineral nitrogen content (Figure 3.14D) were notably higher under conventional management compared to cover crop management. This could be due to the absence of cover crops in conventional systems may result in fewer competing nitrogen sinks, thereby enhancing nitrogen availability in the soil. Overall, these results highlight the complex interplay between management practices and soil nitrogen dynamics, underscoring the potential benefits of cover crop management in mitigating nitrogen losses while maintaining or even enhancing soil organic carbon levels.

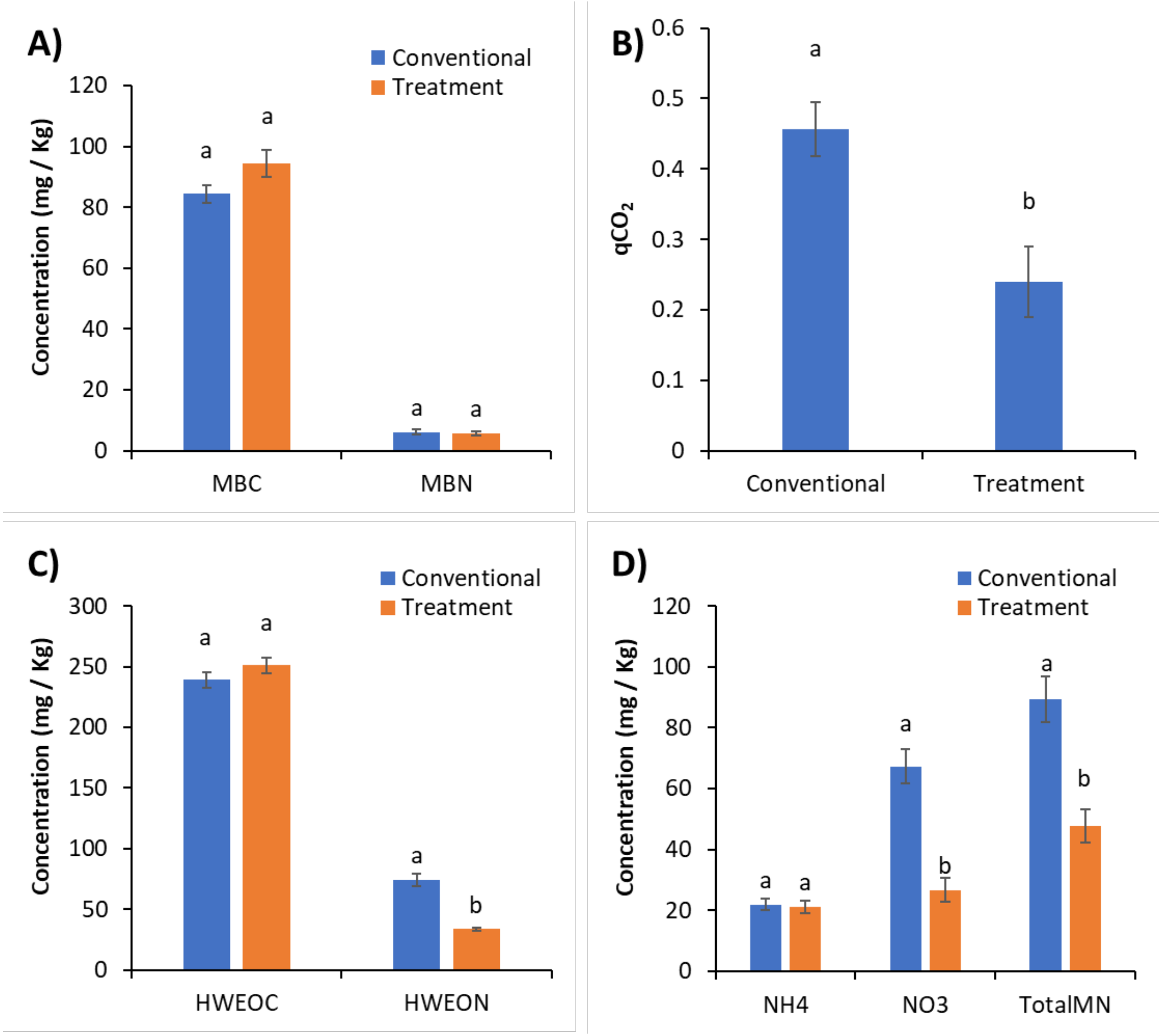


Figure 3.14. Soil biological properties; MBC and MBN (A), and microbial qCO_2 (B), and chemical properties; HWEOC and HWEON concentration (C) and soil mineral nitrogen pool (D) at the initial stage under two management practices. Treatment = Cover crop management. Vertical bars represent standard error. In each soil parameter, the means with the same letter did not differ at the 5% level of significance.

3.3.2.2 The Effect of Moisture Stress on Coil Biological Properties

3.3.2.2.1 Cumulative CO_2 Emission

During the resistance phase, cumulative CO_2 emissions were consistently lower under both stress treatments compared to the control for both conventional and cover crop management practices (Figure 3.15A and 3.15B). However, by the end of the resilience phases, there were no significant differences in cumulative CO_2 emissions between stress conditions and the control for either soil management approach (Figure 3.15A and 3.15B). This suggests that microbial respiration suppression in response to moisture stress. However, convergence of emissions during the resilience phase indicates microbial recovery once stress is alleviated.

Soil managed with cover crops exhibited increased resistance and resilience to moderate drought stress in contrast to severe stress and both stress levels under conventional soil management. This increase was statistically significant for the resistance index. Nonetheless, there was no significant difference observed for the resilience index across both stress levels under either conventional soil management or soil managed with cover crops (Figure 3.16). Soil managed with cover crops exhibits increased resistance to moderate drought stress compared to severe stress and conventional soil management, potentially due to enhanced soil health and microbial activity. Conversely, no significant difference in resilience indices between stress levels or management practices implies similar recovery capabilities once stress is removed. These findings underscore the importance of cover cropping in mitigating moisture stress impacts on microbial respiration and highlight the need for sustainable soil management practices to ensure ecosystem resilience in the face of climate change.

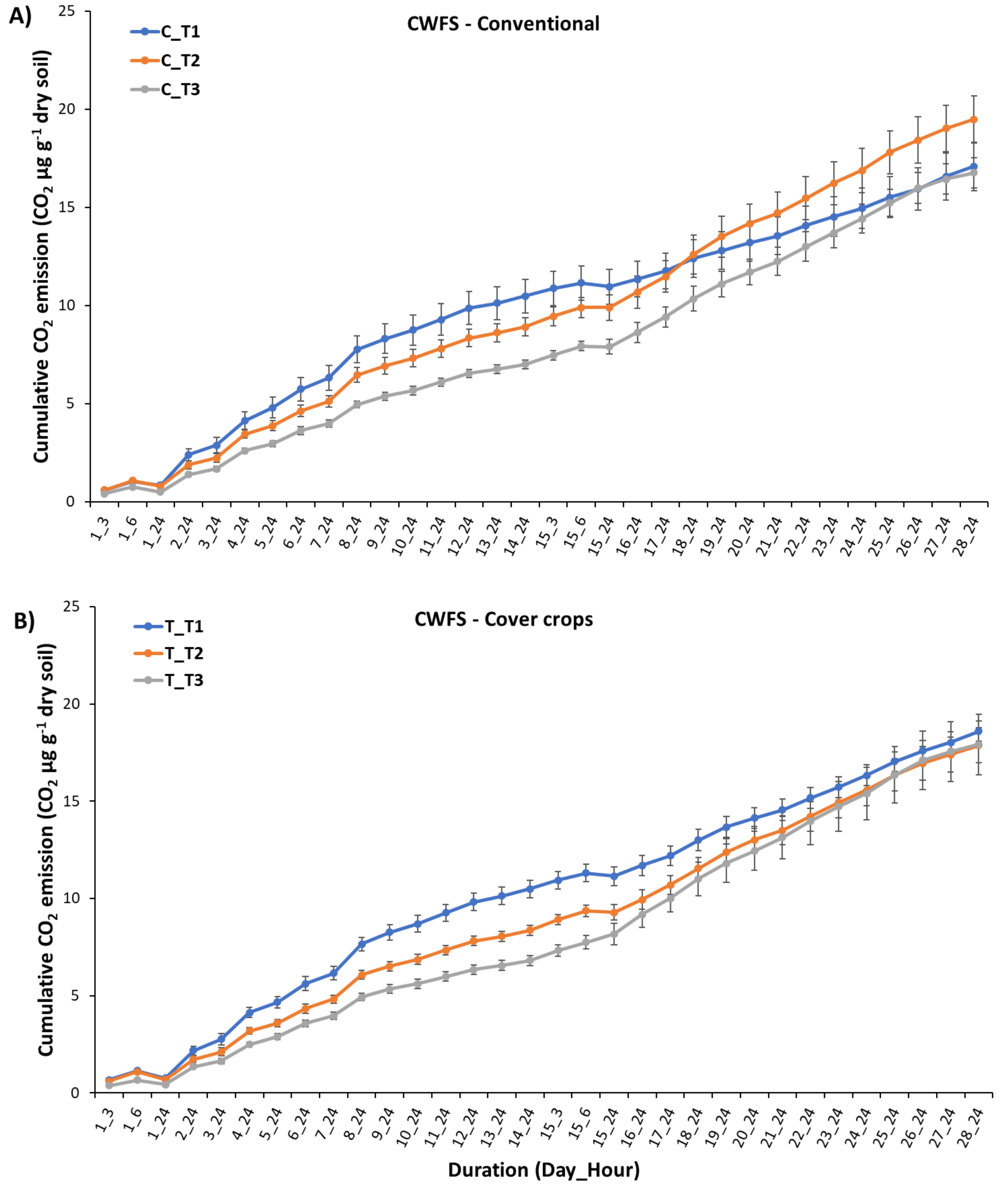


Figure 3.15. Effect of moisture stresses on cumulative CO_2 emissions (CO_2 µg g^{-1} dry soil) in conventionally managed soil (A) and Soil management with cover crops (B) in CWFS . C = conventionally managed soil, T = Soil management with cover crops, T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error.

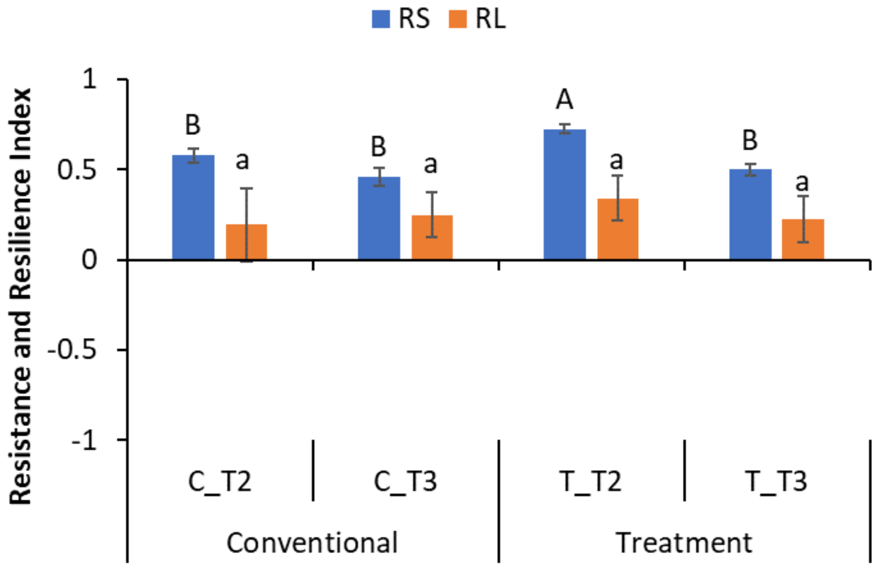


Figure 3.16. Resistance (RS) and Resilience (RL) indices of microbial respiration under moisture stress levels in conventionally managed soil and soil managed with cover crops in CWFS . RS = Resistance index, RL = Resilience index, T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS), Conventional = Conventionally managed soil. Treatment = soil management with cover crops. Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

3.3.2.2.2 Substrate-induced Respiration

Measurement of substrate-induced respiration using Galactose, Glucose, and Xylose indicated no significant difference among the water stress treatments within each management practice at the end of the resistance phase (Figure 3.17, Table 3.3). However, within conventional management practices, there was a notable variation in microbial functional diversity, with lower metabolic rates observed for the severe stress treatment (C_T3) compared to the no-stress treatment (C_T1) at the end of the resilience phase (Figure 17, Table 3). Conversely, soil under cover crop management exhibited no difference in functional diversity for both stress treatments (T_T2 and T_T3) compared to the no-stress treatment (T_T1) (Figure 3.17, Table 3.3).

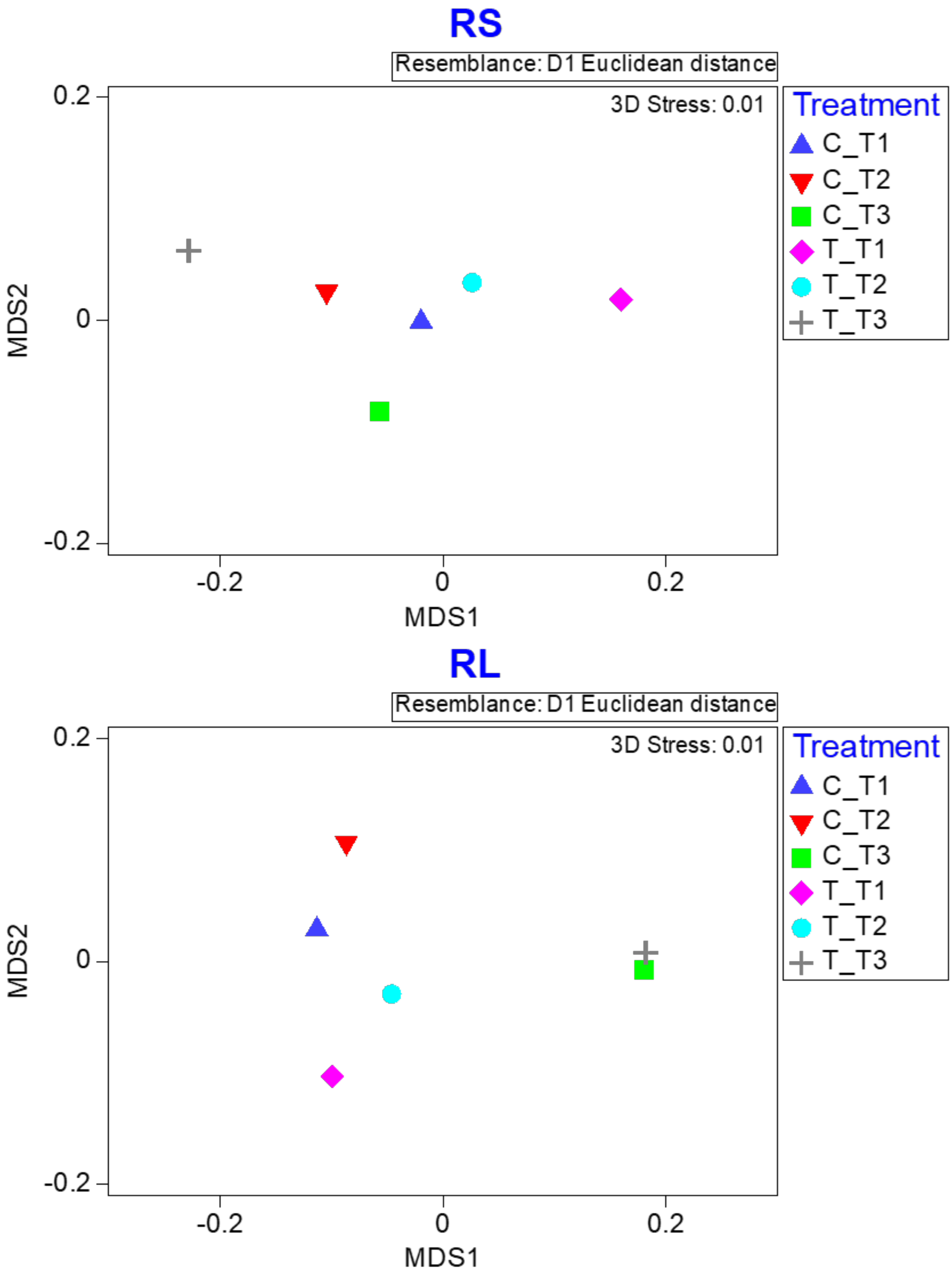


Figure 3.17. Ordinations of bootstrap averages of substrate induced respiration data under water stress treatments for conventional and cover crop management history. Two-dimensional metric multi-dimensional scaling ordinations (mMDS) of 50 bootstrap sample averages for each treatment and the empirical approximate 95% confidence region, based on Euclidean distance. The distance between the points corresponds to the functional diversity of the microbes in particular treatment: the shorter the distance, the higher similarity between the treatments. C = conventionally managed soil, T = cover crop management, T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS).

Table 3.3. Pairwise tests from PERMANOVA on Euclidean distance of substrate induced respiration data of microbial population in response to water stress treatments.

Group	RS		RL	
	Distance	P(MC)	Distance	P(MC)
C_T1, C_T2	0.25	0.53	0.21	0.29
C_T1, C_T3	0.35	0.74	0.33	0.01
T_T1, T_T2	0.30	0.35	0.38	0.65
T_T1, T_T3	0.41	0.10	0.42	0.08

The shorter the distance, the higher similarity between treatments. C = conventionally managed soil, T = Cover crop management, T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS).

No significant differences were observed in the resistance and resilience indexes regarding the utilisation rates of individual substrates (such as Galactose, Glucose, and Xylose) under moderate stress conditions in conventional management, and for both stress levels under cover crop management (figure 3.18). However, the resistance index for Xylose utilisation was found to be lower under severe stress treatment in conventional management, whereas the resilience index was higher for Xylose. Overall, the resistance index of substrate utilisation for moderate stress treatment (T_T2) under cover crop management was higher compared to severe stress treatment (C_T3) under conventional management (Figure 3.18). However, there was no significant difference found between treatments for the resilience indexes.

The substrate-induced respiration results show that under moderate water stress, microbial communities maintain similar metabolic rates regardless of management method. However, in conventional management, severe stress leads to lower microbial diversity and metabolic rates. Conversely, cover crop systems show consistent microbial diversity and metabolic rates under stress. The lower resistance index for Xylose in conventional management under severe stress suggests reduced microbial ability to use this substrate. Yet, these microbes show higher resilience, meaning they recover better after stress. Overall, cover crop systems seem to better support microbial activity under stress compared to conventional methods, highlighting the importance of management practices in shaping microbial responses to stress.

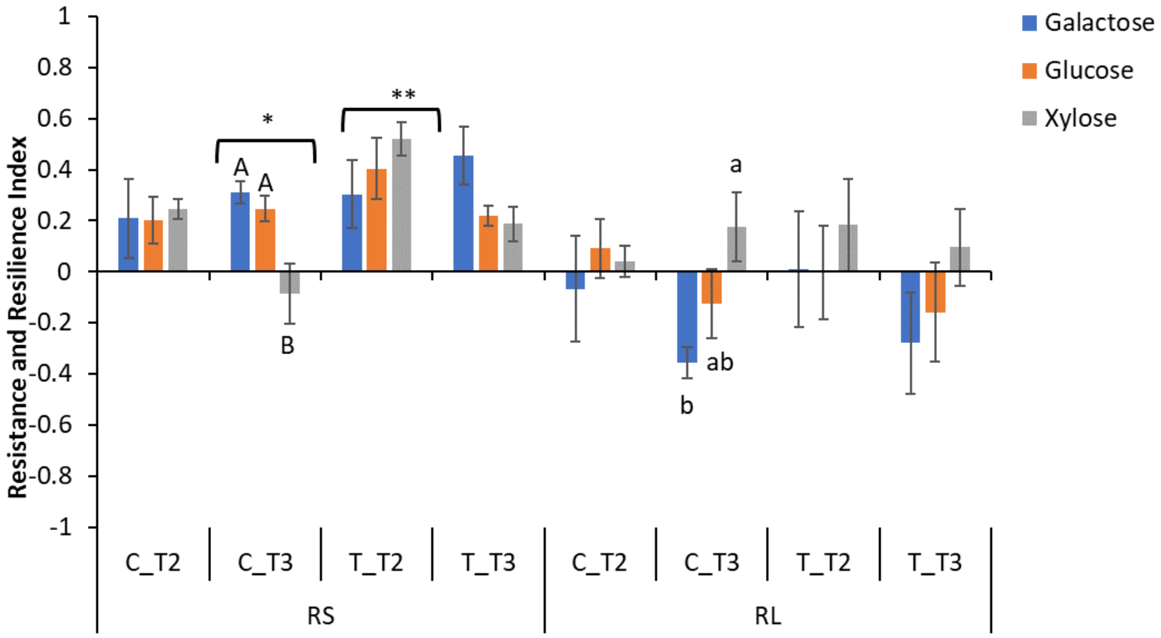


Figure 3.18. Resistance (RS) and Resilience (RL) indices of microbial substrate induced respiration under moisture stress levels in conventionally managed soil and soil managed with cover crops in CWFS. RS = Resistance index, RL = Resilience index, T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS), Conventional = Conventionally managed soil. Treatment = soil management with cover crops. Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience. The overall mean differences of the indices between the treatments are indicated as "*" and "**"

3.3.2.2.3 Soil Microbial Biomass Carbon and Nitrogen, and Metabolic Quotient

At the end of the resistance phase in both management systems, lower MBC content was observed under stress treatments compared to the respective control (C_T1 and T_T1). Specifically, MBC content for the no-stress treatment under cover crop management (T_T1) was significantly higher overall compared to the moderate stress treatment (T_T2) and both stress treatments under conventional management (C_T2 and C_T3) (Figure 3.19A). However, there were no notable differences observed for MBC at the end of the resilience phase (Figure 3.19A). In terms of MBN content, it was significantly higher in no-stress treatment compared to the stress treatments at the end of the resistance phase for both management practices. At the end of the resilience phase, no notable differences were observed between the treatments under each management practice. However, cover crop management exhibited increased MBN content compared to conventional management for all the treatments (Figure 3.19B). Resistance and resilience index of the MBC and MBN was not significantly difference for both stress levels under two management systems (Figure 3.20A and 3.20B).

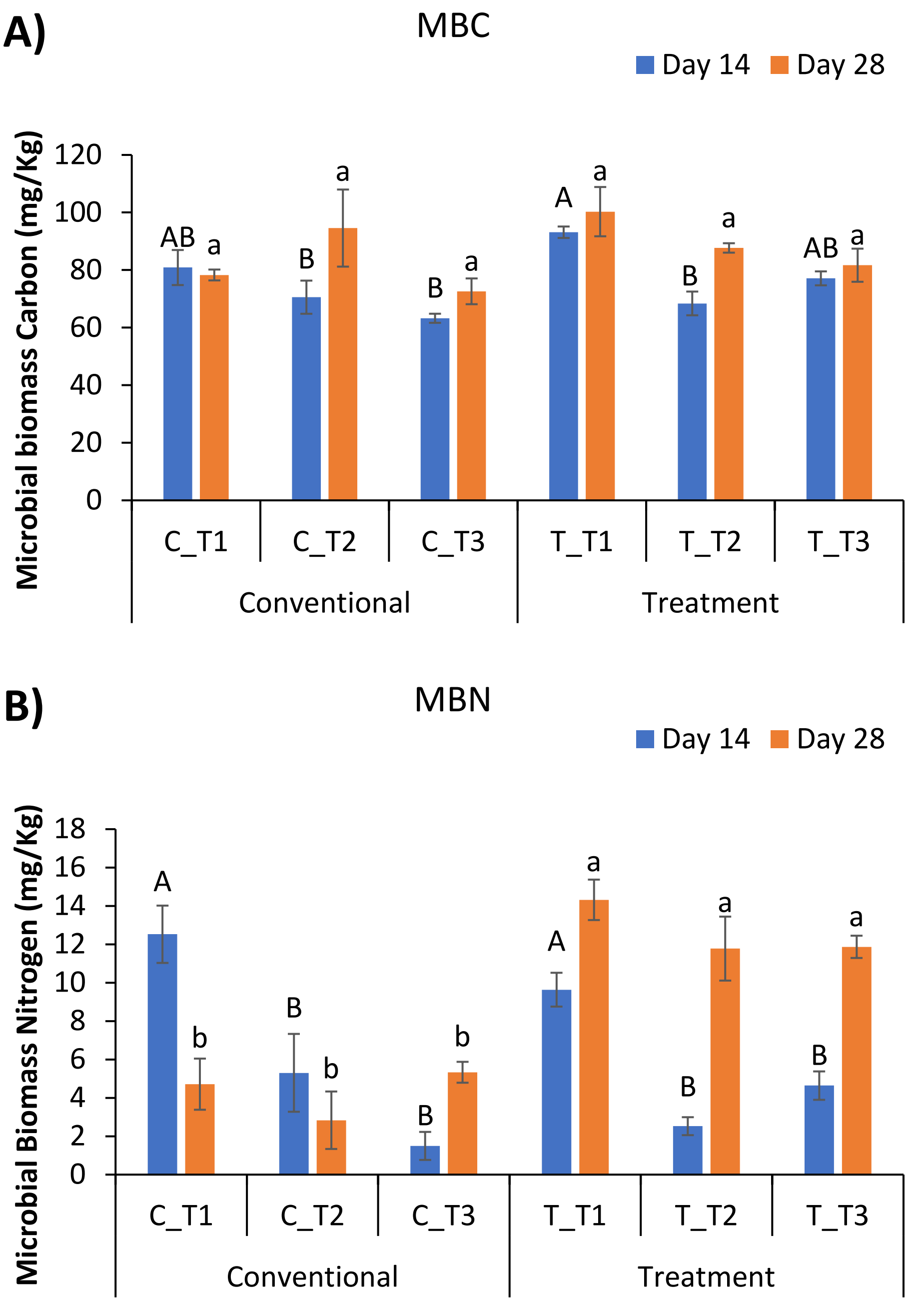


Figure 3.19. Effect of soil management on MBC (A) and MBN (B) concentrations during the incubation period for different water stress treatments in CWFS. C = conventionally managed soil, T = Soil management with cover crops, T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of

the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

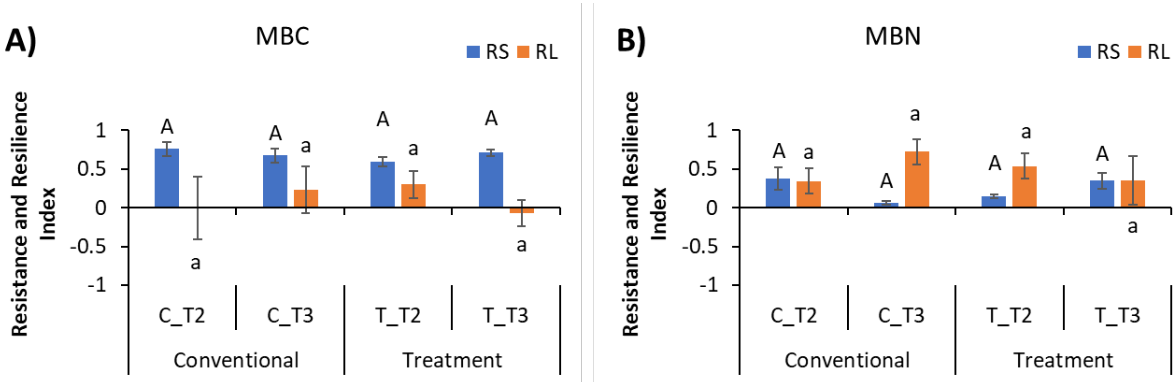


Figure 3.20. The resistance and resilience indices of MBC (A) and MBN (B) between treatments under different management practices in CWFS. C = conventionally managed soil, T = Soil management with cover crops. T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

At the end of both the resistance and resilience phases, there were no significant differences observed in microbial qCO_2 between treatments for both management practices (Figure 3.21A). Similarly, the resistance and resilience indices for both moderate and severe stress showed no significant differences under either management practice (Figure 3.21B). However, overall, lower qCO_2 values and higher resistance and resilience index values were observed under cover crop management.

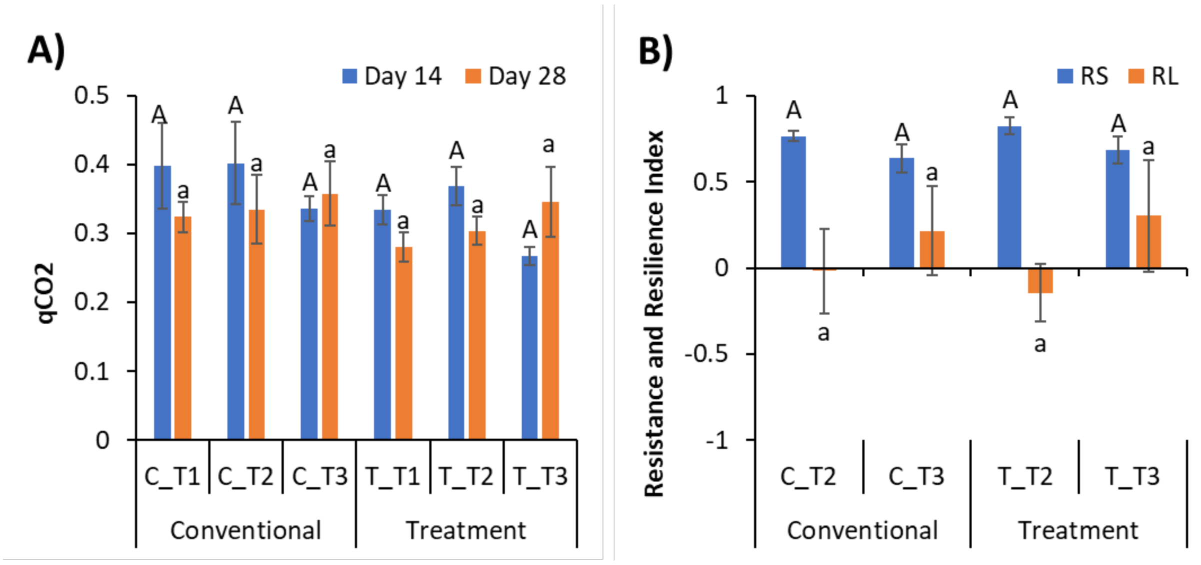


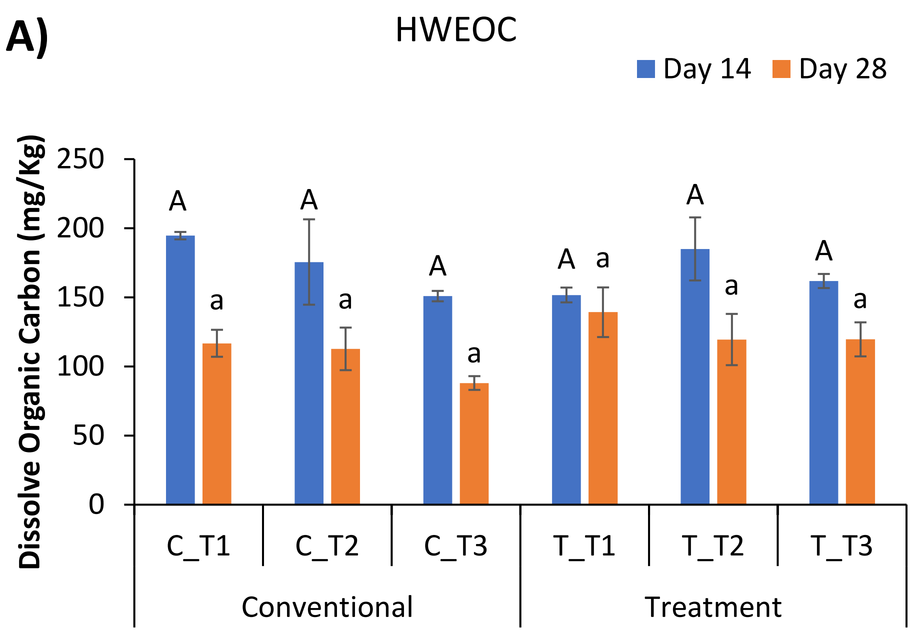
Figure 3.21. Effect of soil management microbial qCO_2 (A) and resistance (RS) and resilience (RL) index (B) during the incubation period for different water stress treatments in CWFS. C = conventionally managed soil, T = Soil management with cover crops, T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

The observed differences in MBC and MBN content between stress treatments and control groups under both cover crop and conventional management systems can be attributed to the distinct impacts of stress on soil microbial communities and the protective role of cover crops. During the resistance phase, where stressors were actively applied, the lower MBC and MBN content in stressed treatments compared to controls indicates a reduction in microbial biomass likely due to stress-induced limitations on microbial growth and activity. Cover crop management showed higher MBC and MBN content in the no-stress treatment compared to stress treatments and conventional management, indicating its potential in maintaining microbial biomass under favourable conditions. However, during the resilience phase, when stressors were removed, microbial communities likely underwent recovery, leading to comparable MBC and MBN levels across treatments. Further, the increased MBN content under cover crop management across all treatments at the end of the resilience phase further suggests the beneficial effects of this management practice on nitrogen availability for microbial communities. Additionally, the lack of significant differences in resistance and resilience indices for MBC and MBN suggests a consistent response of microbial communities to stress across management practices, indicating resilience in microbial functioning. The absence of significant differences in microbial qCO_2 and resistance/resilience indices for moderate and severe stress under both management practices suggests a similar metabolic response regardless of stress severity or management approach. However, the overall trend of lower qCO_2 values and higher resistance and resilience index values under cover crop management highlights the potential benefits of this system in promoting microbial stability and activity, likely attributed to enhanced soil quality and resource availability provided by cover crops.

3.3.2.3 The Effect of Moisture Stress on Soil Chemical Properties

3.3.2.3.1 Hot Water Extractable Organic Carbon and Nitrogen

The concentrations of HWEOC remained unaffected by the stress treatments across both management approaches at the conclusion of both the resistance and resilience phases (Figure 3.22A). In contrast, the content of HWEON was notably higher for all treatments under conventional management compared to those under cover crop management (Figure 3.22B). Furthermore, the resistance and resilience indices did not exhibit significant differences among the stress treatments under the two management practices (Figure 3.23A and 3.23B).



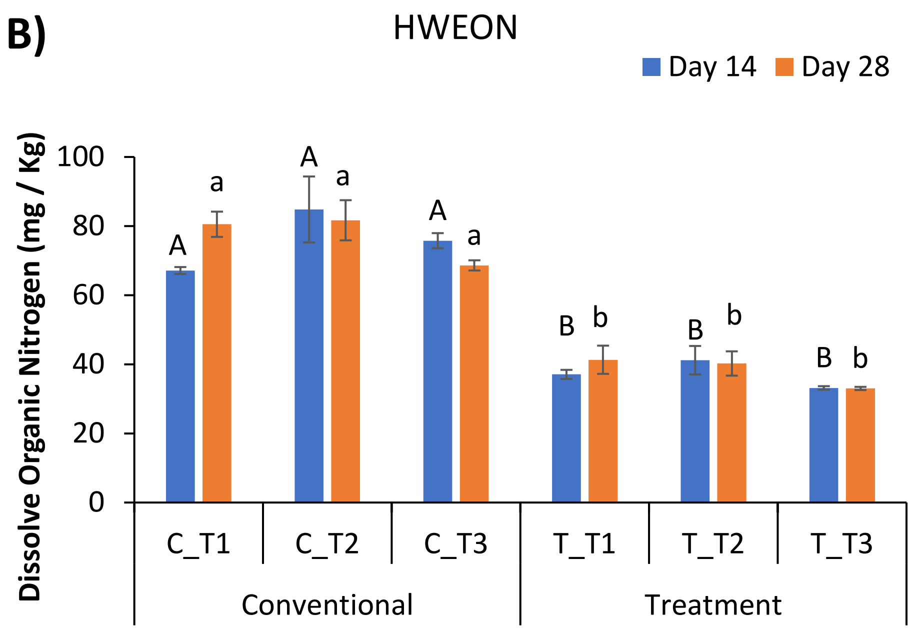


Figure 3.22. Effect of soil management on HWEOC (A) and HWEON (B) concentrations during the incubation period for different water stress treatments in CWFS. C = conventionally managed soil, T = Soil management with cover crops, T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

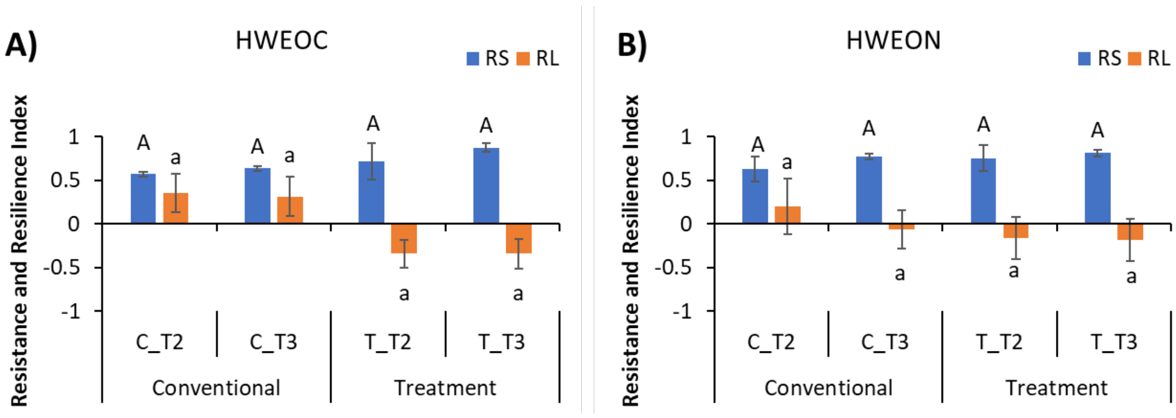


Figure 3.23. The resistance and resilience indices of HWEOC (A) and HWEON (B) between treatments under different management practices in CWFS. C = conventionally managed soil, T = Soil management with cover crops. T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

The observed patterns in the concentrations of HWEOC, HWEON, the resistance and resilience indices can be attributed to the differential effects of cover crop management and conventional management on soil nutrient dynamics and microbial activity. The consistent HWEOC concentrations across stress treatments and management approaches suggest minimal alterations in soil organic carbon availability, possibly due to stable organic matter inputs or microbial processes that mitigate stress impacts. Conversely, the lower HWEON content under cover crop management indicates potential retention or immobilisation of nitrogen within the soil-plant system, reflecting enhanced nitrogen cycling or reduced leaching losses compared to conventional management.

3.3.2.3.2 Soil Mineral Nitrogen

There were no notable differences in mineral nitrogen content (NH4+ and NO3-) between treatments under conventional and cover crop management approaches at the end of the resistance and resilience phases. However, cover crop management exhibits lower mineral nitrogen content in all the treatments compared to conventional management approach (Figure 3.24 A). No significance difference was noted in the resistance and resilience indices between moderate and severe stress treatments across both management approaches. Nevertheless, under cover crop management, there were higher resistance index values for both stress levels (T_T2 and T_T3) and a greater resilience value for severe stress treatment (T_T3) compared to conventional management (Figure 3.24B).

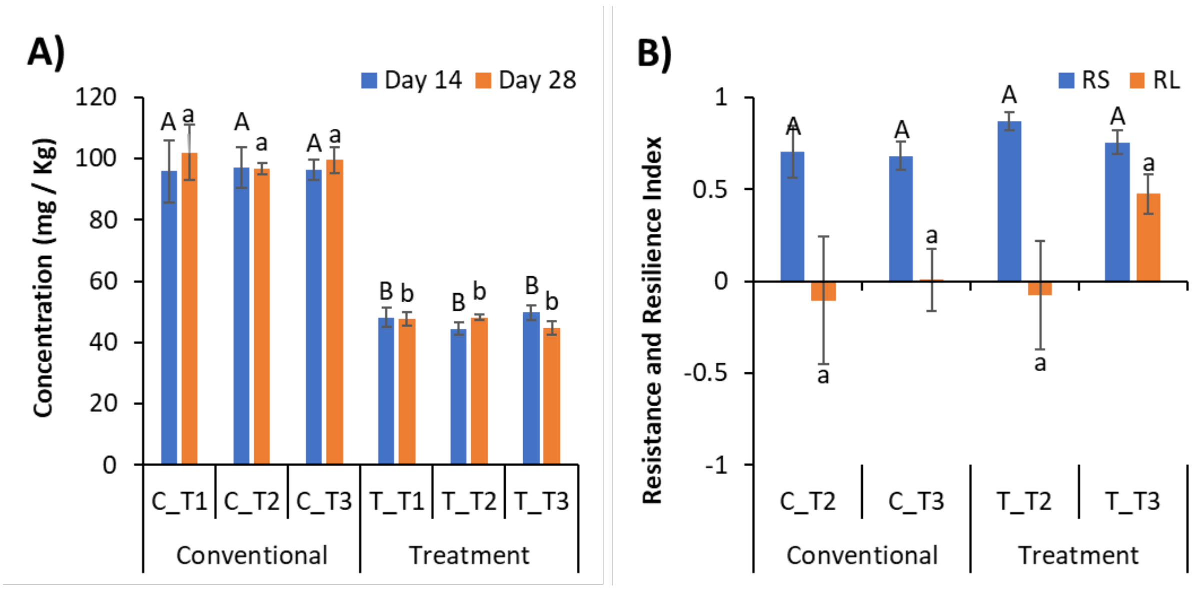


Figure 3.24. Effect of soil management on total mineral nitrogen concentrations (A) and resistance and resilience index for total mineral nitrogen (B) during the incubation period for different water stress treatments in CWFS. C = conventionally managed soil, T = Soil management with cover crops, T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

The absence of significant differences in mineral nitrogen content between treatments under each management approach by the end of the resistance and resilience phases suggests comparable nitrogen availability dynamics within each system. However, by the end of the resilience phase, overall cover crop management exhibited lower mineral nitrogen values compared to conventional management. This discrepancy may stem from differences in nitrogen utilisation and retention mechanisms between the two approaches. Cover crop management likely facilitated increased nitrogen uptake by cover crops or enhanced nitrogen retention within the soil-plant system, leading to reduced mineral nitrogen levels compared to conventional management. This finding highlights the potential of cover crops to effectively manage nitrogen dynamics and promote soil health and resilience over time.

3.3.3 Department of Primary Industries and Regions, South Australia

3.3.3.1 Soil Biological and Chemical Properties at the Initial Stage

Microbial biomass carbon and nitrogen contents were notably higher in soil treated with lime compared to conventionally managed soil. This difference was particularly significant for MBC (Figure 3.25A). However, there was no marked difference in the microbial qCO2 between the two management practices (Figure 3.25B). These results suggest that lime treatment effectively boosts MBC and MBN levels, potentially enhancing soil health and nutrient cycling, while exerting no distinct impact on microbial metabolic activity. The HWEOC content exhibited a notable increase in lime-treated soil compared to soil under conventional management (Figure 3.25C). Conversely, there were no significant differences observed in HWEON (Figure 3.25C) or mineral nitrogen contents (Figure 3.25D) between the two management practices. The higher HWEOC content in lime-treated soil may be attributed to enhanced decomposition of organic matter facilitated by increased microbial activity stimulated by lime application. The lack of differences in HWEON and mineral nitrogen contents between the management practices suggests that lime treatment primarily affects carbon dynamics rather than nitrogen availability in the soil.

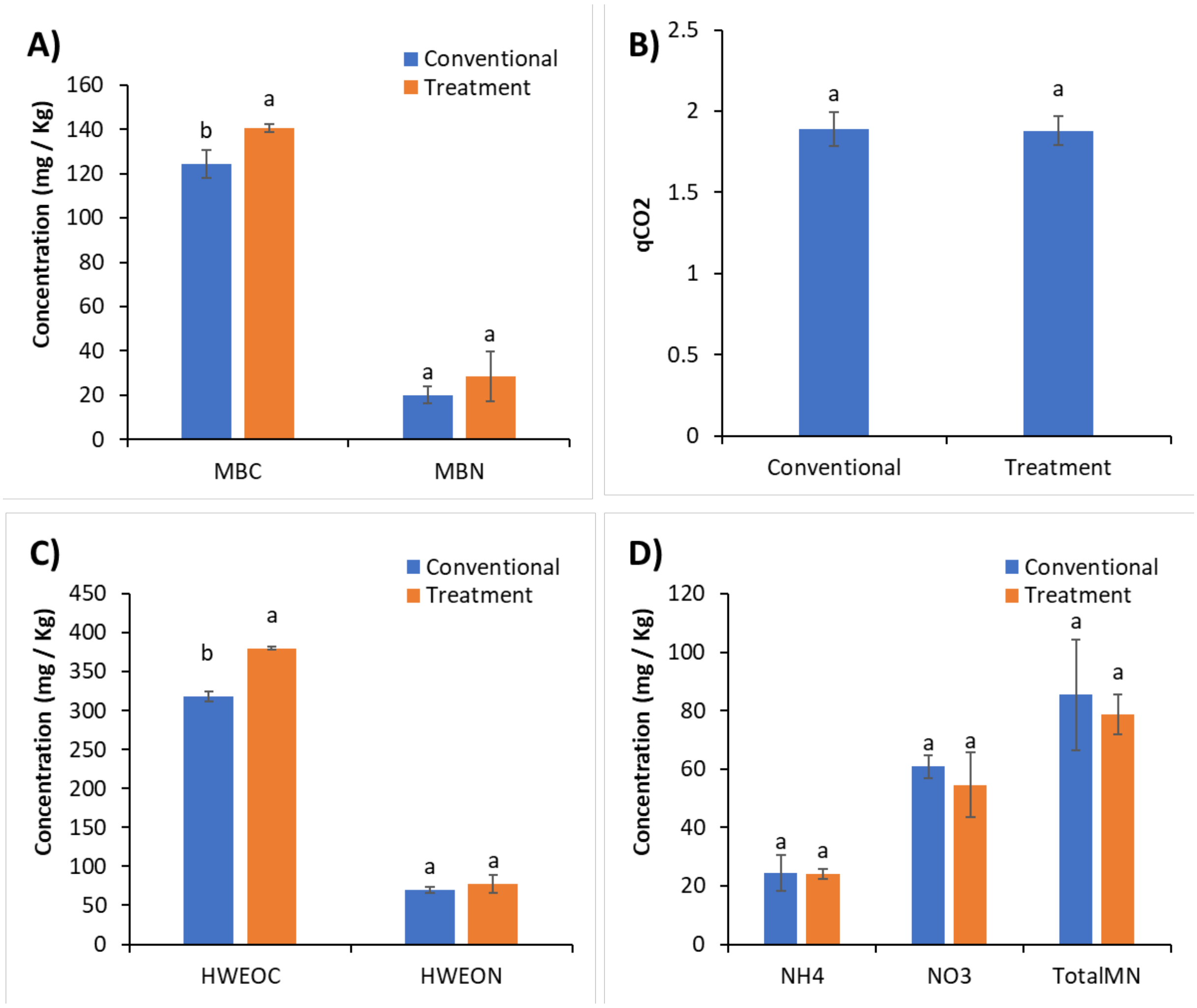


Figure 3.25. Soil biological properties; MBC and MBN (A), and microbial qCO_2 (B), and chemical properties; HWEOC and HWEON concentration (C) and soil mineral nitrogen pool (D) at the initial stage under two management practices. Treatment = Lime application. Vertical bars represent standard error. In each soil parameter, the means with the same letter did not differ at the 5% level of significance.

3.3.3.2 The Effect of Moisture Stress on Soil Biological Properties

3.3.3.2.1 Cumulative CO_2 Emission

In conventionally managed soil, there was no noticeable variance in cumulative CO_2 emission between moderate (C_T2) and severe (C_T3) stress treatments compared to the control (C_T1) until the fifth day. Subsequently, the severe stress treatment exhibited a significant reduction in cumulative CO_2 compared to the control treatment until the end of the stress period (Day 14).

This suggests a period of relatively stable microbial activity. However, the subsequent significant reduction in CO_2 production under severe stress conditions indicates a shift in microbial dynamics, possibly due to stress-induced limitations in substrate availability or metabolic inhibition. This trend persisted for two days after stress release, suggesting potential prolonged effects on microbial communities or delayed recovery of microbial activity. Following that, no significant differences were noted between the treatments until the end of the resilience phase (Day 28) (Figure 3.26A).

In lime-treated soil, both moderate (T_T2) and severe (T_T3) stress treatments exhibited notably lower cumulative CO_2 values compared to the control (T_T1) treatment from the eighth day of stress application to the sixth day after stress release. This implies an altered microbial response possibly due to lime-induced changes in soil pH or chemical properties. However, following this period, no significant differences were observed in cumulative CO_2 emission between the stress treatments and the control until the end of the resilience phase (Day 28) (Figure 3.26B). The absence of significant differences in CO_2 emissions between stress treatments and the control after the initial stress period suggests potential resilience or recovery of microbial activity, possibly due to the buffering capacity of lime-treated soil or microbial adaptation to stress conditions.

Soil managed with lime treatment exhibited increased resistance and resilience to moderate drought stress in contrast to severe stress and both stress levels under conventional soil management. This increase was statistically significant for the resistance index. Nonetheless, there was no significant difference observed for the resilience index across both stress levels under either conventional soil management or soil managed with lime treatment (Figure 3.27). The findings suggest that lime treatment enhances the soil's ability to withstand and recover from moderate drought stress compared to severe stress and both stress levels under conventional soil management, as evidenced by the cumulative CO_2 emission results. This enhancement is particularly notable in the statistically significant increase in the resistance index, indicating that lime-treated soil exhibits greater resistance to stress-induced changes in CO_2 emission levels. The improved resistance in lime-treated soil may be attributed to factors such as enhanced soil structure, increased water retention capacity, or improved nutrient availability.

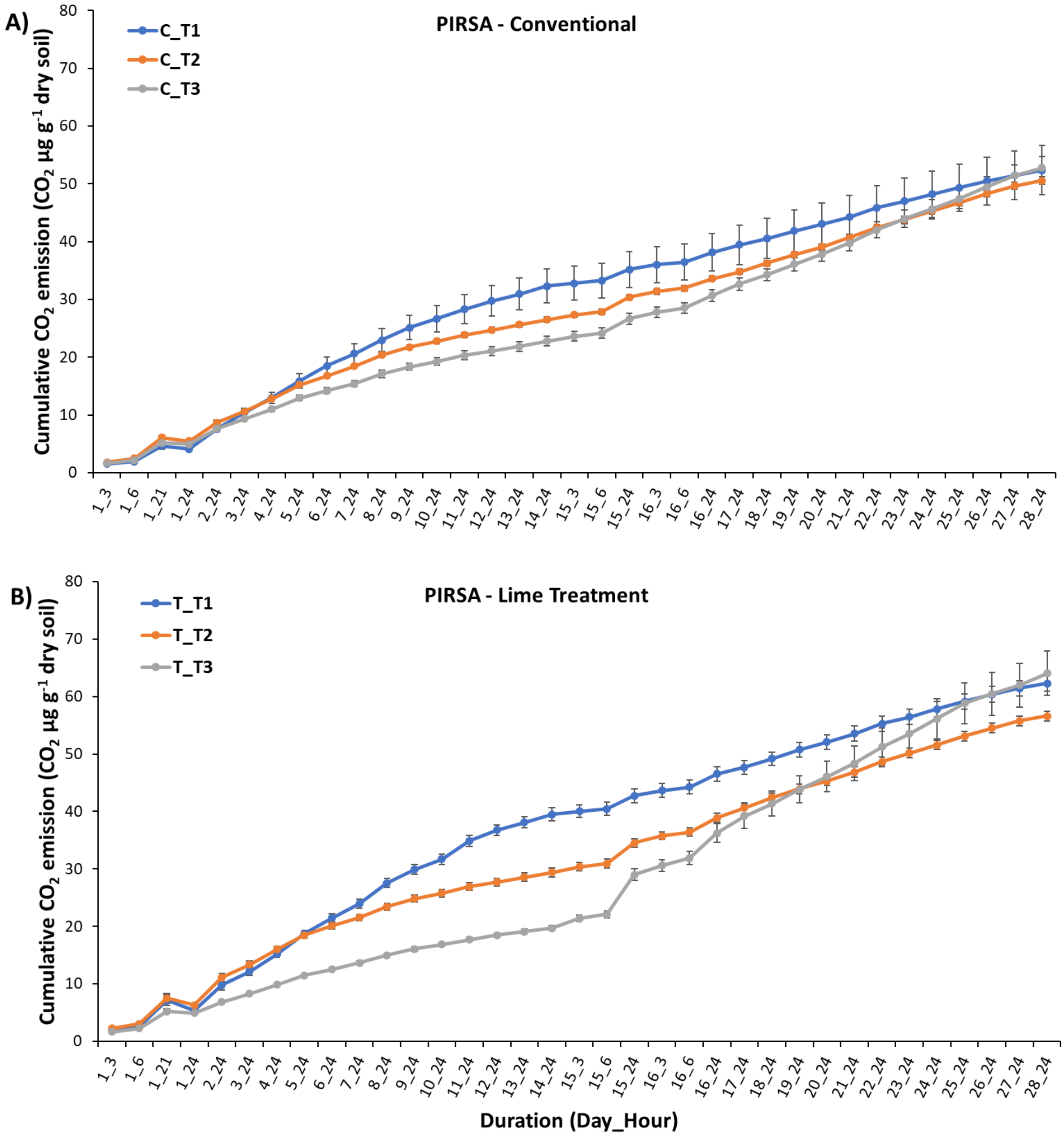


Figure 3.26. Effect of moisture stresses on cumulative CO_2 emissions (CO_2 µg g^{-1} dry soil) in conventionally managed soil (A) and lime-treated soil (B) in PIRSA. C = conventionally managed soil, T = Lime treated soil, T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error.

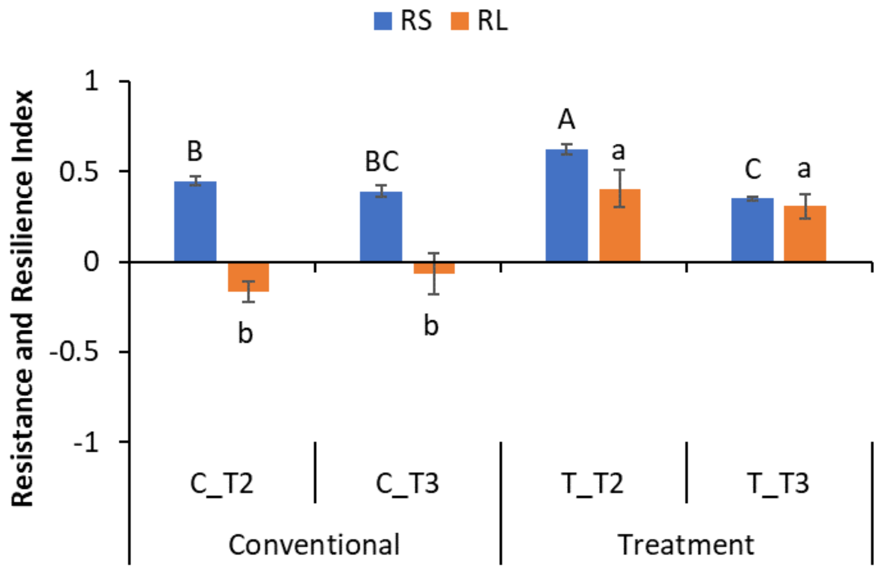


Figure 3.27. Resistance (RS) and Resilience (RL) indices of microbial respiration under moisture stress levels in conventionally managed soil and soil managed with cover crops in PIRSA. RS = Resistance index, RL = Resilience index, T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS), Conventional = Conventionally managed soil. Treatment = Lime treated soil. Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

3.3.3.2.2 Substrate-induced Respiration

Analysis of substrate-induced respiration utilising Fructose and Glucose revealed no notable differences among the water stress treatments within each management practice at the end of the resistance and resilience phases (Figure 3.28, Table 3.4). Additionally, there were no significant variances observed in the resistance and resilience indexes concerning the utilisation rates of individual substrates, such as Glucose and Fructose, under both stress levels in conventionally managed soil and lime-treated soil (Figure 3.29).

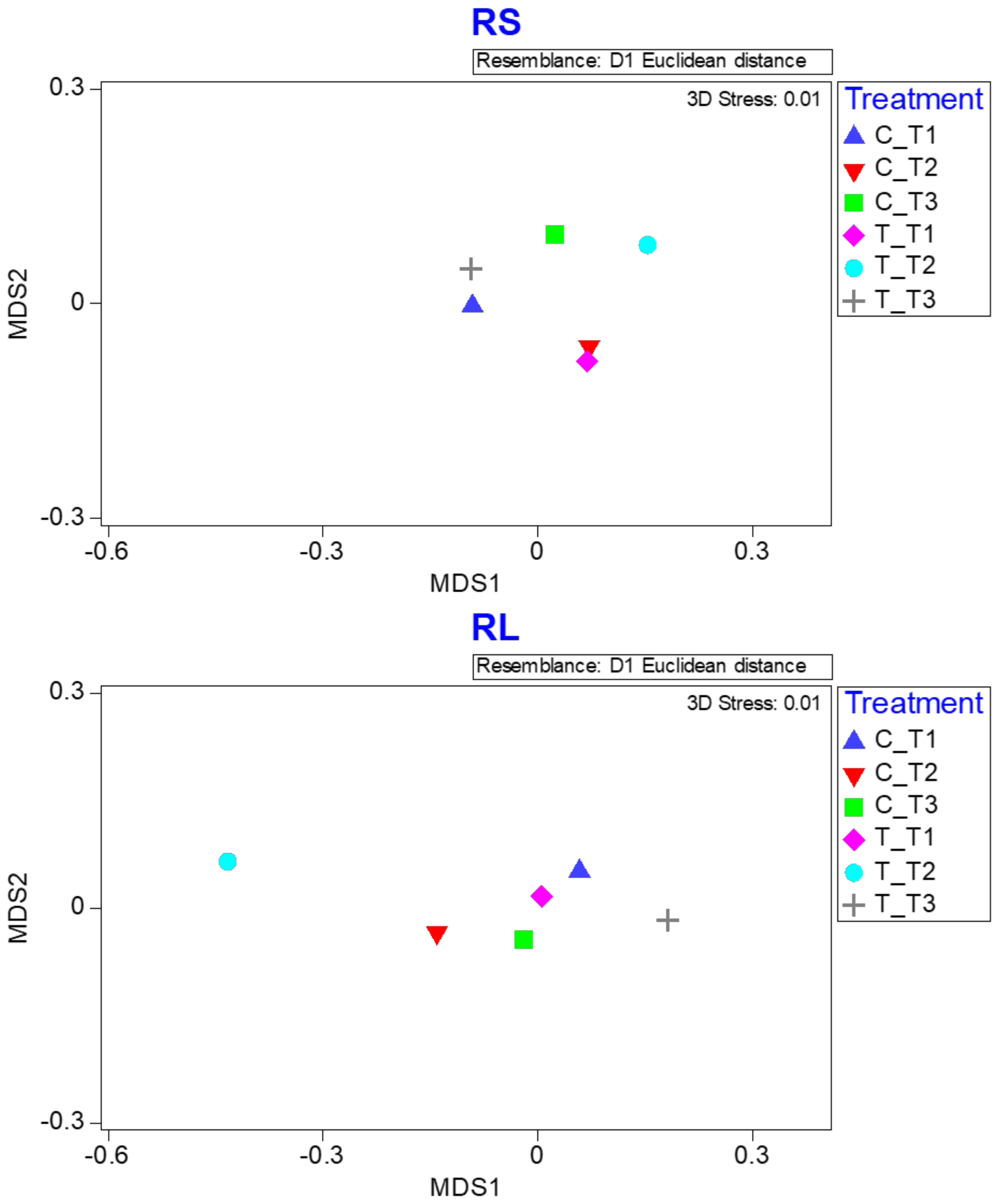


Figure 3.28. Ordinations of bootstrap averages of substrate induced respiration data under water stress treatments for conventional and lime management history. Two-dimensional metric multi-dimensional scaling ordinations (mMDS) of 50 bootstrap sample averages for each treatment and the empirical approximate 95% confidence region, based on Euclidean distance. The distance between the points corresponds to the functional diversity of the microbes in particular treatment: the shorter the distance, the higher similarity between the treatments. C = conventionally managed soil, T = Lime treatment, T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS).

Table 3.4. Pairwise tests from PERMANOVA on Euclidean distance of substrate induced respiration data of microbial population in response to water stress treatments.

Groups	RS		RL	
	Distance	P(MC)	Distance	P(MC)
C_T1, C_T2	0.32	0.29	0.48	0.37
C_T1, C_T3	0.44	0.50	0.46	0.66
T_T1, T_T2	0.58	0.69	0.61	0.12
T_T1, T_T3	0.47	0.44	0.29	0.33

The shorter the distance, the higher similarity between treatments. C = conventionally managed soil, T = Lime treatment, T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS).

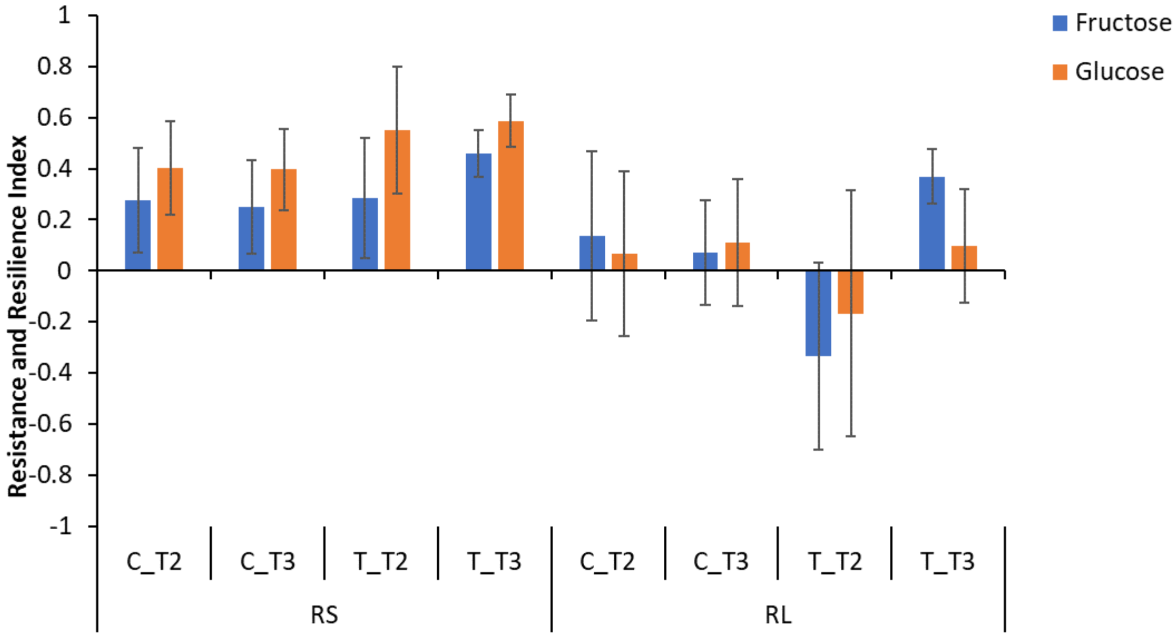


Figure 3.29. Resistance (RS) and Resilience (RL) indices of microbial substrate induced respiration under moisture stress levels in conventionally managed soil and soil managed with cover crops in PIRSA. RS = Resistance index, RL = Resilience index, T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS), Conventional = Conventionally managed soil. Treatment = Lime treated soil. Error bars represent standard error.

3.3.3.2.3 Soil Microbial Biomass Carbon and Nitrogen, and Metabolic Quotient

In conventional management approach, there were no distinct variations in MBC levels between moderate (C_T2) and severe stress (C_T3) treatments when compared to the control treatment (C_T1), during both resistance and resilience phases (Figure 3.30A). This indicating a relatively stable response to stress. However, in lime-treated soil, there was a decrease in MBC under severe (T_T3) stress treatment compared to the control (T_T1) and moderate stress (T_T2) treatments by the end of the resistant phase (Figure 3.30A), suggesting a differential impact of stress in this system. Interestingly, by the end of the resilience phase, there were no significant disparities observed between the control and stress treatments. Moreover, there was an evident increase in MBC content under moderate and severe stress treatments in lime-treated soil compared to conventional management method by the end of the resilience phase. The MBN content was notably reduced under severe stress conditions in both conventional and lime-treated soils by the end of the resistance phase (Figure 3.30B), indicating a general sensitivity of microbial nitrogen dynamics to stress. However, there was no significant difference observed between the stress-treated samples and the control by the end of the resilience phase in either management approach. Interestingly, overall, the lime-treated soil exhibited greater MBN content compared to conventionally managed soil by the end of the resilience phase (Figure 3.30B). This highlighting potential differences in nitrogen cycling dynamics and microbial responses to stress between the two management practices. Resistance and resilience index of the MBC and MBN was not significantly difference for both stress levels under two management systems (Figure 3.31A and 3.31B). Overall, these results underscore the complex and interconnected nature of microbial responses to stress and soil management, influenced by factors such as nutrient availability, soil structure, and microbial community composition.

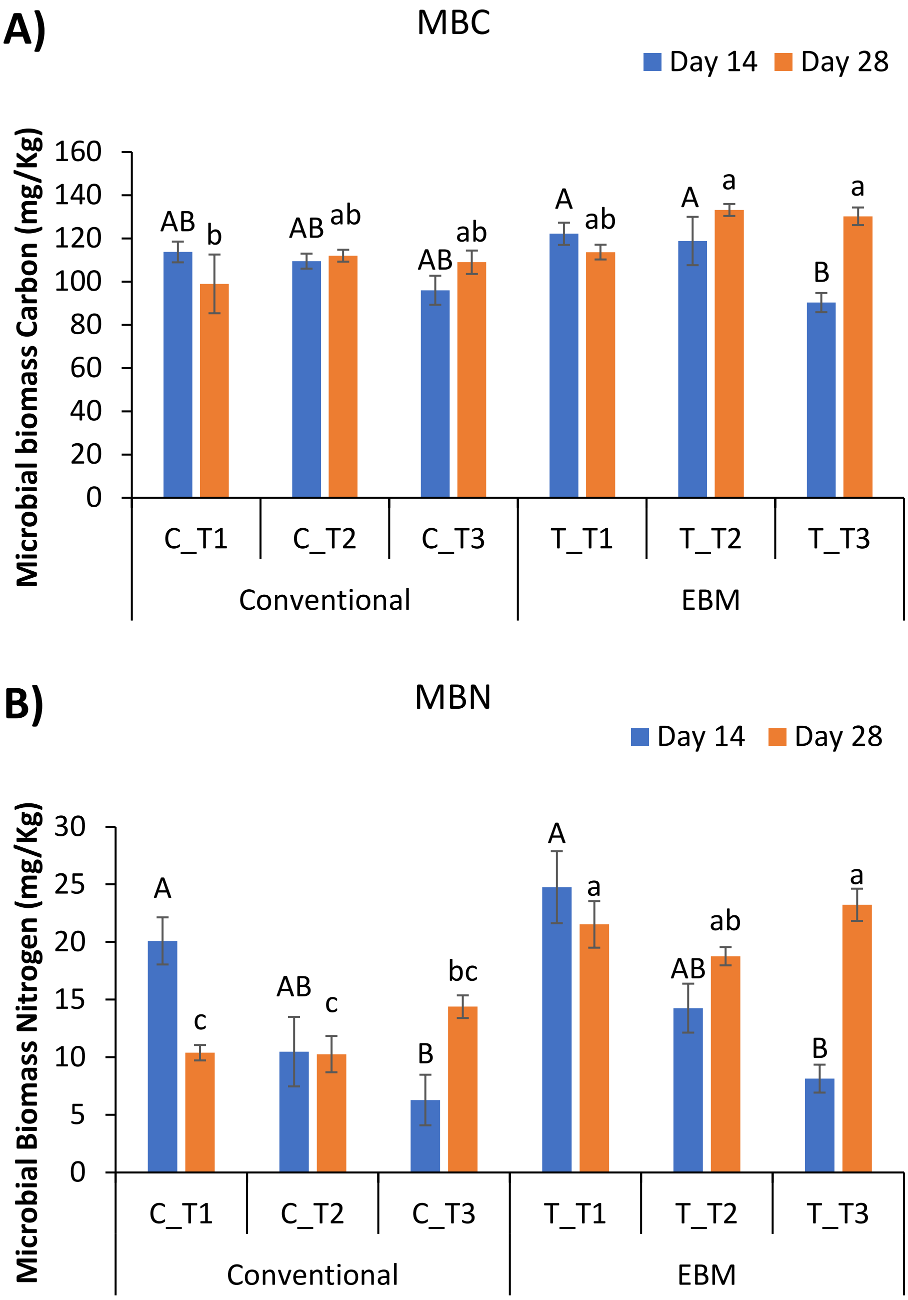


Figure 3.30. Effect of soil management on MBC (A) and MBN (B) concentrations during the incubation period for different water stress treatments in PIRSA. C = conventionally managed soil, T = Lime treated soil. T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

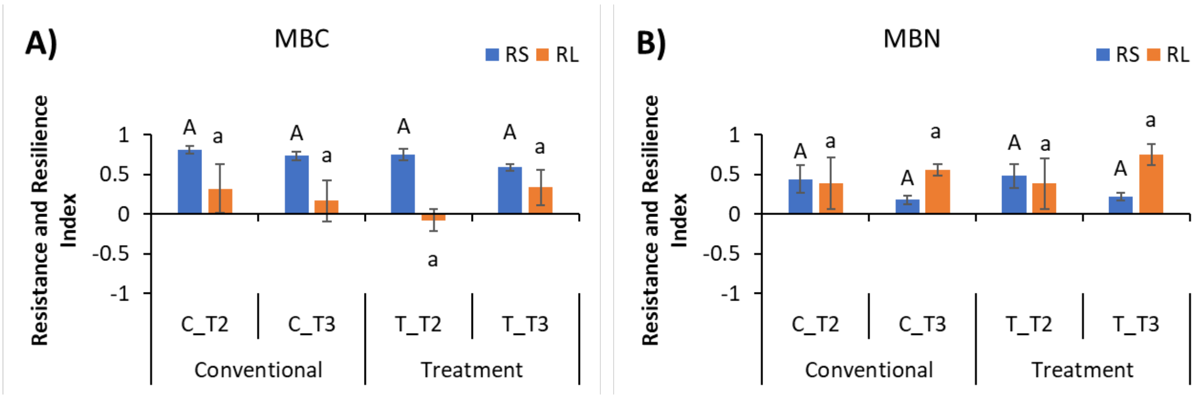


Figure 3.31. The resistance and resilience indices of MBC (A) and MBN (B) between treatments under different management practices in PIRSA. C = conventionally managed soil, T = Lime treated soil. T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

During the resistance phase, under conventional management, severe stress treatment (C_T3) showed a notably lower microbial qCO_2 compared to both the control (C_T1) and moderate stress (C_T3) treatment (Figure 3.32A). Conversely, in lime-treated soil, both moderate (T_T2) and severe stress (T_T3) treatments resulted in lower qCO2 values than the control (T_T1) (Figure 3.32A). However, by the end of the resilience phase, there was no significant difference observed in qCO2 values between the treatments in either management practices (Figure 3.32A). Additionally, within lime-treated soil, moderate stress treatment exhibited the highest resistance index compared to all other treatments. Nonetheless, no significant difference was detected for the resilience index (Figure 3.32B).

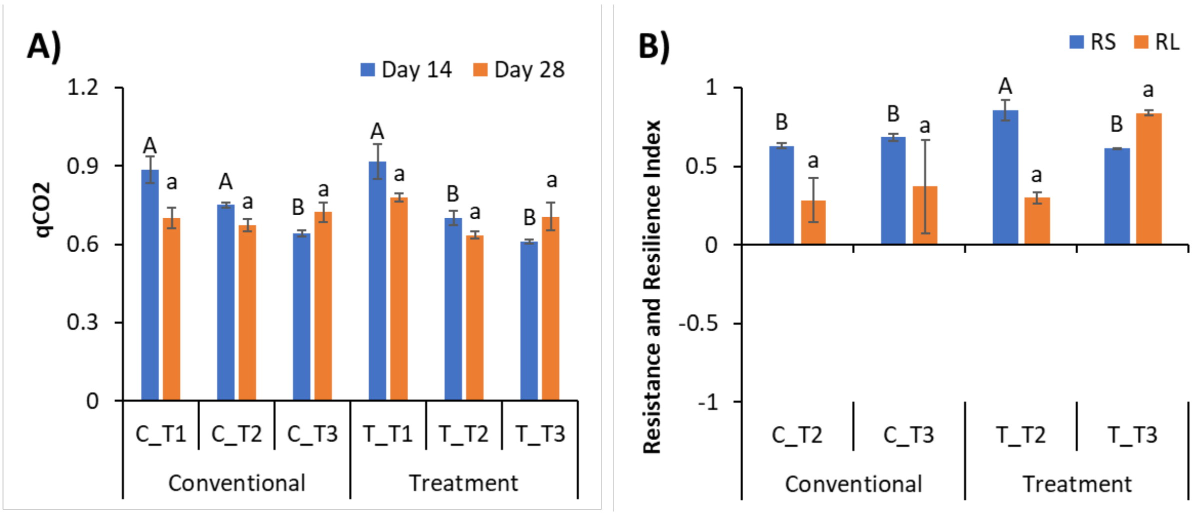


Figure 3.32. Effect of soil management on microbial qCO_2 (A) and resistance and resilience index for qCO_2 (B) in PIRSA. C = conventionally managed soil, T = Lime treated soil. T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

The variations in microbial qCO_2 observed during the resistance phase likely due to differential responses of microbial communities to stress levels and soil management practices. Under conventional management, severe stress treatment induced a significant reduction in qCO_2 compared to control and moderate stress treatment, potentially due to harsh conditions impacting microbial activity. Conversely, in lime-treated soil, both moderate and severe stress treatments led to lower qCO_2 values compared to the control, possibly influenced by altered soil properties affecting microbial metabolism. The lower microbial metabolic activity could be associated with more stable soil conditions or reduced CO_2 emissions, which can be considered as beneficial for developing a sustainable soil. By the resilience phase, no significant difference in qCO_2 values between treatments suggests a capacity for microbial communities to recover from initial stress impacts. Additionally, within lime-treated soil, the highest resistance index for moderate stress treatment suggests enhanced maintenance of metabolic activity, while no difference in resilience index implies similar recovery capabilities among treatments, possibly indicating a threshold effect where stress enhances resilience without significantly affecting long-term recovery.

3.3.3.3 The Effect of Moisture Stress on Soil Chemical Properties

3.3.3.3.1 Hot Water Extractable Organic Carbon and Nitrogen

There were no significant differences in HWEOC and HWEON contents between the control and stress treatments at the end of the resistance phase in either conventionally managed soil or lime-treated soil (Figure 3.33A and 9B). However, during the resilience phase, HWEOC content increased notably for the moderate stress (C_T2) treatment in conventionally managed soil compared to both the control (C_T1) and severe stress (C_T3) treatments, as well as all treatments in lime-treated soil. Similarly, HWEON contents for both control and moderate stress treatments exhibited significant increases compared to the severe stress treatment in conventionally managed soil, as well as all treatments in lime-treated soil (Figure 3.33A and 3.33B). The lower available carbon and nitrogen can be attributed to several interrelated factors. Lime treatment alters soil pH, raising it to more alkaline levels, which can inhibit the activity of certain soil microbes involved in organic matter decomposition and nutrient cycling. This altered microbial activity, combined with changes in soil pH, may lead to reduced breakdown of organic matter and thus lower levels of readily available organic carbon and nitrogen in the soil. This leading to enhanced soil carbon sequestration and long-term soil fertility. Furthermore, while the short-term decrease in these nutrients may seem concerning, it may be offset by the long-term benefits of improved soil pH and fertility, resulting in sustainable and productive agricultural systems.

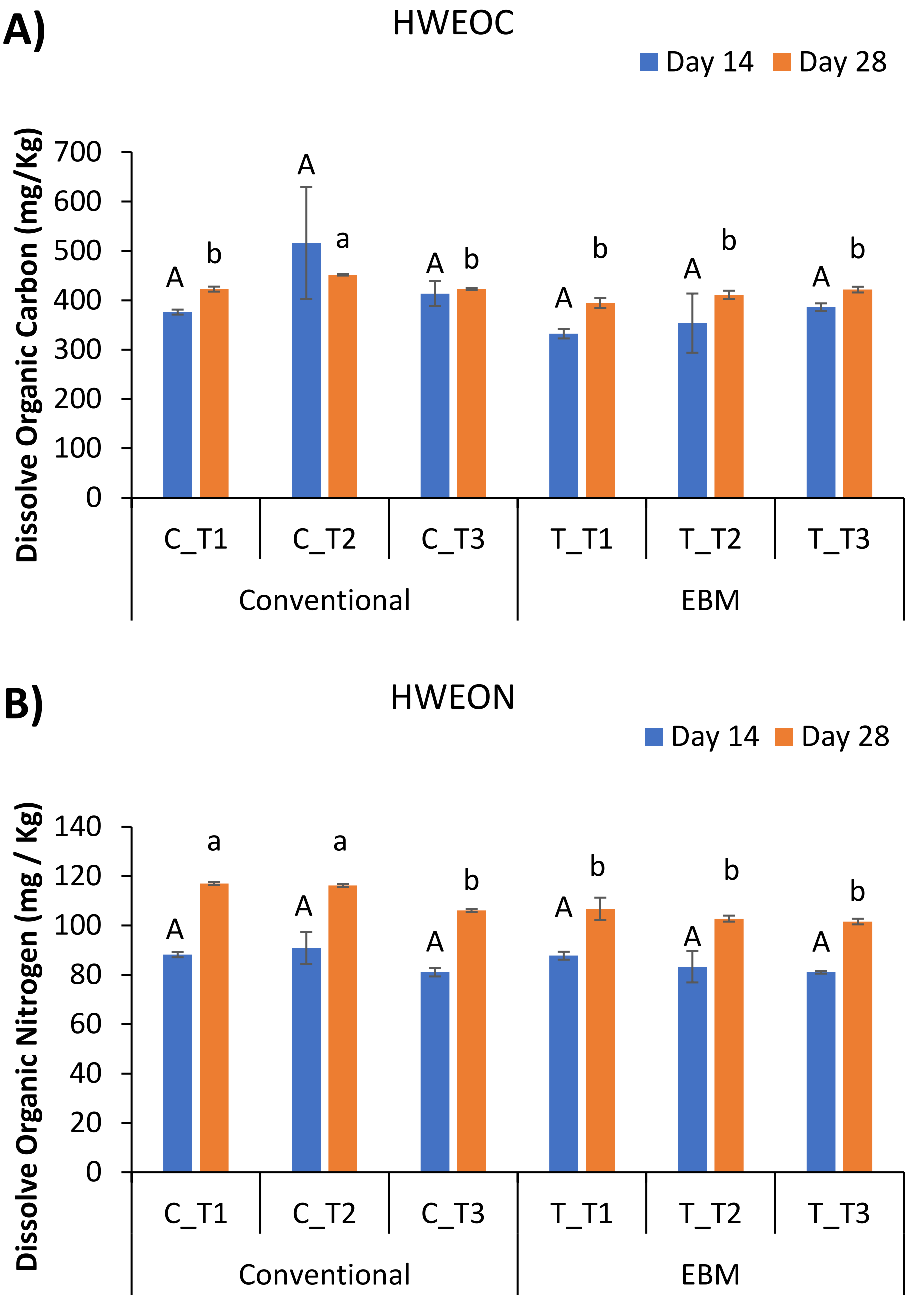


Figure 3.33. Effect of soil management on HWEOC (A) and HWEON (B) concentrations during the incubation period for different water stress treatments in PIRSA. C = conventionally managed soil, T = Lime treated soil. T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

The resistance and resilience indices did not exhibit significant differences among the stress treatments under the two management practices (Figure 34A and 10B). This suggests that the soil's ability to withstand stress and recover from it was not significantly influenced by either conventional management or lime treatment. Despite variations in nutrient levels and other soil parameters, both management practices exhibited similar levels of resilience and resistance to stressors. This finding underscores the robustness and adaptability of soil systems, which can maintain their functionality and stability under different management approaches. It suggests that factors other than nutrient levels, such as soil structure, microbial activity, and plant-soil interactions, may play a more dominant role in determining soil resilience and resistance to stress. Overall, the lack of differences in resistance and resilience indices emphasises the inherent capacity of soils to cope with environmental challenges, regardless of specific management practices.

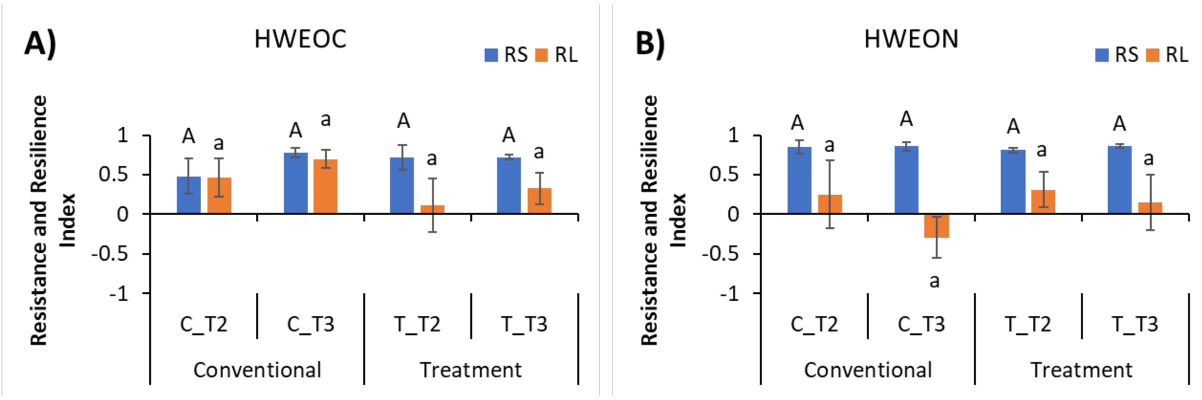


Figure 3.34. The resistance and resilience indices of HWEOC (A) and HWEON (B) between treatments under different management practices in PIRSA. C = conventionally managed soil, T = Lime treated soil. T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

3.3.3.3.2 Soil Mineral Nitrogen

The total mineral nitrogen (NH_4^+ and NO_3^-) content in soil saw a notable decrease under severe stress conditions (C_T3) compared to both control (C_T1) and moderate stress (C_T2) treatments by the end of the resistance phase in conventionally managed soil. Additionally, in lime-treated soil, both moderate (T_T2) and severe stress (T_T3) treatments exhibited reduced total mineral nitrogen contents compared to the control (T_T1) by the end of the resistance phase (Figure 3.35A). However, at the end of the resilience phase, the moderate stress treatment in lime-treated soil did not display a significant difference compared to the control. Conversely, conventionally managed soil exhibited lower total mineral nitrogen contents under both moderate and severe stress treatments in comparison to the control, as well as to both moderate stress and control treatments in lime-treated soil (Figure 3.35A). The resistance indices were notably lower for severe stress treatment than for moderate stress treatment in conventionally managed soil. Conversely, in lime-treated soil, there was no discernible difference between the two stress treatments (Figure 3.35B). Moreover, the highest resilience index was noted for the moderate stress treatment in lime-treated soil (Figure 3.35B).

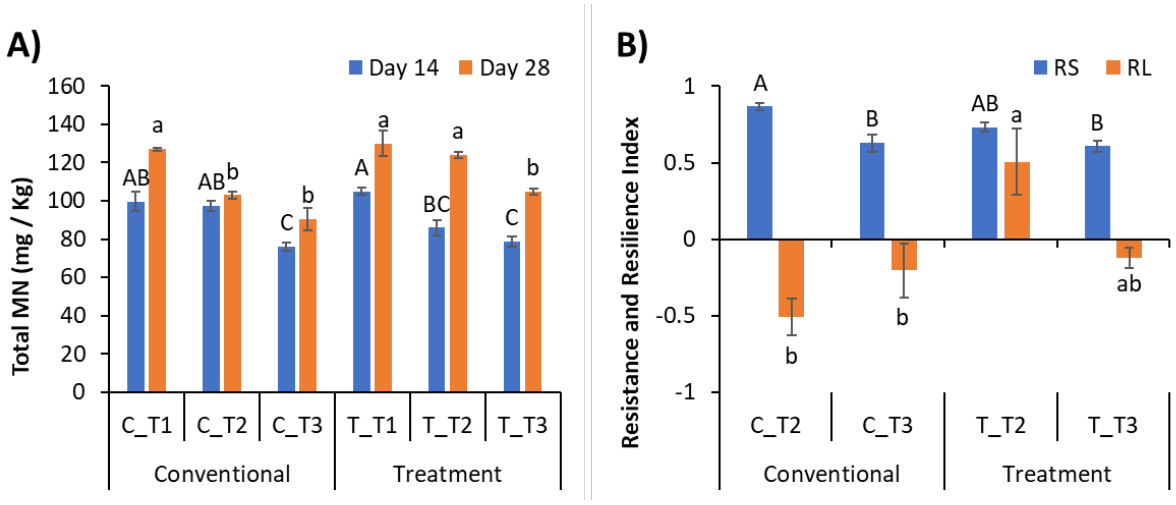


Figure 3.35. Effect of soil management on total mineral nitrogen concentrations (A) and resistance and resilience index for total mineral nitrogen (B) during the incubation period for different water stress treatments in PIRSA. C = conventionally managed soil, T = Lime treated soil, T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

The results indicate that conventionally managed soil experiences a significant reduction in total mineral nitrogen content under severe stress conditions compared to both control and moderate stress treatments, highlighting its susceptibility to severe stress-induced nitrogen loss. Similarly, lime-treated soil exhibits decreased nitrogen content under stress, suggesting that lime treatment alone does not fully mitigate the negative effects of stress on nitrogen availability. Notably, conventionally managed soil shows no recovery in nitrogen availability during the resilience phase, whereas lime-treated soil demonstrates resilience, particularly evident in the moderate stress treatment where nitrogen content came back to levels comparable to the control. This suggests that lime treatment enhances the soil's capacity to recover nitrogen availability after stress events, possibly through alterations in soil pH, microbial activity, buffering capacity, and soil structure. Moreover, lime-treated soil displays consistent resistance indices across moderate and severe stress treatments, indicating its ability to mitigate the impact of severe stress on nitrogen loss, contrasting with conventionally managed soil's greater susceptibility to severe stress. These findings underscore the potential of lime treatment to enhance nitrogen availability and resilience to stress in soil. However, further research is required to reveal the underlying mechanisms comprehensively.

3.3.4 Soils for Life

3.3.4.1 Soil Biological and Chemical Properties at the Initial Stage

There was no significant difference in soil MBC and MBN content between cover crop management (Treatment) and conventional management (Figure 3.36A). Similarly, the microbial qCO_2 showed no notable difference between the two management practices (Figure 3.36B). However, HWEOC content was significantly lower under cover crop management compared to conventional management, while HWEON content was significantly higher under cover crop management (Figure 3.36C). Additionally, the soil mineral nitrogen pool (NH4+, NO3-) was higher in cover crop managed soil than in conventionally managed soil (Figure 3.36D).

The lack of significant difference in soil MBC and MBN content between cover crop and conventional management may be due to the microbial community adapting similarly to both practices, with each providing sufficient organic matter and nutrients. The microbial qCO_2 being comparable suggests that microbial efficiency and activity are similar, likely due to similar environmental conditions and resources. The lower HWEOC under cover crop management might result from cover crops utilising more labile carbon for growth and enhancing microbial decomposition of organic matter. Conversely, the higher HWEON content under cover crop management could be due to cover crops fixing atmospheric nitrogen or mobilising nitrogen from deeper soil layers. Additionally, the higher soil mineral nitrogen pool in cover crop managed soil might be due to improved nitrogen cycling and retention, as cover crops can reduce nitrogen leaching and increase nitrogen availability through fixation and decomposition processes.

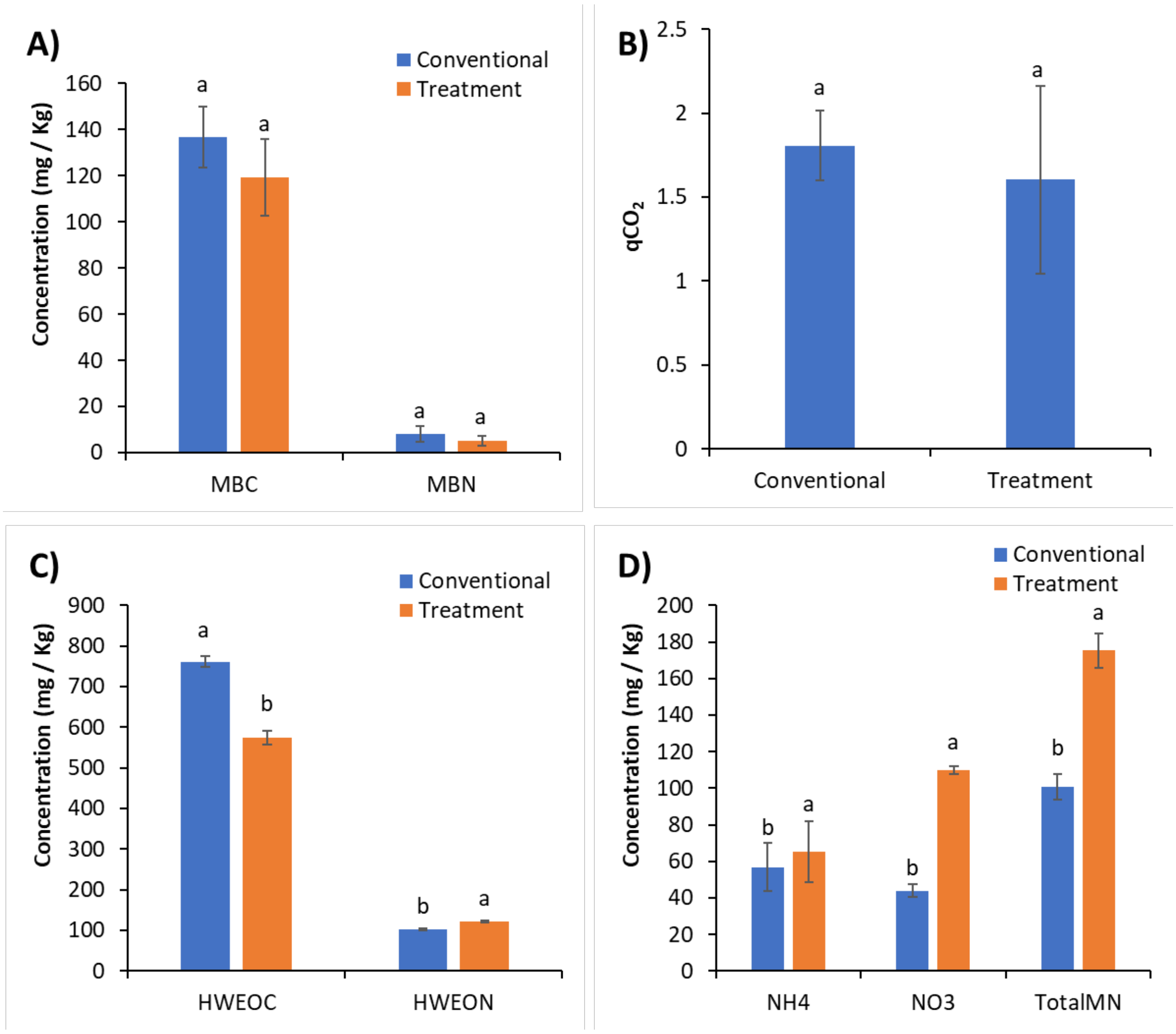


Figure 3.36. Soil biological properties; MBC and MBN (A), and microbial qCO_2 (B), and chemical properties; HWEOC and HWEON concentration (C) and soil mineral nitrogen pool (D) at the initial stage under two management practices. Treatment = Cover crop. Vertical bars represent standard error. In each soil parameter, the means with the same letter did not differ at the 5% level of significance.

3.3.4.2 The Effect of Moisture Stress on Soil Biological Properties

3.3.4.2.1 Cumulative CO_2 Emission

In conventionally managed soil, there was no significant difference in cumulative CO_2 emissions between moderate (C_T2) and severe (C_T3) stress treatments compared to the control (C_T1) during both the resistance and resilience phases (Figure 3.37A). In cover crop managed soil, the moderate (T_T2) stress treatment showed significantly lower cumulative CO_2 emissions compared to the control (T_T1) from the seventh day of stress application until the end of the stress period (Day 14). This trend continued after the stress release until the end of the resilience phase (Day 28). The severe stress treatment (T_T3) exhibited a significant reduction in CO_2 emissions throughout both the resistance and resilience phases (Figure 3.37B). Soil managed with both cover crops and conventional methods (no cover crop) showed increased resistance to moderate drought stress (C_T2 and T_T2) compared to severe stress (C_T3 and T_T3). However, the resilience index did not significantly differ between the two stress levels in either conventional soil management or soil managed with lime treatment (Figure 3.38).

The observed differences in CO_2 emissions between conventionally managed soil and soil managed with cover crops under various stress treatments can be attributed to differences in soil health, microbial activity, and organic matter content. In conventionally managed soil, the lack of significant differences in CO_2 emissions among control, moderate, and severe stress treatments is likely due to lower organic matter and microbial activity, which limits the soil's responsiveness to stress levels. Conversely, in cover crop managed soil, the moderate stress treatment showed significantly lower CO_2 emissions compared to the control during and after the stress period, likely due to enhanced soil structure, moisture retention, and microbial efficiency from the cover crops. Severe stress led to a notable reduction in CO_2 emissions due to decreased microbial activity under extreme conditions. Both soil management practices demonstrated increased resistance to moderate drought stress compared to severe stress, highlighting both management practices can cope with the moderate stress. However, the resilience index, reflecting the soil's recovery capability, did not significantly differ between moderate and severe stress levels in either management system, suggesting that while the initial impact varies, the recovery process is similarly effective across stress severities, likely due to the inherent properties and management practices that aid in resilience.

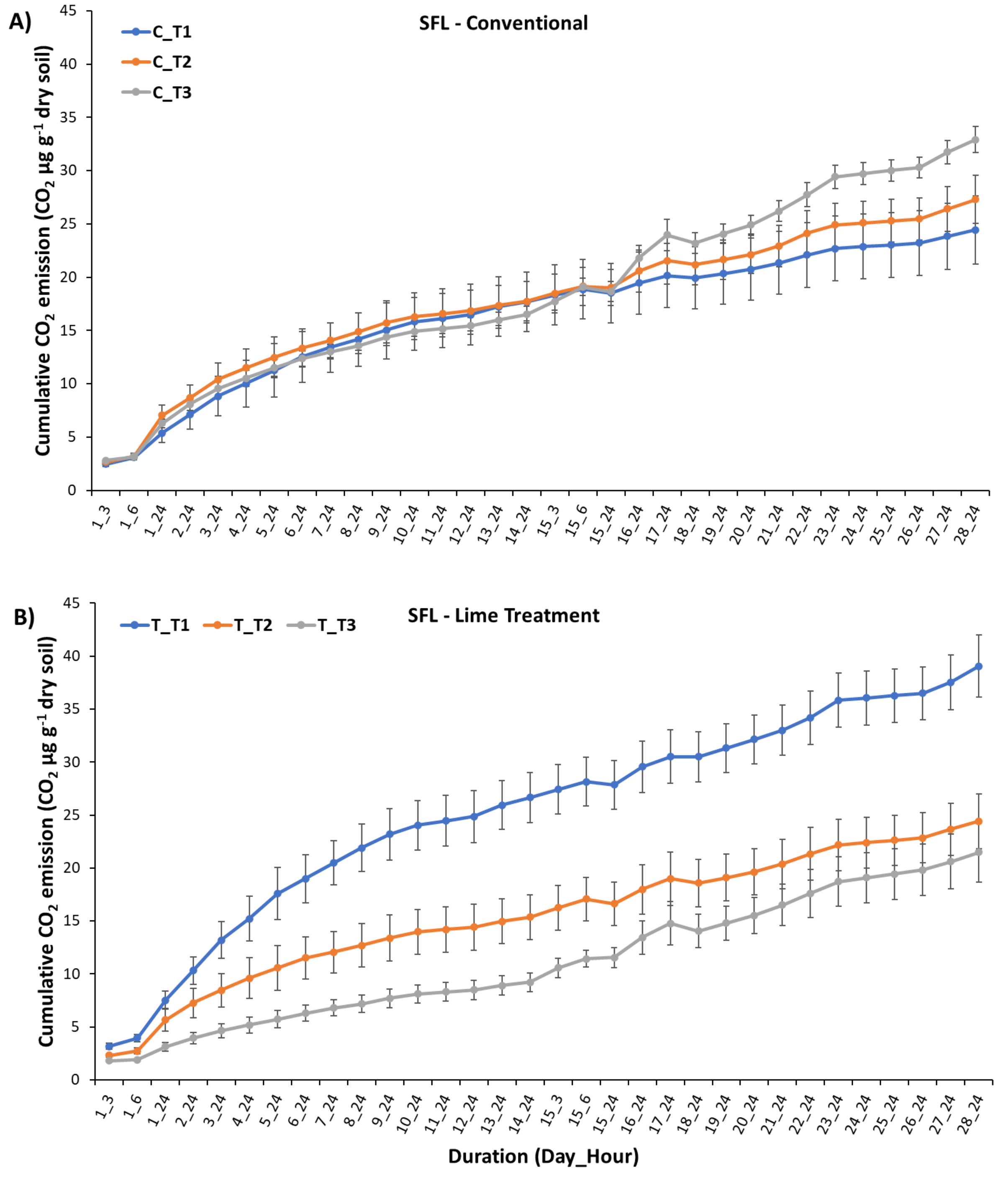


Figure 3.37. Effect of moisture stresses on cumulative CO_2 emissions (CO_2 µg g^{-1} dry soil) in conventionally managed soil (A) and Lime treated soil (B) in SFL. C = No cover crop, T = Cover crop managed soil, T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error.

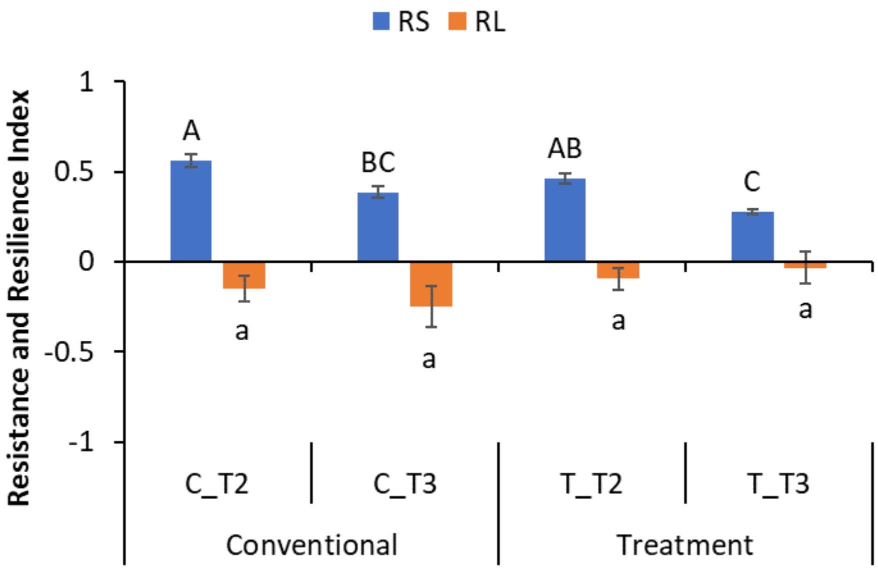


Figure 3.38. Resistance (RS) and Resilience (RL) indices of microbial respiration under moisture stress levels in conventionally managed soil and soil managed with cover crops in SFL. RS = Resistance index, RL = Resilience index, T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS), Conventional = Conventionally managed soil. Treatment = Cover crop. Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

3.3.4.2.2 Substrate-induced Respiration

Analysis of substrate-induced respiration utilising fructose and glucose revealed no notable differences among the water stress treatments (C_T1 and C_T3) within conventionally managed soil at the end of the resistance (RS) and resilience (RL) phases (Figure 3.39, Table 3.5). However, under cover crop managed soil, the moderate stress treatment (T_T2) showed a significant difference in microbial functionality (i.e., substrate utilisation) compared with the control (C_T1) at the end of the resistance phase. By the end of the resilience phase, no notable differences in microbial functionality were observed for the water stress treatments under cover crop managed soil (Figure 3.39, Table 3.5). Additionally, the resistance and resilience index for fructose utilisation was higher for the moderate stress treatment (T_T2) under cover crop managed soil compared with the moderate stress treatment (C_T2) under conventionally managed soil. Conversely, for glucose utilisation, the resistance index was higher under the moderate stress treatment in conventionally managed soil (C_T2) compared with the moderate stress treatment under cover crop managed soil (T_T2) (Figure 3.40).

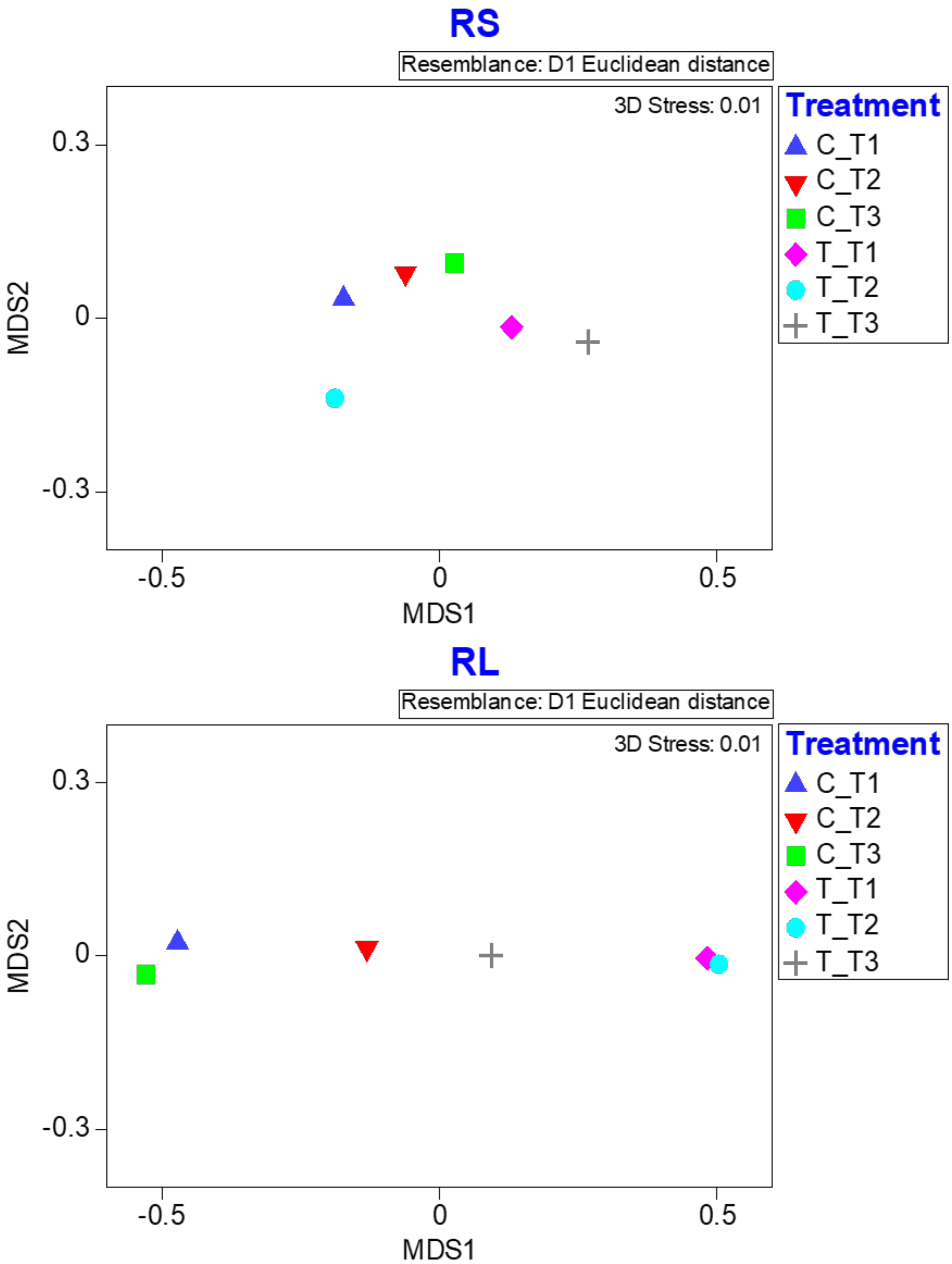


Figure 3.39. Ordinations of bootstrap averages of substrate induced respiration data under water stress treatments for conventional and lime management history. Two-dimensional metric multi-dimensional scaling ordinations (mMDS) of 50 bootstrap sample averages for each treatment and the empirical approximate 95% confidence region, based on Euclidean distance. The distance between the points corresponds to the functional diversity of the microbes in particular treatment: the shorter the distance, the higher similarity between the treatments. C = No cover crop, T = Cover crop managed soil, T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS).

Table 3.5. Pairwise tests from PERMANOVA on Euclidean distance of substrate induced respiration data of microbial population in response to water stress treatments.

Groups	**RS**		**RL**	
	Distance	**P(MC)**	**Distance**	**P(MC)**
C_T1, C_T2	0.39	0.55	0.48	0.06
C_T1, C_T3	0.38	0.18	0.14	0.12
T_T1, T_T2	0.45	0.02	0.61	0.85
T_T1, T_T3	0.23	0.08	0.77	0.23

The shorter the distance, the higher similarity between treatments. C = No cover crop, T = Cover crop managed soil, T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS).

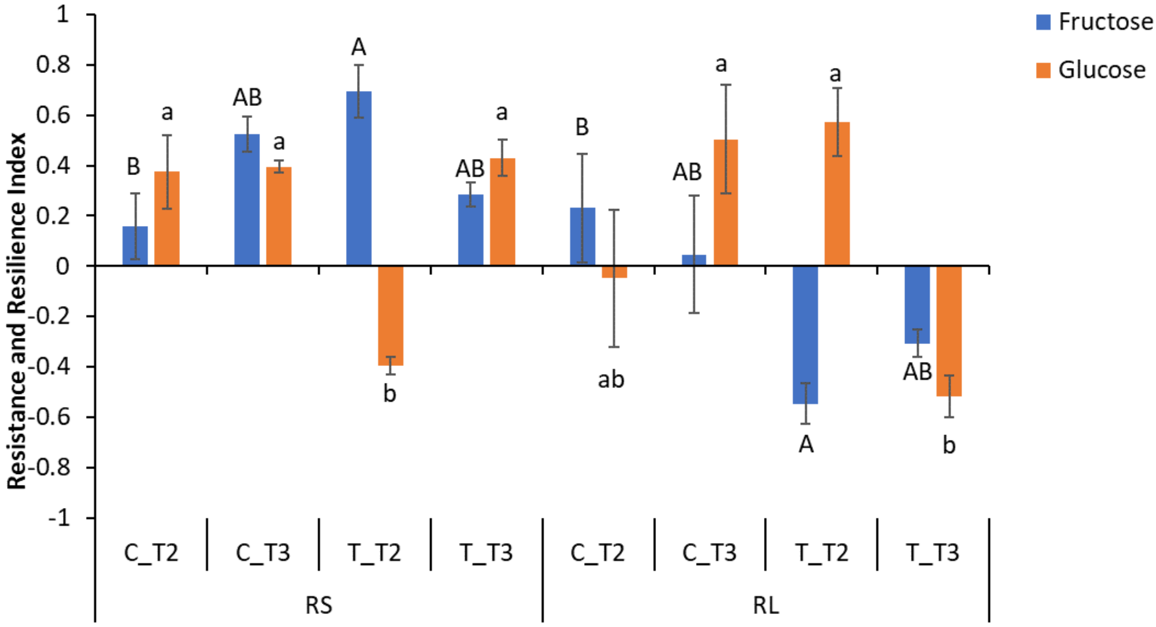


Figure 3.40. Resistance (RS) and Resilience (RL) indices of microbial substrate induced respiration under moisture stress levels in conventionally managed soil and soil managed with cover crops in SFL. RS = Resistance index, RL = Resilience index, T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS), Conventional = No cover crop, Treatment = Cover crop managed soil. Error bars represent standard error.

The observed results highlight the impact of soil management practices on microbial activity and substrate utilisation under water stress. In conventionally managed soil, no notable differences in substrate-induced respiration with fructose and glucose were observed among the water stress treatments (C_T1 and C_T3) at the end of both the resistance (RS) and resilience (RL) phases, suggesting that conventional management does not significantly alter microbial functionality in response to water stress. However, under cover crop managed soil, the moderate stress treatment (T_T2) showed a significant difference in microbial functionality compared to the control (C_T1) at the end of the resistance phase, indicating enhanced microbial activity due to improved soil structure and organic matter from cover crops. By the end of the resilience phase, these differences diminished, suggesting soil system stabilisation. Furthermore, the resistance and resilience index for fructose utilisation was higher in cover crop managed soil (T_T2) compared to conventionally managed soil (C_T2), demonstrating better maintenance and recovery of microbial functionality. Conversely, the resistance index for glucose utilisation was higher under moderate stress in conventionally managed soil (C_T2) compared to cover crop managed soil (T_T2), possibly due to differences in microbial community composition and metabolic pathways.

3.3.4.2.3 Soil Microbial Biomass Carbon and Nitrogen, and Metabolic Quotient

MBC content under cover crop management did not significantly differ between moderate (T_T2) and severe (T_T3) water stress treatments compared to the control (T_T1) at the end of the stress period. However, in conventionally managed soil, there was a significant reduction in MBC content under severe water stress (C_T3) compared to both the control (C_T1) and moderate (C_T2) water stress treatments (Figure 3.41A). At the end of the resilience phase, no significant differences in MBC content were observed across all treatments in both management approaches (3.41A). MBN content was significantly higher in the severe water stress treatment under cover crop management at the end of the stress period. In contrast, under conventional management, the no stress treatment had the highest MBN content compared to the stress treatments (3.41B). Similar to MBC, MBN content did not show significant differences between treatments in either management practice at the end of the resilience phase (3.41B). Additionally, the resistance and resilience indices of MBC and MBN for the moderate and severe stress treatments did not show significant differences under both management approaches (3.42A and B).

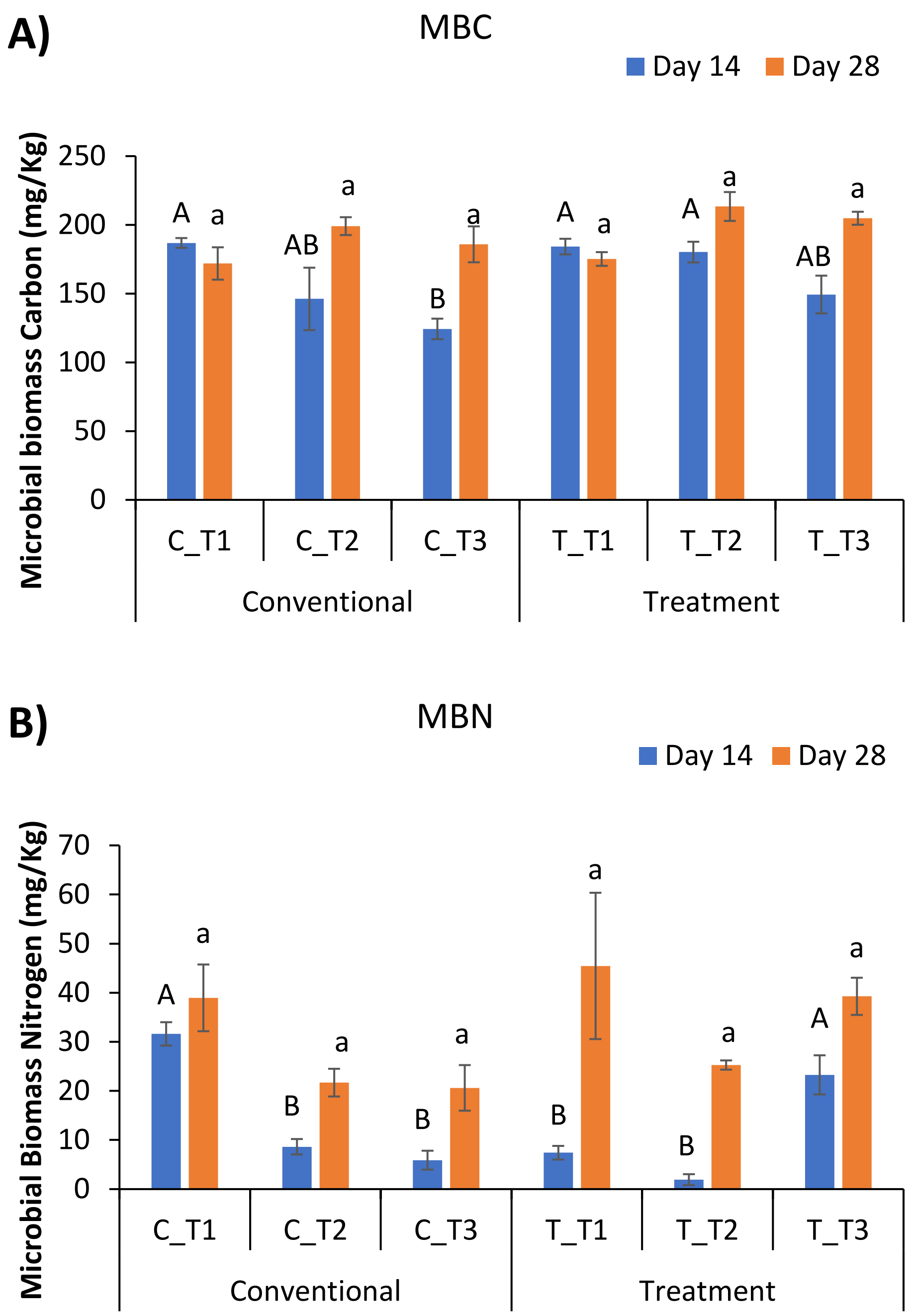


Figure 3.41. Effect of soil management on MBC (A) and MBN (B) concentrations during the incubation period for different water stress treatments in SFL. C = No cover crop, T = Cover crop managed soil. T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

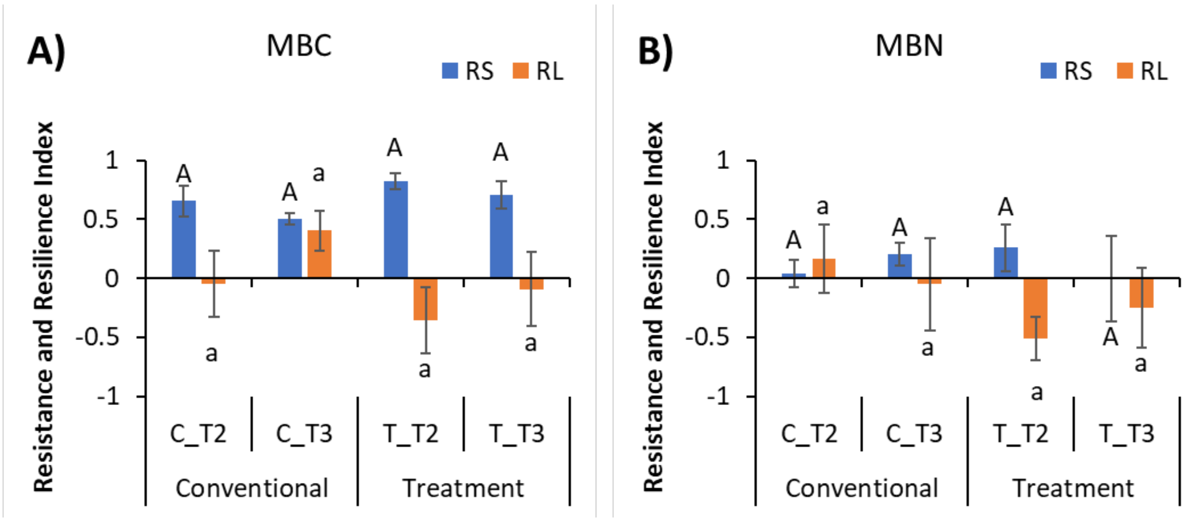


Figure 3.42. The resistance and resilience indices of MBC (A) and MBN (B) between treatments under different management practices in SFL. C = No cover crop, T = Cover crop managed soil. T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

The observed differences in MBC and MBN content under different water stress and management regimes could be attributed to several factors. Under cover crop management, the stable MBC levels across stress treatments suggest that the cover crop might have provided a consistent microbial habitat and nutrient availability, buffering the effects of water stress. In contrast, conventional management practices may have exacerbated the impact of severe water stress on MBC, possibly due to reduced organic matter inputs or disrupted soil structure, limiting microbial activity and biomass accumulation. The higher MBN content under severe water stress in cover crop systems could reflect enhanced nitrogen cycling or allocation strategies under stress conditions, supported by the cover crop's influence on soil nitrogen dynamics. Conversely, in conventionally managed soils, the lack of stress induced MBN increase might indicate a less responsive nitrogen cycling system or limited availability of organic nitrogen sources. Overall, the resilience phase results suggest that both management systems had similar capacities to recover MBC and MBN levels after stress, indicating potential adaptive mechanisms within microbial communities to regain biomass and nutrient cycling functions once stress is alleviated.

The microbial qCO_2 exhibited a significant increase in the severe water stress (C_T3) treatment compared to the no stress (C_T1) treatment in conventionally managed soil by the end of the stress period (Figure 3.43A). In contrast, under cover crop management, both moderate (T_T2) and severe stress (T_T3) treatments led to a significant decrease in the microbial qCO_2 compared to the no stress treatment (T_T1) (Figure 3.43A). By the end of the resilience phase, there were no marked differences in the microbial qCO_2 between stress treatments under either management approach (Figure 3.43A). Additionally, the resistance and resilience indices for the microbial qCO_2 did not indicate significant differences between stress treatments in either management approach (Figure 3.43B).

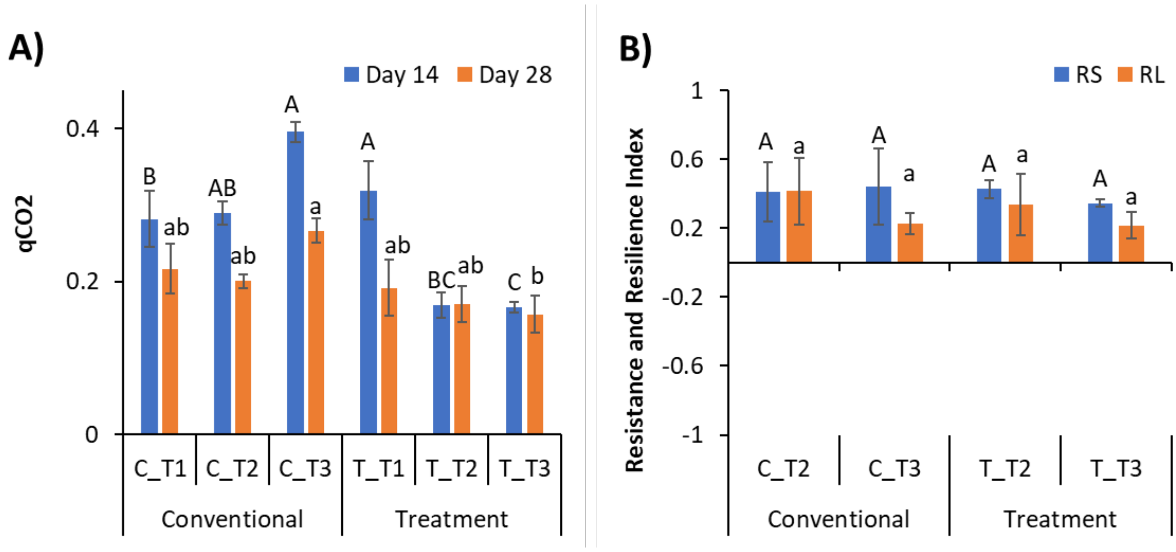


Figure 3.43. Effect of soil management on microbial qCO_2 (A) and resistance and resilience index for qCO_2 (B) in SFL. C = No cover crop, T = Cover crop managed soil. T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

The variation in microbial qCO_2 across different water stress and management can be attributed to distinct microbial responses to environmental pressures. In conventionally managed soil, the elevated qCO_2 under severe water stress suggests a heightened metabolic demand per unit biomass, possibly due to stress-induced shifts in microbial community composition or increased energy expenditure to cope with adverse conditions. Conversely, under cover crop management, the reduced qCO_2 in response to stress treatments indicates a more stable microbial community with lower metabolic stress, potentially supported by improved soil structure, nutrient availability, and microbial habitat provided by cover crops. The convergence of qCO_2 values during the resilience phase across all stress treatments under both management approaches suggests microbial communities' resilience in restoring metabolic equilibrium once stressors are removed, reflecting adaptive mechanisms in response to varying environmental pressures. These findings underscore the pivotal role of management practices in shaping microbial metabolic dynamics and resilience in agroecosystems facing fluctuating water availability.

3.3.4.3 The Effect of Moisture Stress on Soil Chemical Properties

3.3.4.3.1 Hot Water Extractable Organic Carbon and Nitrogen

The HWEOC content significantly increased in the severe water stress treatment (C_T3) under conventional management compared to the no stress treatment (C_T1) at the end of the stress period. However, under cover crop management, there was no significant difference in HWEOC between the water stress treatments (T_T2, T_T3) and the no stress treatment (T_T1) (Figure 3.44A). By the end of the resilience phase, no notable differences were observed between treatments in either management approach (3.44A). The hot water extractable total nitrogen (HWETN) content did not show a notable difference between control and stress treatments under conventional management at the end of the resistance period (Figure 3.44B). However, under cover crop management, the moderate stress treatment exhibited the lowest HWETN content compared to the severe stress and no stress treatments. By the end of the resilience phase, the moderate stress treatments under both conventional and cover crop management had the lowest HWETN content (3.44B). Overall, HWETN contents were higher in all treatments under cover crop management compared to conventional management.

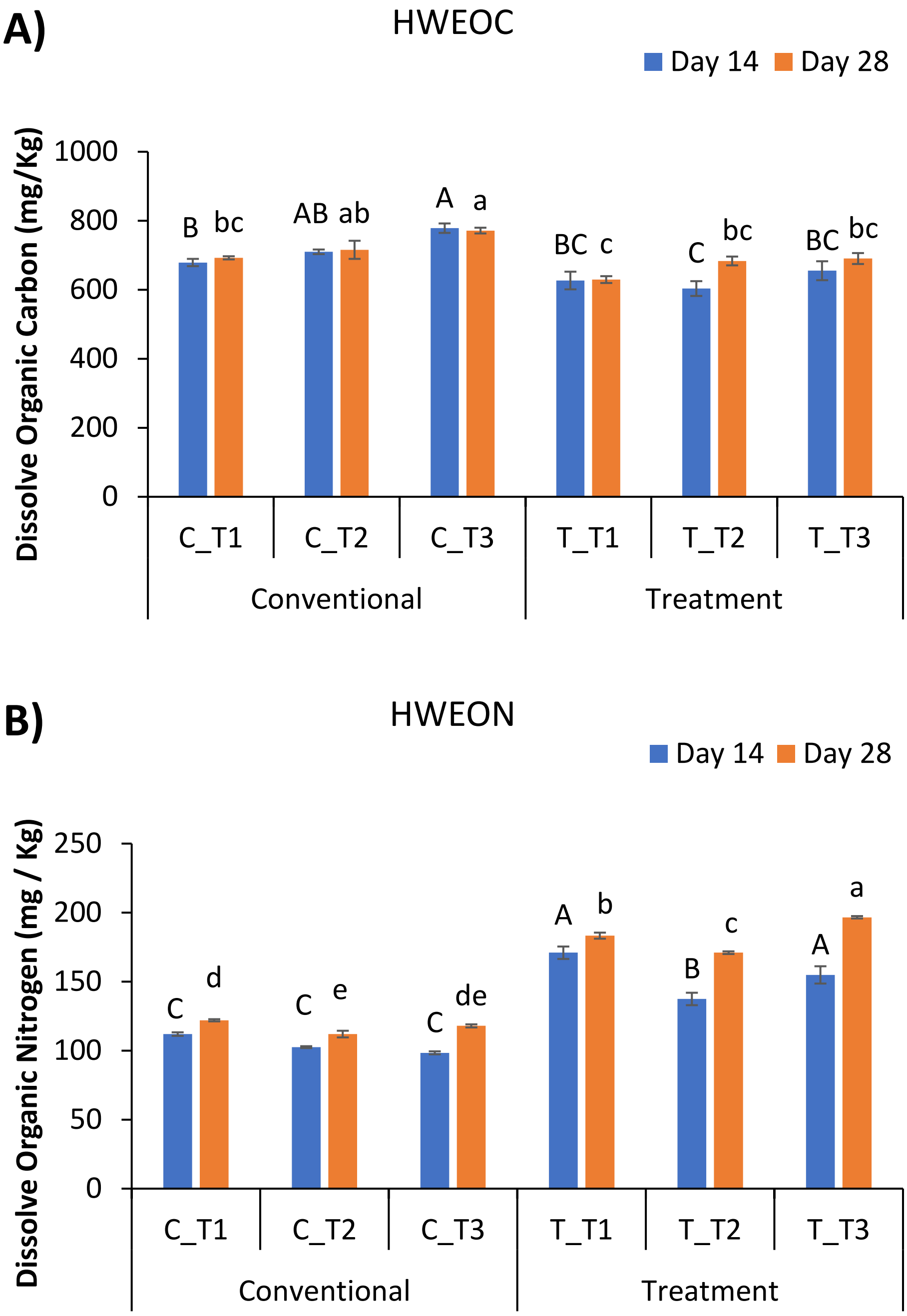


Figure 3.44. Effect of soil management on HWEOC (A) and HWEOC (B) concentrations during the incubation period for different water stress treatments in SFL. C = No cover crop, T = Cover crop managed soil. T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

The resistance index of HWEOC was significantly higher for the severe water stress treatment (T_T3) under cover crop management compared to conventional management (C_T3). However, for moderate stress treatments (T_T2 and C_T2), there was no notable difference in HWEOC between the two management approaches (3.45A). The resistance index for HWEON did not show a significant difference between management approaches for the stress treatments (3.45B). The resilience index for the moderate water stress treatment of both HWEOC (3.45A) and HWEON (3.45B) under cover crop management was significantly higher than for the severe water stress treatment. Under conventional management, there was no significant difference in the resilience index of HWEOC between moderate and severe stress treatments (3.45A), but the resilience index of HWETN was significantly higher in severe water stress treatment compared to moderate water stress treatment (3.45B).

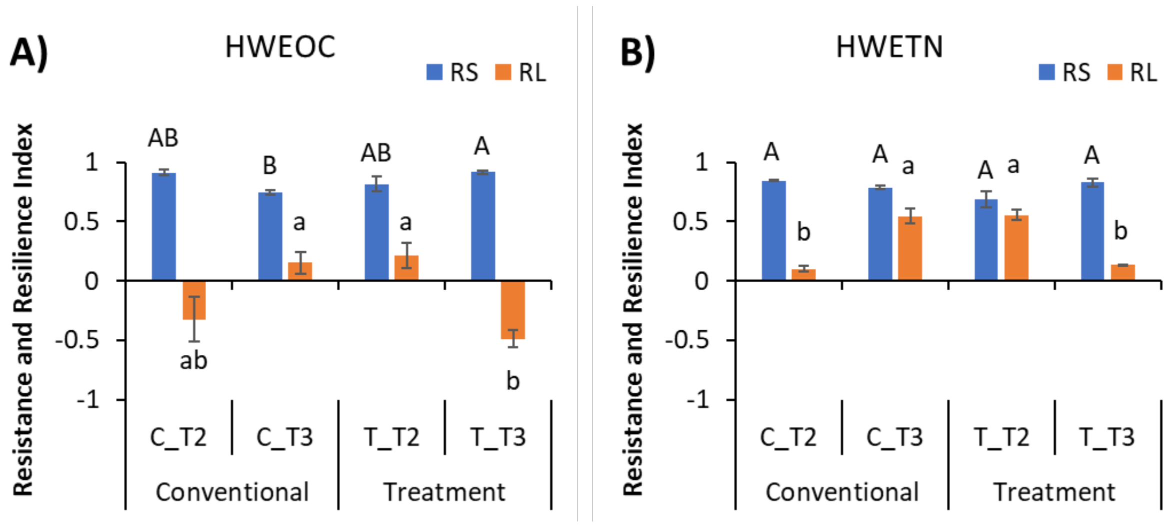


Figure 3.45. The resistance and resilience indices of HWEOC (A) and HWETN (B) between treatments under different management practices in SFL. C = No cover crop, T = Cover crop managed soil. T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

The observed differences in HWEOC and HWETN are due to the distinct impacts of conventional and cover crop management on soil health and microbial activity. Under conventional management, severe water stress likely induces a surge in microbial activity upon rehydration, increasing HWEOC, while cover crops provide continuous organic inputs that stabilise HWEOC levels regardless of stress. The higher overall HWETN under cover crops is attributed to increased organic matter and microbial diversity, which buffer nitrogen dynamics against stress. The resistance and resilience indices reflect the superior soil structure and microbial resilience provided by cover crops, enhancing recovery and stability of HWEOC and HWETN, especially under moderate stress. Conversely, conventional management's less diverse microbial community and lower organic matter content result in less stable nutrient dynamics and a different stress response profile.

3.3.4.3.2 Soil Mineral Nitrogen

No significant difference in total mineral nitrogen content (NH_4^+ and NO_3^-) was observed between the no-stress treatments (C_T1 and T_T1) and the moderate (C_T2 and T_T2) and severe water stress (C_T3 and T_T3) treatments at the end of the resistance phase (Day 14) under both soil management practices (Figure 3.46A). Overall, an increase in total mineral nitrogen content was observed for all treatments under cover crop management compared to the respective treatments under conventional management. By the end of the resilience phase (Day 28), the moderate water stress treatment under both management methods showed the lowest total mineral nitrogen content compared to the severe stress and no stress treatments. Similar to the stress period, after the stress release, cover crop management showed a higher total mineral nitrogen content compared to conventional management (Figure 3.46A). Additionally, the resistance and resilience indices for total mineral nitrogen content did not show a notable difference under cover crop management. However, under conventional management, the severe water stress treatment showed a lower resistance index and a higher resilience index compared to the moderate stress treatment (Figure 3.46B).

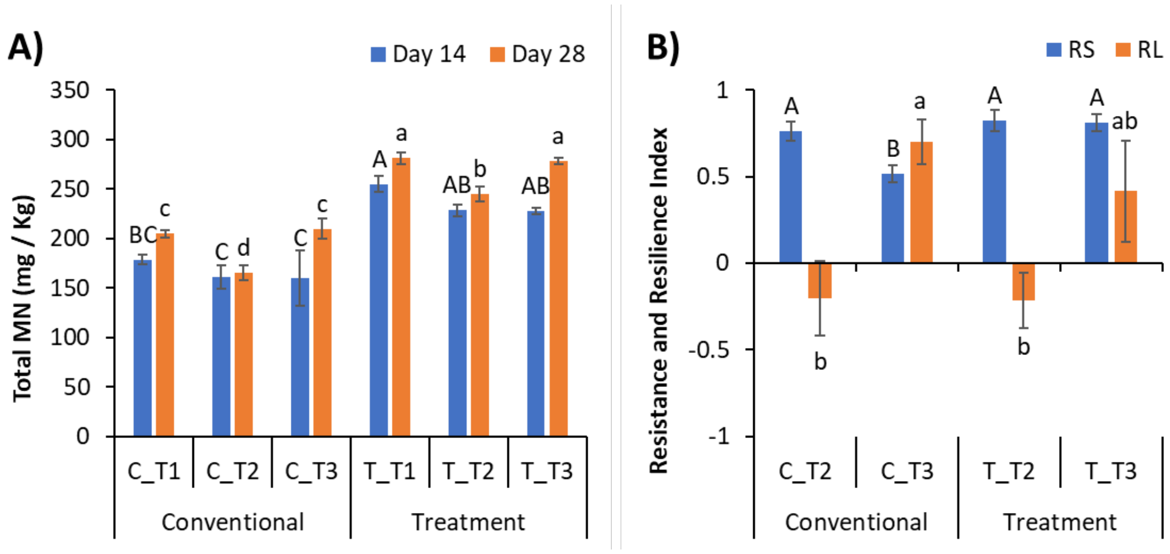


Figure 3.46. Effect of soil management on total mineral nitrogen concentrations (A) and resistance and resilience index for total mineral nitrogen (B) during the incubation period for different water stress treatments in SFL. C = No cover crop, T = Cover crop managed soil. T1 = no stress (35% WFPS), T2 = moderate stress (15% WFPS), T3 = severe stress (7.5% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

The observed differences in total mineral nitrogen content under varying water stress and soil management practices can be attributed to several factors. Cover crop management enhances soil structure and microbial activity, leading to higher nitrogen mineralisation and retention compared to conventional management, which lacks these benefits. During the resistance phase, the balance between mineralisation and immobilisation under moderate and severe stress might prevent significant changes in nitrogen content. By the resilience phase, moderate stress potentially causes more leaching and denitrification upon rewetting, resulting in the lowest nitrogen content. In contrast, cover crops consistently support microbial recovery and nitrogen release from decomposing organic matter, maintaining higher nitrogen levels post-stress. Conventional management shows lower resistance under severe stress due to greater disruption of microbial and plant activity, but higher resilience as microbial communities rebound more significantly when conditions improve.

3.4 Conclusion

In conclusion, the collective findings from investigations into soil responses to stress conditions and diverse management practices underscore the pivotal role of strategic soil management in promoting resilience and nutrient dynamics within ecosystems. The Bednar method emerges as an effective strategy for mitigating the adverse effects of environmental stressors, maintaining stable microbial processes, and enhancing MBC and MBN concentrations, particularly under moderate stress. Similarly, cover crop management demonstrates promise in bolstering soil resilience and microbial functioning, evident through increased resistance and resilience indices, and improved nutrient retention under moisture stress conditions. Moreover, lime treatment shows significant potential in enhancing soil health and resilience to drought stress, offering insights into its capacity to mitigate nitrogen loss and facilitate nutrient cycling. These findings collectively emphasise the importance of sustainable soil management practices in fostering resilient ecosystems amidst climate change challenges and highlight the need for further research to refine and optimise these strategies for long-term environmental conservation and agricultural sustainability.

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CHAPTER 4: IMPACT OF SOIL COMPACTION AND ITS ALLEVIATION ON SOIL MICROBIOME RESILIENCE AND SOIL HEALTH IN AGRICULTURAL SYSTEMS

4.1 Abstract

The study investigated the effects of soil compaction stress and tillage intensity on soil respiration, microbial biomass, and nutrient dynamics. Results from the soil incubation experiment revealed no significant differences in cumulative CO_2 emissions between compaction treatments and controls under both minimum (DiBella) and excessive (Cauchi) tillage management during the resistance phase, indicating that initial soil response to compaction is not heavily influenced by tillage intensity. Following stress removal, minimal tillage exhibited no significant differences until Day 25, whereas severe stress under minimal tillage resulted in lower CO_2 emissions by Day 28. Moderate stress under minimum tillage had the highest respiration resistance index, whereas severe stress under excessive tillage had the lowest. Resilience indices did not significantly differ among treatments in either tillage regime. Substrate-induced respiration showed no significant differences among compaction treatments by the end of the resilience phase, suggesting microbial adaptation over time. Microbial biomass carbon (MBC) and nitrogen (MBN) content varied, with moderate compaction under minimum tillage enhancing MBC, while excessive tillage generally resulted in lower MBC. Microbial metabolic quotient (qCO_2) was higher under excessive tillage due to disrupted soil structure. Hot water extractable organic carbon (HWEOC) and nitrogen (HWETN) content were more affected by compaction in minimum tillage, with better recovery during the resilience phase. Total mineral nitrogen content did not differ significantly between compaction treatments and controls during the resistance phase but was higher in moderate stress treatments under minimum tillage. The study underscores the complex interactions between tillage practices, compaction stress, and soil microbial and nutrient dynamics, highlighting the importance of balanced tillage for soil health.

4.2 Introduction

In agricultural systems, the soil microbiome is a diverse and dynamic community comprising bacteria, fungi, archaea, protozoa, and other microorganisms. These microorganisms play intricate roles in soil ecosystems, influencing nutrient cycling, organic matter decomposition, and plant health. However, compaction stress, stemming from various agricultural practices such as tillage, machinery operations, and livestock trampling, can profoundly influence the intricate web of soil microorganisms, macrofauna, and plant roots that constitute the soil biome (Abdul Rahman et al. 2021; Kabzems and Haeussler 2005). Therefore, compaction stress can profoundly alter the structure and function of the soil microbiome, impacting its resilience and resistance to environmental disturbances. Soil resistance measures soil's ability to withstand (i.e., maintain its functions / immediate response to stress) environmental and anthropogenic pressure (de Vries et al. 2012; Isbell et al. 2015). Soil resilience is the ability to recover soil's functional and structural integrity after disturbance (i.e., recovery potential after stress removal) (Griffiths et al. 2008; Li et al. 2019; Quinlan et al. 2016; Schwalm et al. 2017). Hence, understanding and managing compaction stress is essential for maintaining the functional and structural integrity of soil ecosystems in agricultural landscapes.

Soil compaction will ultimately be influenced on other physical and chemical properties in soil including water content, air or water permeability, and strength (Jung et al. 2010). This compression restricts the movement of air and water within the soil, creating anaerobic conditions in compacted zones. Anaerobic microenvironments inhibit the growth and activity of aerobic microorganisms, such as bacteria and fungi, which rely on oxygen for respiration (Longepierre et al. 2021). Consequently, the abundance and diversity of aerobic microbial communities may decline in compacted soils, leading to disruptions in essential soil processes. Such as organic matter decomposition, nutrient mineralisation, and nitrogen fixation, ultimately affecting plant nutrient availability and productivity (Hartmann et al. 2014; Weisskopf et al. 2010).

Moreover, compaction stress can disrupt soil microaggregates and microbial habitats, affecting microbial biomass and community composition (Ouyang 2016). Microaggregates provide sheltered microenvironments where microorganisms can thrive, but compaction can crush these aggregates, exposing microorganisms to harsher conditions. Additionally, compaction can seal off soil pores, limiting microbial access to organic matter substrates and reducing microbial activity (Mattila and Vihanto 2024). This alteration in habitat structure can lead to shifts in microbial community composition, with potential consequences for nutrient cycling dynamics and soil fertility.

Furthermore, compaction stress influences the rhizosphere, the zone of soil surrounding plant roots where interactions between roots and soil microorganisms occur. Soil compaction restricts root growth and alters root architecture, impacting root exudation patterns and rhizosphere microbial communities (Frene et al. 2024; Wendel et al. 2022). Root exudates, such as sugars, organic acids, and amino acids, serve as energy sources for soil microbes and influence microbial community composition and activity (Ma et al. 2022; Wen et al. 2022). Therefore, compaction-induced changes in root exudation can alter the competitive dynamics between beneficial and pathogenic microorganisms in the rhizosphere, potentially affecting plant health and nutrient uptake. In summary, the intricate interactions between compaction stress and the soil microbiome have profound implications for soil health and agricultural productivity. Understanding these dynamics is essential for developing management practices that promote soil resilience and resistance to compaction stress, thereby sustaining the functionality of soil ecosystems in agricultural landscapes.

To explore these complex interactions further, we have conducted an experiment to investigate how the application and subsequent release of compaction stress affect the resistance and resilience of soil chemical and biological properties. This study aims to provide a deeper understanding of how temporary compaction influences the soil microbiome and its associated processes. By systematically applying and then alleviating compaction stress, we can observe the immediate impacts on microbial community structure, nutrient cycling, and soil physical properties. Through detailed analysis of soil samples, we assess changes in microbial biomass, and functional potential, as well as alterations in soil chemical attributes such as soil organic carbon and nutrient availability. The insights gained from this experiment are crucial for developing agricultural management strategies that mitigate the adverse effects of soil compaction, enhancing soil health, and promoting sustainable agricultural productivity.

4.3 Methodology

4.3.1 Sampling Site and Soil Preparation

Soil samples were collected from two agricultural sites with different management histories, including varied tillage practices, at Herbert Cane Productivity Services Ltd (HCPSL) (Table 4.1). The soil type belongs to a silty loam texture, with an acidic pH ranging between 3.78 to 4.09, and a clay content ranging between 10.81 to 12.75 (Table 4.1). The collected soil samples were passed through a 2.0 mm stainless steel sieve to remove impurities and then stored at 4°C until analysis. To restore microbial activity, the cooled samples were incubated at 25°C for two weeks.

Table 4.1. Management history and soil properties between sites

Description	Site 1 (Cauchi Fallow – CF)	Site 2 (DiBella 0135A 1-1)
Management history	This block has been excessively tilled, no stubble conservation practices (aligns with a traditional sugarcane farming system (Conventional management)	This block has been under controlled traffic, minimum tillage, a large mixed fallow cover crop during the fallow period and all cane stubbles retained (Ecosystem-based management)
Soil texture	Silty loam	Silty loam
Sand (%)	31.15	20.70
Silt (%)	58.04	66.55
Clay (%)	10.81	12.75
Soil pH	3.78	4.09
Electrical Conductivity	53.00	65.33

4.3.2 Arranging Soil in 96-well Plates

We investigated soil biological resistance and resilience under two different soil management practices using 96-well deep-well plates. The use of 96-well deep-well plates allows for simultaneous testing of multiple soil samples, enabling the analysis of a broad range of conditions and treatments efficiently. Furthermore, the standardised well format ensures precise measurements and facilitates the replication of experiments, thereby increasing the reliability of results. Deep-well plates provide controlled conditions, including soil moisture levels and nutrient availability, allowing researchers to simulate compaction stress under controlled laboratory settings. Using a filling device, soil samples were carefully added to the deep-wells as per the pre-prepared layout (Figure 4.1). At each instance of soil addition, the weight of both the empty plate and the plate filled with soil was recorded. This data collection is crucial for calculating the average weight of the soil present in each well, which is required for subsequent steps such as applying different levels of compaction stress.

01	Control (T1)		Medium Stress (T2)		Severe Stress (T3)		Control (T1)		Medium Stress (T2)		Severe Stress (T3)	
	1	2	3	4	5	6	7	8	9	10	11	12
	R	R	R	R	R	R	R	R	R	R	R	R
A	T1_1	T1_9	T2_1	T2_9	T3_1	T3_9	T1_1	T1_9	T2_1	T2_9	T3_1	T3_9
B	T1_2	T1_10	T2_2	T2_10	T3_2	T3_10	T1_2	T1_10	T2_2	T2_10	T3_2	T3_10
C	T1_3	T1_11	T2_3	T2_11	T3_3	T3_11	T1_3	T1_11	T2_3	T2_11	T3_3	T3_11
D	T1_4	T1_12	T2_4	T2_12	T3_4	T3_12	T1_4	T1_12	T2_4	T2_12	T3_4	T3_12
E	T1_5	T1_13	T2_5	T2_13	T3_5	T3_13	T1_5	T1_13	T2_5	T2_13	T3_5	T3_13
F	T1_6	T1_14	T2_6	T2_14	T3_6	T3_14	T1_6	T1_14	T2_6	T2_14	T3_6	T3_14
G	T1_7	T1_15	T2_7	T2_15	T3_7	T3_15	T1_7	T1_15	T2_7	T2_15	T3_7	T3_15
H	T1_8	T1_16	T2_8	T2_16	T3_8	T3_16	T1_8	T1_16	T2_8	T2_16	T3_8	T3_16

Figure 4.1. Layout of experimental units and positioning of stress treatments in a 96-Well Deep-Well plate for soil respiration measurement.
Green shading = soil from conventional management (excessive tillage), orange shading = soil from ecosystem-based management practice (minimum tillage). A-H = Rows in the 96-well plate, 1-12 = Columns in the 96-well plate, T1= control, T2= medium stress, T3=severe stress. R= Respiration.

4.3.3 Imposing Compaction Stress Treatments

To establish different compaction levels for our experiment, we maintained three distinct conditions to achieve control (no stress), moderate, and severe levels of stress in two management practices (Table 4.2). The compaction stress was applied with the help of a compaction device which was developed based on the configuration of the 96-well deep-well plates (Figure 4.2).

Table 4.2. Different levels of compaction stress treatments

Treatment	Compaction Stress
T1	Control (0.9 g/cm^3)
T2	Medium compaction (1.1 g/cm^3)
T3	Severe compaction (1.2 g/cm^3)

The impact of soil compaction was assessed by first calculating the soil height (H) in the deep-well after compression using the following formula:

H (mm) = [(W/BD) * (4.2/1.2)] * 10

Where, W is the average weight of the soil present in the well (g), BD is the anticipated bulk density (gcm^{-3}), 4.2 is the height of the well (cm), and 1.2 is the volume of the well (cm^3).

Subsequently, the nail depth inside the plate was adjusted using a screw mechanism (Figure 4.2) The required millimetres (mm) to be lifted were calculated by considering the initial nail depth, measured to be 29 mm when fully inserted into the well, and subtracting the calculated value of H from this depth. This subtraction provided specific mm to be lifted. Finally, the nail bar (Figure 4.2) was pressed using the handle to apply different levels of compaction stress, ensuring proper adjustment based on the calculated height to achieve consistent and accurate compaction levels.

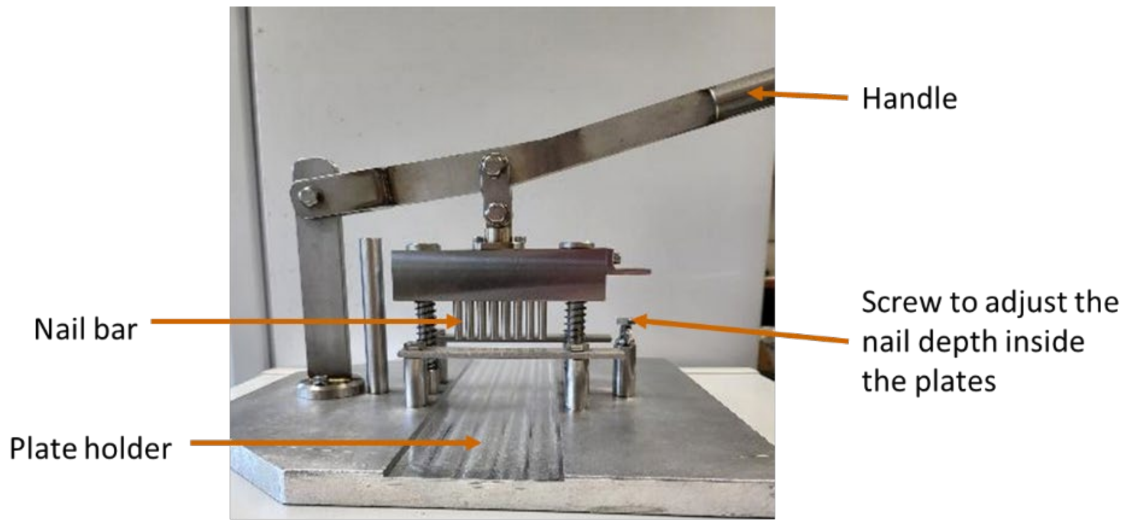


Figure 4.2. Compaction device design for apply different levels of compaction stresses in 96-Well deep-well plates.

4.3.4 Assessing Resistance and Resilience

A 96-well deep-well plate subjected to compaction stress treatments was placed in a sealed container along with a beaker of soda lime and water. The soda lime absorbed CO_2 produced by soil microbes during incubation, maintaining a controlled environment and preventing measurement interference. The water in the beaker ensured consistent soil moisture, essential for accurate research results. The containers were incubated at 25°C for 14 days. After this period, half of the 96-well plates were removed and stored at 4°C for up to two weeks for further analysis, marking the end of the resistance phase. In the remaining plates, drought stress was alleviated by loosening the soil, and incubation continued for another 14 days. After the full 28-day incubation period, soil samples were extracted from the plates for analysis, representing the end of the resilience phase. Daily respiration measurements were recorded using a microplate reader throughout the incubation period. A separate set of plates was maintained specifically for the respiration measurements.

4.3.5 Analysis of Soil Biological Properties

4.3.5.1 Soil Respiration Measurements

We measured microbial respiration using the MicroResp™ technique, which assesses short-term changes in soil respiration in response to various external factors, such as stress treatments and management practices (Campbell et al. 2003). This technique has been effectively used to detect post-disturbance changes in soil functional capacity (Gartzia-Bengoetxea et al. 2016) and to assess microbial functional recovery in environmental plantings (Amarasinghe et al. 2021). To measure the CO_2 produced during respiration calorimetrically, we employed an indicator-gel medium to capture the emitted CO_2. This colorimetric method relies on pH changes in a bicarbonate solution in quasi-equilibrium with the headspace of the MicroResp wells. Detection plates were prepared by combining an indicator dye, cresol red (12.5 ppm, wt/wt), potassium chloride (150 mM), and sodium bicarbonate (2.5 mM) in 150 µl of 1% Noble agar per well. The agar and indicator solutions were prepared separately and combined at a 1:2 ratio (agar: indicator) before use. These plates were stored in sealed plastic boxes with wet paper towels or a beaker of water and soda lime to prevent desiccation and reaction with atmospheric CO_2 until they were used. To calculate respiration, we measured the colour change of the detection plate as absorbance at 570 nm immediately before and after the incubation using a microplate reader (Burton et al. 2010; Campbell et al. 2003). Microbial respiration was monitored over 28 days, covering both the resistance and resilience phases. Detection plates were replaced before saturation, and initial absorbance was measured each time a new detection plate was used.

4.3.5.2 Substrate-induced Respiration

Carbon and amino acid substrate solutions were prepared based on their water solubility. After preparation, the required volume of each solution was filter-sterilised and stored in sterile tubes at 4°C. At the start of the experiment, as well as at the end of the resistance and resilience phases, 30 µl of each prepared substrate were added to the soil in the deep-well plates. Basal respiration was measured by adding 30 µl of sterilised water. Carbon utilisation was determined using the same method as for respiration measurement.

4.3.5.3 Microbial Biomass

Soil MBC and MBN contents were measured using the fumigation-extraction method with an EC conversion factor of 2.64 (Vance et al. 1987) and an En conversion factor of 2.22 (Jenkinson 1988) as described by (Liu et al. 2018). Briefly, four replicates of 4 g of each treatment were fumigated with ethanol-free chloroform for 24 hours at 25°C in a sealed desiccator in the dark at the beginning, and end of the resistance and resilience phases. The non-fumigated subsamples of 4 g of each soil were stored at 4°C during this period. The following day, after removing the beaker of chloroform, the desiccator was evacuated four times to remove the chloroform from the soils. Both fumigated and non-fumigated subsamples were extracted with freshly prepared 0.5 M K_2SO_4 at a 1:4 ratio and filtered. The filtrate was analysed using a Shimadzu TOC-VCSH/CSN total organic carbon and nitrogen analyser (Shimadzu Scientific Instruments, Japan) to calculate the concentrations of dissolved organic carbon and total nitrogen in fumigated and unfumigated samples.

4.3.6 Analysis of Soil Chemical Properties

The concentration of HWEOC and HWETN in the samples was determined following the method described by Chen et al. (2000). Briefly, 4.0 g of fresh soil was combined with 40 mL of water in a capped falcon tube and incubated at 70°C for 18 hours. The tubes were then agitated on an end-over-end shaker for 15 minutes and filtered through Whatman 42 filter paper (Whatman Ltd., Maidstone, UK). The filtrates were analysed for concentrations of extractable organic carbon and total nitrogen using a SHIMADZU TOC-VCPH TOC/N analyzer (Shimadzu, Kyoto, Japan). Soil mineral nitrogen was extracted with 2M KCl at a 1:4 ratio of soil to extractant using an end-over-end shaker, followed by filtration through Whatman 42 filter paper after one hour of shaking (Rayment and Lyons 2011). Concentrations of ammonium nitrogen ($NH4^{+}$-N) and nitrate nitrogen (NO^{3-}-N) were measured using a SEAL AA3 Continuous Segmented Flow Analyzer (SEAL Analytical Limited, USA). All results were expressed on an oven-dry basis.

4.3.7 Calculating Resistance and Resilience

The resistance (RS) and resilience (RL) indexes are calculated using the following formula (Orwin and Wardle 2004):

$$\text{Resistance (RS)} = 1 - [(2 \times |D_{rs}|) / (C_{rs} + |D_{rs}|)]$$

$$\text{Resilience (RL)} = [(2 \times |D_{rs}|) / (|D_{rs}| + |D_{rl}|)] - 1$$

Where, D_{rs} is the absolute difference between soil response (i.e., respiration) value in the control treatment (C_{rs}) and the stressed treatment at the end of the RS phase. D_{rl} is the difference between soil response (i.e., respiration) value in the control treatment and the stressed treatment at the end of the RL phase. The values of the RS and RL indices range from -1 to +1. A value of +1 signifies maximum RS (no impact of stress) or RL (complete recovery from stress), while lower values indicate reduced RS or RL of soil microbial biomass and respiration in response to drought stress. Negative values of the RS index indicate a change greater than 100% in the response variable compared to the control condition. If the absolute value of D_{rl} exceeds that of D_{rs}, the RL index will be negative. Mathematically, the RL index for the control condition is nearly +1, but it is considered as +1 in the RL equation.

4.3.8 Data Analysis

Differences in soil chemical and biological properties between the compaction stress treatments under conventional management practices (excessive tillage) and ecosystem-based management practice (minimum tillage) were analysed using R (RCoreTeam 2023). Differences between patterns of microbial metabolic function profiles were tested with permutational analysis of variance (PERMANOVA) and visualised using multidimensional scaling (MDS) of bootstrap averages. Monte Carlo P–values were used to detect significant differences among management practices. MDS and PERMANOVA were run in PRIMER v7 with PERMANOVA+ (Amarasinghe et al. 2021; Strickland et al. 2017).

4.4 Results and Discussion

4.4.1 Soil Biological and Chemical Properties at the Initial Stage

The application of minimum tillage practices (DiBella) resulted in a higher MBC content compared to soil managed with excessive tillage (Cauchi) (Figure 4.3A). However, there was no significant difference in MBN content between excessive and minimum tillage management practices (Figure 4.3A). The microbial qCO_2 was lower under minimum tillage management than under excessive tillage management, though this difference was not statistically significant (Figure 4.3B). HWEOC increased under minimum tillage practices compared to excessive tillage management. Conversely, HWETN content did not show a notable difference between the two management practices (Figure 4.3C). Within the soil mineral nitrogen pool, NH_4^+-N was significantly higher under excessive tillage management, while NO_3^--N was higher under minimum tillage management (Figure 4.3D). However, the total mineral nitrogen content did not differ between the two tillage management systems.

The observed differences between minimum and excessive tillage practices can be attributed to several factors. Minimum tillage tends to maintain better soil structure and aggregation, enhancing MBC due to stable habitats and improved organic matter retention. It also retains more surface residue, providing continuous organic matter for microbial growth, and better soil moisture, creating a favourable environment for microbial activity and carbon storage. Conversely, excessive tillage disrupts soil structure, accelerates organic matter decomposition, and leads to rapid soil drying, reducing MBC.

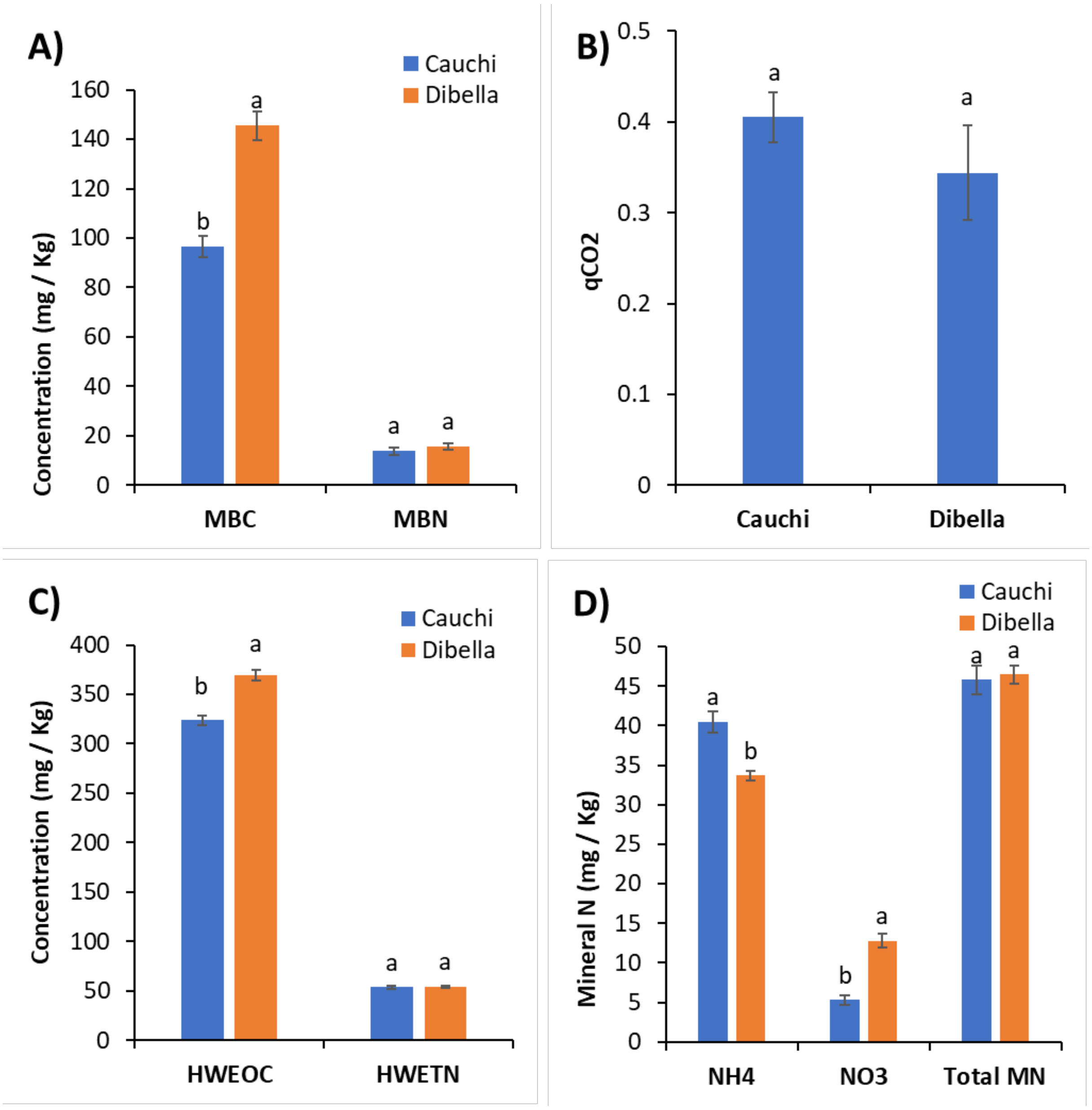


Figure 4.3. Soil biological properties; MBC and MBN (A), and microbial qCO_2 (B), and chemical properties; HWEOC and HWEON concentration (C) and soil mineral nitrogen pool (D) at the initial stage under two management practices. Cauchi = Excessive tillage practices, DiBella = Minimum tillage practices. Vertical bars represent standard error. In each soil parameter, the means with the same letter did not differ at the 5% level of significance.

The lower microbial qCO_2 under minimum tillage suggests more efficient microbial use of carbon, while excessive tillage stresses microbial communities, increasing their metabolic rates. The increase in HWEOC under minimum tillage is likely due to the preservation of labile organic compounds, whereas excessive tillage accelerates their decomposition. Regarding soil mineral nitrogen, NH4+-N is higher under excessive tillage due to enhanced mineralisation, while NO3--N is higher under minimum tillage, possibly due to slower conversion processes. Despite these differences, total mineral nitrogen content remains similar between the two tillage practices, indicating that overall nitrogen availability is unaffected by the tillage method.

4.4.2 The Effect of Moisture Stress on Soil Biological Properties

4.4.2.1 Cumulative CO_2 Emission

The results of the soil incubation study indicate that cumulative CO_2 emissions did not significantly differ between compaction stress treatments (C_T2, C_T3, D_T2, and D_T3) and their respective controls (C_T1 and D_T1) under both minimum (DiBella) and excessive (Cauchi) tillage management during the resistance phase. This suggests that the soil's initial response to compaction stress is not strongly influenced by the level of tillage management. After the compaction stress was lifted at 14 days, excessive tillage management continued to show no significant differences between treatments, indicating a consistent response to stress removal. In contrast, under minimum tillage, the trend of no significant difference persisted until Day 25. By the end of the resilience phase (Day 28), however, the severe stress treatment under minimum tillage exhibited lower cumulative CO_2 emissions compared to both the control and medium stress, suggesting that severe compaction stress may have a longer-term impact on soil microbial activity or soil structure, which affects CO_2 emissions.

The moderate stress treatment (D_T2) under minimum tillage management exhibited the highest respiration resistance index, compared to the severe stress treatment (D_T3) as well as both the medium (C_T2) and severe stress treatments (C_T3) under excessive tillage management (Figure 4.5). The severe stress treatment under excessive tillage management showed the lowest resistance index. The resilience index did not significantly differ between stress treatments under excessive tillage management. However, under minimum tillage management, the severe stress treatment exhibited a higher resilience index compared to the moderate stress treatment.

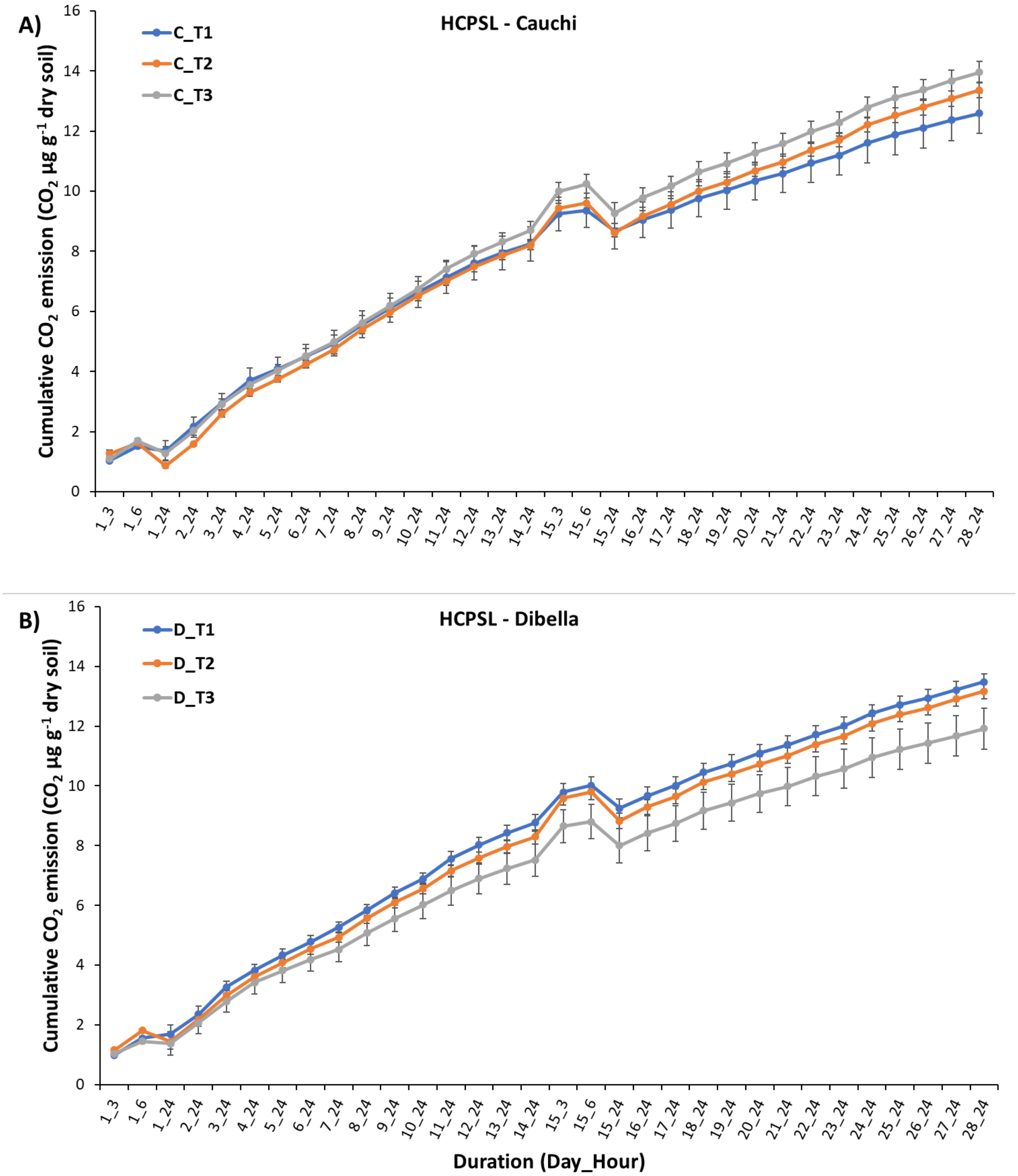


Figure 4.4. Effect of moisture stresses on cumulative CO_2 emissions (CO_2 µg g^{-1} dry soil) in conventionally managed soil; excessive tillage, no stubble management (A) and improved soil management; minimum tillage, stubble management (B) in HCPSL. C = conventionally managed soil, D = Improved soil management, T1 = no stress (BD = 0.9 gcm^{-3}), T2 = moderate stress (BD = 1.1 gcm^{-3}), T3 = severe stress (BD = 1.2 gcm^{-3}). Error bars represent standard error.

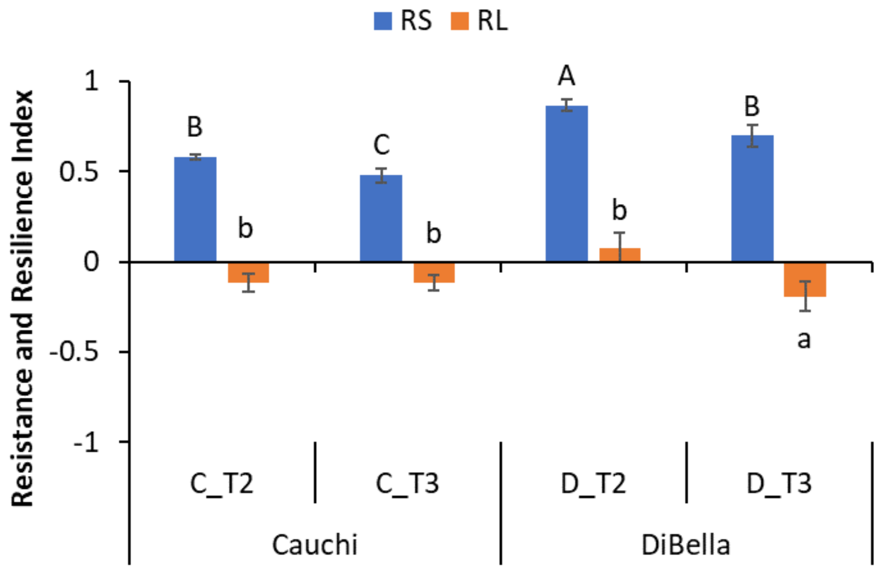


Figure 4.5. Resistance (RS) and Resilience (RL) indices of microbial respiration under moisture stress levels in conventionally managed soil and soil managed with cover crops in in HCPSL. RS = Resistance index, RL = Resilience index, T2 = moderate stress (BD = 1.1 gcm^{-3}), T3 = severe stress (BD = 1.2 gcm^{-3}). Cauchi = excessive tillage. DiBella = minimum tillage. Error bars represent standard error. Means of the treatments the same capital letter is not different at the 95% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 95% level of significance for the resilience.

The observed differences in the respiration resistance and resilience indices can be attributed to the impacts of tillage intensity and compaction stress on soil structure and microbial activity. In minimum tillage management, the soil structure remains relatively undisturbed, allowing for a stable microbial environment, which likely explains the high respiration resistance index under moderate stress (D_T2). In contrast, excessive tillage disrupts soil structure and reduces microbial habitat stability, leading to lower resistance indices, especially under severe stress (C_T3). The severe stress treatment under excessive tillage shows the lowest resistance index due to compounded negative effects on soil aeration and microbial activity. While the resilience index does not vary significantly among stress treatments under excessive tillage, the higher resilience index for severe stress under minimum tillage suggests that less disturbed soils can recover more effectively from severe stress, maintaining microbial activity and soil health.

4.4.2.2 Substrate-induced Respiration

The measurement of substrate-induced respiration using raffinose and trehalose revealed no significant differences among the compaction stress treatments within each management practice by the end of the resistance and resilience phases (Figure 4.6, Table 4.3). Notably, the difference between drought stress treatments and the control (no-stress) decreased by the end of the resilience phase compared to the end of the resistance phase under both tillage management practices, except for the moderate stress treatment in the minimum tillage management approach (Table 4.3). However, the resistance and resilience indices for substrate utilisation did not significantly differ among the stress treatments under both excessive and minimum tillage management approaches (Figure 4.7).

The observed results in substrate-induced respiration using raffinose and trehalose can be attributed to several factors. Soil microorganisms may adapt to stress conditions over time, reducing differences in respiration rates among compaction stress treatments by the end of the resilience phase. Shifts in microbial community structure and composition can lead to similar substrate utilisation across different stress treatments. Tillage practices impact soil structure, aeration, and moisture retention, influencing microbial activity, but excessive tillage may disrupt microbial habitats. The specific nature of raffinose and trehalose utilisation might remain stable if the relevant microbial community is resilient. The intensity and duration of stress, soil organic matter, and nutrient availability also play crucial roles.

Table 4.3. Pairwise tests from PERMANOVA on Euclidean distance of substrate induced respiration data of microbial population in response to water stress treatments.

Groups	RS		RL	
	Distance	P(MC)	Distance	P(MC)
C_T1, C_T2	0.77	0.16	0.45	0.41
C_T1, C_T3	0.71	0.24	0.38	0.67
D_T1, D_T2	0.44	0.74	0.49	0.24
D_T1, D_T3	0.55	0.79	0.44	0.25

The shorter the distance, the higher similarity between treatments. C = Cauchi (Severe tillage management), D = DiBella (Minimum tillage management), T1 = no stress (BD = 0.9 gcm^{-3}), T2 = moderate stress (BD = 1.1 gcm^{-3}), T3 = severe stress (BD = 1.2 gcm^{-3}).

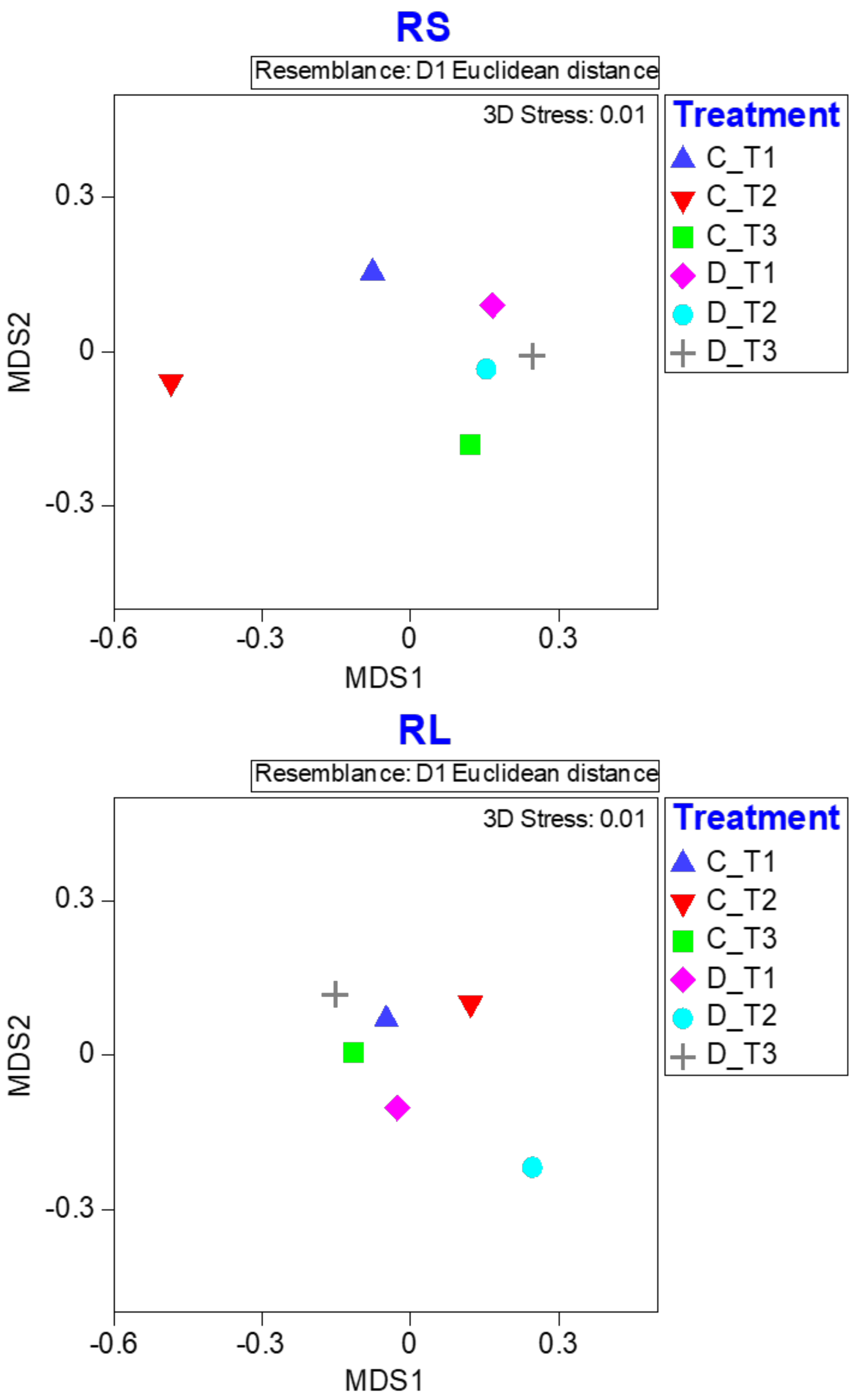


Figure 4.6. Ordinations of bootstrap averages of substrate induced respiration data under compaction stress treatments for excessive and minimum tillage management history. Two-dimensional metric multi-dimensional scaling ordinations (mMDS) of 50 bootstrap sample averages for each treatment and the empirical approximate 95% confidence region, based on Euclidean distance. The distance between the points corresponds to the functional diversity of the microbes in particular treatment: the shorter the distance, the higher similarity between the treatments. C = Cauchi (Severe tillage management), D = DiBella (Minimum tillage management), T1 = no stress (BD = 0.9 gcm^{-3}), T2 = moderate stress (BD = 1.1 gcm^{-3}), T3 = severe stress (BD = 1.2 gcm^{-3}).

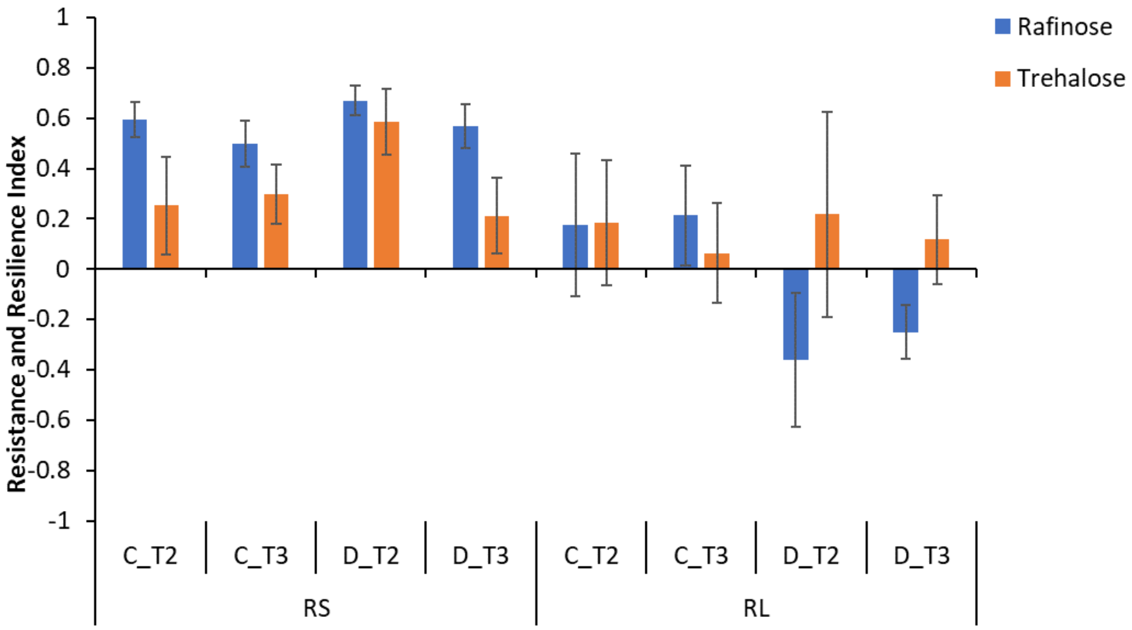


Figure 4.7. Resistance (RS) and Resilience (RL) indices of microbial substrate induced respiration under moisture stress levels in excessive tillage management history and minimum tillage management history in HCPSL. RS = Resistance index, RL = Resilience index, C = Cauchi (Severe tillage management), D = DiBella (Minimum tillage management), T2 = moderate stress (BD = 1.1 gcm^{-3}), T3 = severe stress (BD = 1.2 gcm^{-3}). Error bars represent standard error.

4.4.2.3 Soil Microbial Biomass Carbon and Nitrogen

At the end of the resistance phase, MBC content was significantly higher in the moderate compaction stress treatment (D_T3) compared to the control (D_T1) and severe stress treatment (D_T2) under the minimum tillage management approach and across all treatments under the severe tillage management approach (C_T1, C_T2, and C_T3) (Figure 4.8A). Additionally, under severe tillage management, all treatments exhibited the lowest MBC content, with no significant differences observed between the moderate (C_T2) and severe stress (C_T3) treatments and the control (C_T1) (Figure 4.8A). However, at the end of the resilience phase, there were no notable differences in MBC content between the treatments under both management approaches, although the control and stress treatments under minimum tillage treatments showed higher MBC values (Figure 4.8A).

At the end of the resistance phase, MBN content was higher in the control (C_T1 and D_T1) and moderate stress (C_T2 and D_T2) treatments compared to the severe stress treatments for both excessive and minimum tillage approaches (Figure 4.8B). This trend persisted under the excessive tillage management approach at the end of the resilience phase, with the control and moderate stress treatments showing the highest MBN contents, while the severe stress treatment had the lowest. However, under the minimum tillage management approach, the moderate stress treatment exhibited the lowest MBN content compared to the control and severe stress treatments (Figure 4.8B). The resistance and resilience indices of MBC (Figure 4.9A) and MBN (Figure 4.9B) under moderate and severe compaction stress treatments did not exhibit significant differences across both tillage management histories.

The observed differences in MBC and MBN content can be attributed to several factors. Moderate compaction under minimum tillage likely enhances soil structure, improving water retention and aeration, which promotes microbial growth, while also increasing organic matter accessibility without the detrimental effects of severe compaction. Minimum tillage reduces soil disturbance, preserving soil aggregates and organic matter, creating a more stable environment for microbes. Conversely, severe tillage disrupts soil structure, increases organic matter decomposition, and enhances soil erosion, leading to the lowest MBC content across all treatments. Over time, microbial communities may adapt to these conditions, stabilising MBC content during the resilience phase. Higher MBN content in control and moderate stress treatments is likely due to optimal nutrient conditions, balanced soil moisture, and aeration, supporting greater microbial activity. Severe stress treatments likely limit nutrients and create anaerobic conditions, reducing microbial activity and nitrogen mineralisation. Additionally, under minimum tillage, the reduced disturbance allows organic matter to accumulate, providing a steady carbon source for microbes, leading to higher MBC values, while unexpected low MBN content in the moderate stress treatment during the resilience phase could result from a temporary imbalance in nitrogen cycling or a shift in microbial community composition.

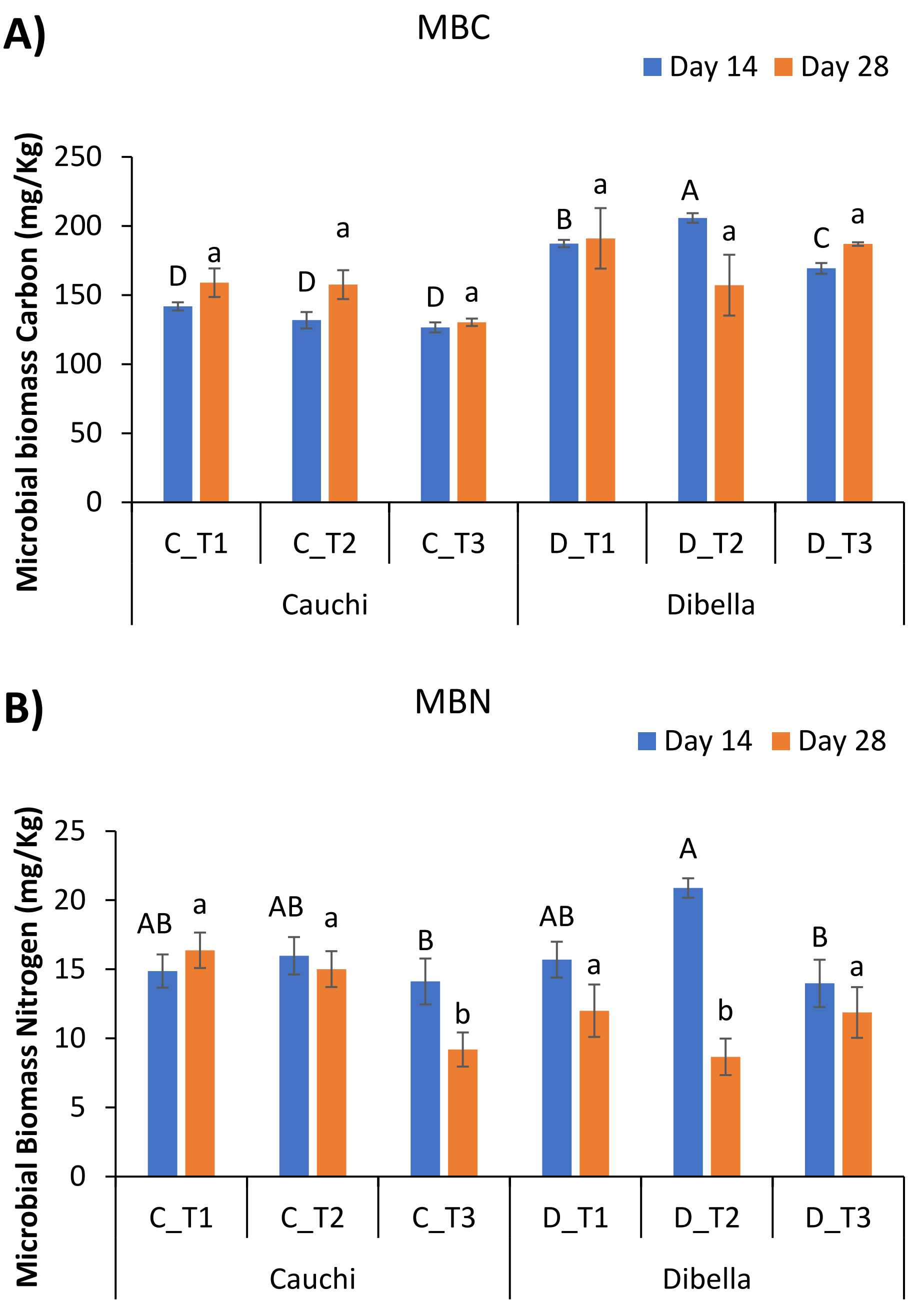


Figure 4.8. Effect of soil management on MBC (A) and MBN (B) concentrations during the incubation period for different water stress treatments in HCPSL. C = Cauchi (Severe tillage management), D = DiBella (Minimum tillage management), T1 = no stress (BD = 0.9 gcm^{-3}), T2 = moderate stress (BD = 1.1 gcm^{-3}), T3 = severe stress (BD = 1.2 gcm^{-3}). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

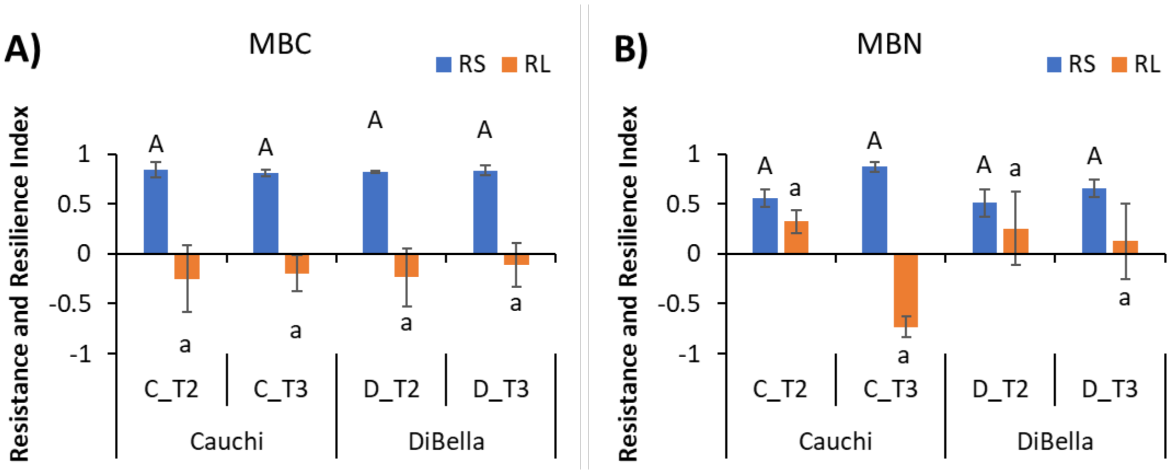


Figure 4.9. The resistance and resilience indices of MBC (A) and MBN (B) between treatments under different management practices in HCPSL. C = Cauchi (Severe tillage management), D = DiBella (Minimum tillage management), T2 = moderate stress (BD = 1.1 gcm^{-3}), T3 = severe stress (BD = 1.2 gcm^{-3}). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

4.4.2.4 Microbial Metabolic Quotient

At the end of the resistance phase, the microbial qCO_2 did not significantly differ between the medium (C_T2 and D_T2) and severe (C_T3 and D_T3) compaction stress treatments and the control treatment (C_T1 and D_T1) under both excessive tillage (Cauchi) and minimum tillage (DiBella) management histories (Figure 4.10A). However, qCO2 values for the compaction treatments under excessive tillage were significantly higher than those under minimum tillage. By the end of the resilience phase, qCO2 showed no significant difference between the control and compaction stress treatments for either tillage management history. Notably, the severe compaction stress treatment under minimum tillage had a lower qCO2 compared to the same treatment under excessive tillage (Figure 4.10A). Despite these observations, the resistance and resilience indices for the compaction stress treatments did not show significant differences between the two tillage management histories (Figure 4.10B).

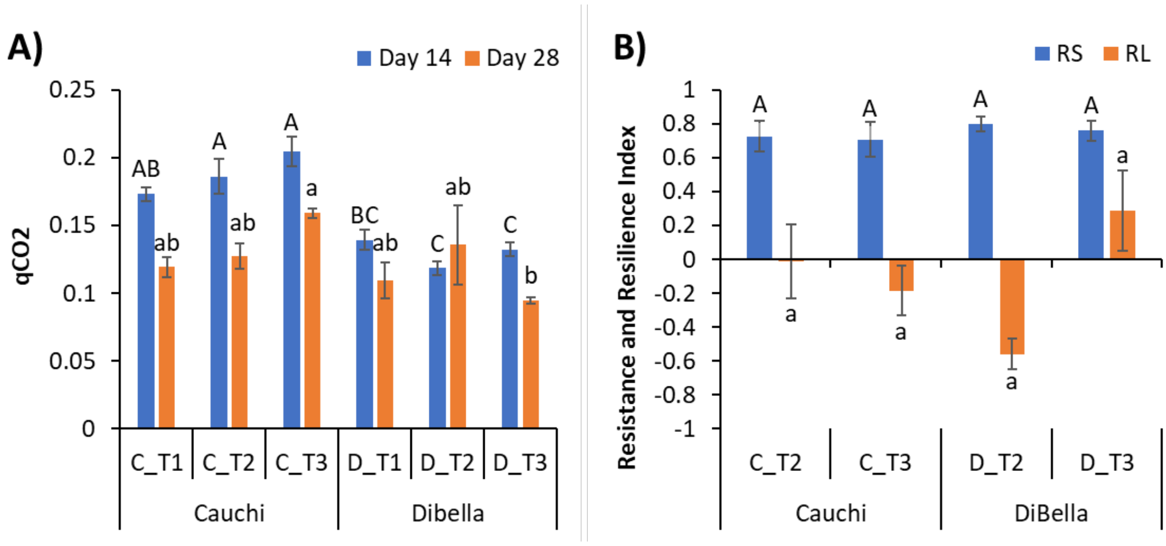


Figure 4.10. Effect of soil management microbial qCO_2 (A) and resistance and resilience index of qCO_2 (B) during the incubation period for different compaction stress treatments in HCPSL. C = Cauchi (Severe tillage management), D = DiBella (Minimum tillage management), T1 = no stress (BD = 0.9 gcm^{-3}), T2 = moderate stress (BD = 1.1 gcm^{-3}), T3 = severe stress (BD = 1.2 gcm^{-3}). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

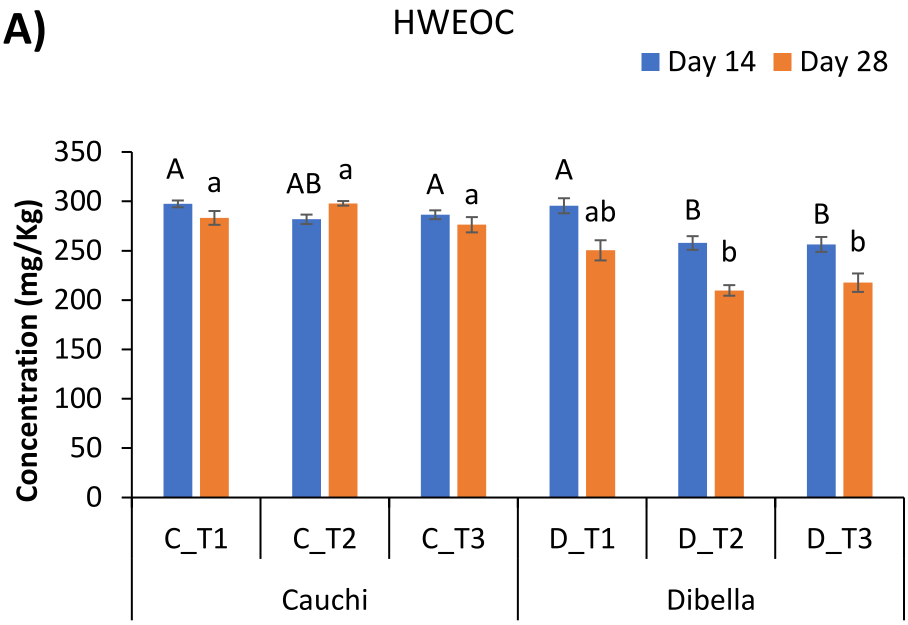
The observed results can be attributed to several underlying factors related to soil biology and physical structure influenced by different tillage practices. Excessive tillage disrupts soil structure, reducing aggregate stability and porosity, which can lead to increased soil compaction under stress, limiting oxygen availability and resulting in higher qCO_2 as microbes expend more energy to survive. In contrast, minimum tillage maintains better soil structure and porosity, mitigating the effects of compaction stress and leading to lower qCO_2 values. Additionally, excessive tillage alters microbial community composition, favouring stress-tolerant species with higher metabolic rates, while minimum tillage supports a diverse microbial community that is more efficient and resilient. The preservation of soil organic matter and better nutrient retention under minimum tillage further enhance microbial efficiency, contributing to lower qCO_2. The severe compaction stress under minimum tillage shows a lower qCO_2 due to better initial soil structure and higher organic matter content, helping microbes cope more effectively compared to excessive tillage. These complex interactions between soil management practices, microbial activity, and soil physical properties explain the differences in qCO_2 and the resistance and resilience indices under different tillage histories.

4.4.3 The Effect of Moisture Stress on Soil Chemical Properties

4.4.3.1 Hot Water Extractable Organic Carbon and Nitrogen

The HWEOC content did not show a notable difference between the medium (C_T2) and severe (C_T3) compaction stress treatments compared to the control (C_T1) treatment under excessive tillage management history at the end of both the resistance and resilience phases (Figure 4.11A). In contrast, under minimum tillage management history, HWEOC content decreased in the medium (D_T2) and severe (D_T3) compaction stress treatments compared to the control (D_T1) at the end of the resistance phase (Figure 4.11A). However, by the end of the resilience phase, there was no notable difference in HWEOC content between the stress treatments under minimum tillage management history (Figure 4.11A).

The HWETN did not display significant differences between stress treatments at the end of the resistance phase, regardless of whether the soil had a history of excessive or minimum tillage (Figure 4.11B). Notably, there was an increase in HWETN content under control and medium compaction stress treatments within the minimum tillage management approach, compared to the compaction stress treatments under the excessive tillage management approach (Figure 4.11B). By the end of the resilience phase, the severe compaction stress treatment resulted in the lowest HWETN content when compared to the control and medium compaction stress treatments within the excessive tillage management approach. In contrast, the minimum tillage management history showed no significant difference in HWETN content between the treatments (Figure 4.11B). Additionally, the resistance and resilience indices of HWEOC and HWETN for moderate and severe compaction stress did not show significant differences across the two tillage management histories (Figure 4.12 A and B).



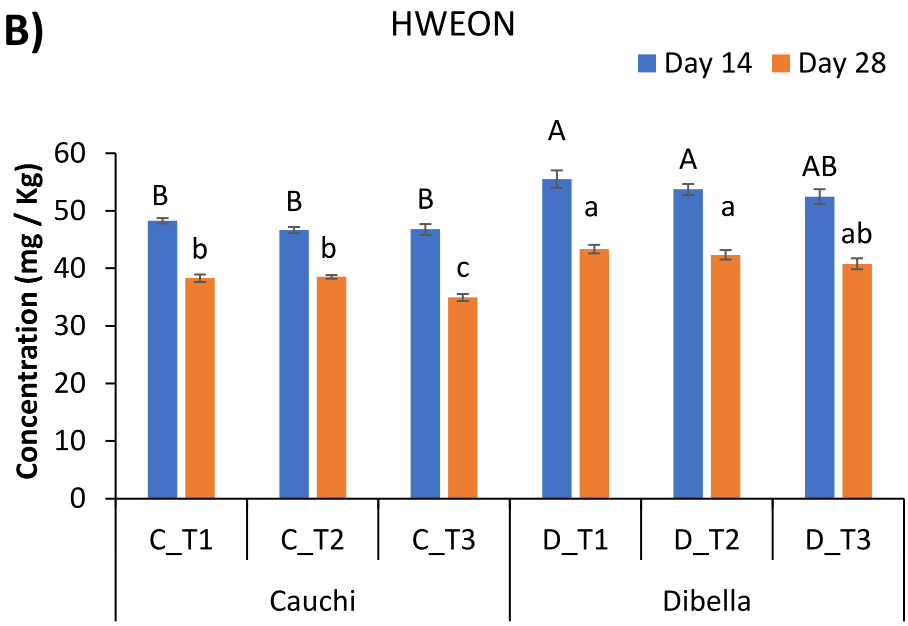


Figure 4.11. Effect of soil management on HWEOC (A) and HWEON (B) concentrations during the incubation period for different compaction stress treatments in HCPSL. C = Cauchi (Severe tillage management), D = DiBella (Minimum tillage management), T1 = no stress (BD = 0.9 gcm^{-3}), T2 = moderate stress (BD = 1.1 gcm^{-3}), T3 = severe stress (BD = 1.2 gcm^{-3}). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

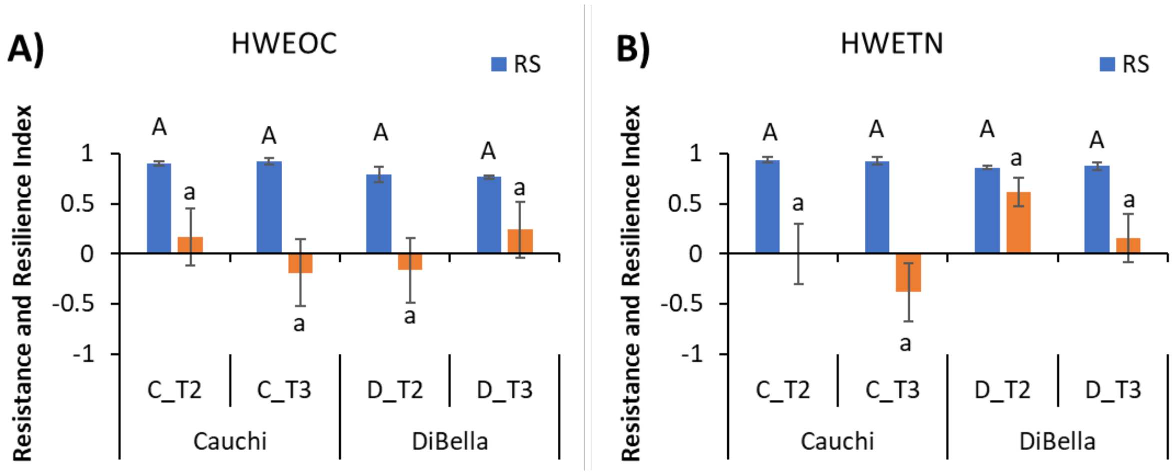


Figure 4.12. The resistance and resilience indices of HWEOC (A) and HWETN (B) between treatments under different management practices in HCPSL. C = Cauchi (Severe tillage management), D = DiBella (Minimum tillage management), T2 = moderate stress (BD = 1.1 gcm^{-3}), T3 = severe stress (BD = 1.2 gcm^{3}). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

The observed differences in HWEOC and HWETN content under varying tillage and compaction stress treatments can be attributed to several factors. Soils with a history of excessive tillage may develop a tolerance to compaction due to continuous disturbance, leading to a more homogenised structure and even organic matter distribution, which buffers the impact on HWEOC levels. Conversely, minimum tillage systems, which maintain a more intact soil structure and stratified organic matter, are more vulnerable to disruption by compaction, causing a notable decrease in HWEOC during the resistance phase. However, these soils show better recovery in the resilience phase due to more robust soil microbial activity and organic matter dynamics. For HWETN, the lack of short-term sensitivity to compaction across tillage histories suggests that nitrogen cycling is initially less affected by compaction. The higher HWETN content under minimum tillage in the resistance phase indicates better nitrogen mineralisation and microbial activity in less disturbed soils. In the resilience phase, excessively tilled soils under severe compaction show reduced HWETN, likely due to long-term impacts on root growth and microbial activity, while minimum tillage systems demonstrate better recovery. The similar resistance and resilience indices across tillage histories indicate that, despite initial differences, both systems eventually achieve comparable equilibrium states in terms of HWEOC and HWETN, emphasising the need for balanced tillage practices to enhance soil health and productivity.

4.4.3.2 Soil Mineral Nitrogen

The total mineral nitrogen (NH_4^+ – N and NO_3^- – N) content in soil managed with both excessive (Cauchi) and minimum tillage (DiBella) practices showed no significant differences between compaction stress treatments and the control at the end of the resistance phase (Figure 4.13A). However, the control (D_T1) and moderate compaction stress (D_T2) treatments under minimum tillage had higher total mineral nitrogen content compared to the control (C_T1) and severe compaction stress (C_T3) treatments under excessive tillage (Figure 4.13A). In the resilience phase, the minimum tillage management also showed no notable differences in total mineral nitrogen content between compaction stress treatments and the control. In contrast, under excessive tillage, the severe compaction stress treatment resulted in lower total mineral nitrogen content than the moderate compaction stress treatment (Figure 4.13A). No significant differences were observed in the resistance and resilience indices for total mineral nitrogen content between the moderate and severe stress treatments under both tillage management methods (Figure 4.13B).

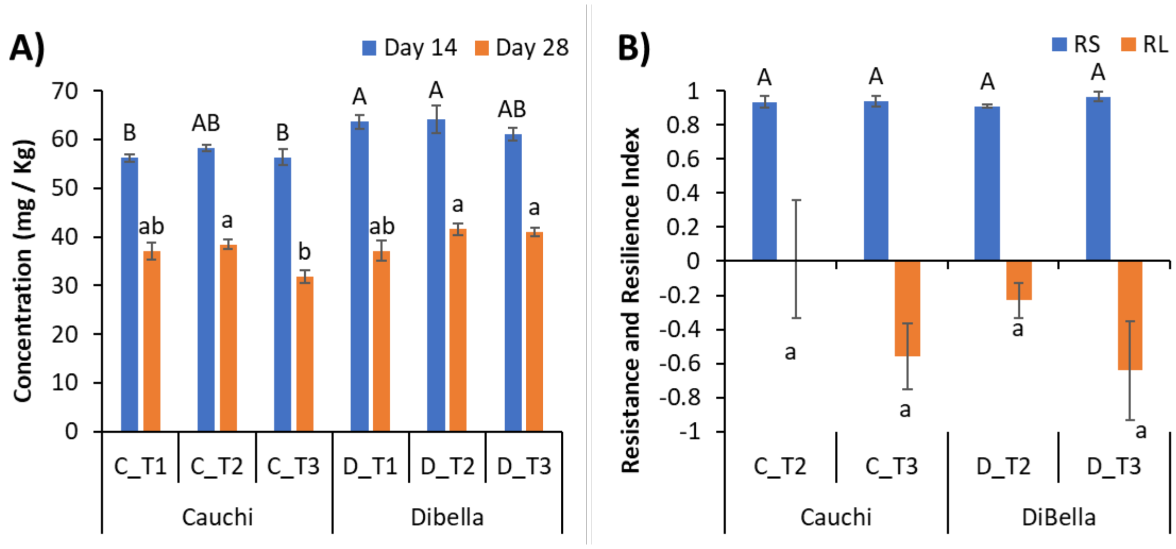


Figure 4.13. Effect of soil management on total mineral N (A) and resistance and resilience index of total mineral N (B) during the incubation period for different compaction stress treatments in HCPSL. C = Cauchi (Severe tillage management), D = DiBella (Minimum tillage management), T1 = no stress (BD = 0.9 gcm^{-3}), T2 = moderate stress (BD = 1.1 gcm^{-3}), T3 = severe stress (BD = 1.2 gcm^{-3}). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

The observed results can be attributed to the different impacts of tillage practices on soil structure and nutrient dynamics. Minimum tillage, which disturbs the soil less, tends to preserve soil structure, enhance microbial activity, and maintain higher organic matter levels. This likely resulted in more stable total mineral nitrogen (NH_4^+ – N and NO_3^- – N) levels across different compaction treatments. In contrast, excessive tillage disrupts soil structure, which can lead to greater nitrogen losses through processes like leaching and denitrification, particularly under severe compaction stress. This disruption is exacerbated under severe compaction stress, leading to significantly lower total mineral nitrogen content compared to moderate stress. The lack of significant differences in the resistance and resilience indices between moderate and severe stress treatments under both tillage methods suggests that while excessive tillage reduces overall nitrogen levels, it does not differentially affect the soil's ability to resist or recover from compaction stress.

4.5 Conclusion

In conclusion, the results of this soil incubation study highlight the complex interplay between compaction stress and tillage management on soil CO_2 emissions, microbial activity, and nutrient dynamics. Despite initial similarities in cumulative CO_2 emissions across stress treatments and tillage practices, the long-term impacts of severe compaction stress under minimum tillage were evident, demonstrating reduced microbial activity and altered soil structure. Moderate compaction under minimum tillage appeared to enhance soil resilience, supporting higher microbial biomass and more efficient nutrient cycling. In contrast, excessive tillage consistently disrupted soil structure and microbial habitats, leading to lower resistance and resilience indices, particularly under severe stress conditions. The study also revealed that MBC and MBN, as well as substrate-induced respiration, adapted over time, reflecting microbial community shifts and soil structure recovery. The findings underscore the importance of adopting balanced tillage practices to maintain soil health, enhance microbial efficiency, and sustain nutrient availability, ultimately promoting sustainable agricultural productivity.

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CHAPTER 5: EVALUATING THE EFFECTS OF DROUGHT AND COMPACTION STRESS ON SOIL RESISTANCE, RESILIENCE, AND PLANT GROWTH: A POT EXPERIMENT

5.1 Abstract

This study explores the impact of various soil management practices on microbial and nutrient dynamics, focusing on microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), respiration, metabolic quotient (qCO_2), enzyme activities, nitrogen content and plant growth under different stress conditions. Soils from diverse management histories, including cover cropping, minimum tillage, conventional tillage, lime treatments, Bednar treatment along with corresponding conventional management approaches were analysed. The results indicate that ecosystem-based practices such as cover cropping and minimum tillage generally enhance microbial resilience, improve nutrient availability, and support better plant growth compared to conventional and excessively tilled soils. Specifically, cover cropping increased microbial biomass and enzyme activity, particularly under moderate stress conditions, while minimum tillage improved soil structure and nutrient retention. Additionally, shoot nitrogen content was consistently higher in cover cropping soils, while conventional soils showed higher shoot carbon content under stress. Lime treatment stabilised soil pH and microbial activity, although its effects varied with stress severity. Contrary to initial assumptions, Bednar treatments did not negatively impact plant productivity, although they showed variable effects on microbial dynamics and enzyme activities. Overall, sustainable soil management practices are crucial for enhancing soil health, microbial efficiency, and crop productivity, demonstrating their role in maintaining resilient and productive agricultural systems.

5.2 Introduction

Drought is one of the most significant environmental stressors, profoundly affecting both soil health and plant growth. The increasing frequency and intensity of drought events, driven by climate change, cause substantial challenges to agricultural productivity and ecosystem stability (Sivakumar 2021; Trenberth et al. 2015). Extended periods of drought can lead to severe soil degradation, characterised by loss of soil moisture, increased erosion, and diminished organic matter content. These changes disrupt the soil's physical structure, hinder microbial activity, and impair nutrient cycling, ultimately reducing the soil's capacity to support healthy plant growth (Ahluwalia et al. 2021; Muller and Bahn 2022).

For plants, drought stress can lead to a series of physical problems. Water scarcity limits the ability of plants to photosynthesise effectively, leading to reduced growth rates, stunted development, and lower yields (Fahad et al. 2017; Kaur et al. 2021). Prolonged drought conditions can also induce oxidative stress in plants, damaging cellular structures and functions, which further compromises plant health and productivity (Blum 2010; Zia et al. 2021). Additionally, drought stress can weaken plants, making them more susceptible to diseases and pests, compounding the negative impacts on crop production and ecosystem services.

In this context, understanding the impacts of drought on soil properties and plant development is critical for developing effective strategies in agricultural and environmental management (Lehmann et al. 2020; Thakur et al. 2021). Research in this area aims to elucidate the mechanisms by which drought affects soil-plant systems, identify soil management practices that enhance resistance and resilience to drought, and develop sustainable agricultural practices that mitigate the adverse effects of water scarcity (Ghadirnezhad Shiade et al. 2023). Such knowledge is essential for ensuring food security, maintaining ecosystem services, and adapting to the challenges posed by a changing climate.

Soil resistance and resilience are key concepts in this field of study, integral to understanding and managing the impacts of environmental stressors on soil health and plant growth. Soil resistance refers to the ability of soil to withstand external pressures without undergoing significant degradation, maintaining its structure, porosity, and nutrient availability under stresses such as drought, compaction, and erosion (de Vries et al. 2012; Isbell et al. 2015). High soil resistance is crucial for sustaining agricultural productivity and ecosystem functions, ensuring that soil can support plant roots, retain water, and provide essential nutrients even under adverse conditions (Seybold et al. 1999; Smith et al. 2022; Wada and Toyota 2007). Conversely, soil resilience is the capacity of soil to recover its functional and structural integrity after experiencing stress, involving the re-establishment of soil structure, restoration of microbial communities, and resumption of nutrient cycling and water retention capabilities (Griffiths et al. 2008; Li et al. 2019; Quinlan et al. 2016; Schwalm et al. 2017). This resilience is vital, particularly in the context of recurring or prolonged stress events like droughts, which can impact soil and plant health over time (Seybold et al. 1999).

In agricultural settings, enhancing soil resistance and resilience can lead to more stable crop yields despite climatic uncertainties. This can be achieved through practices such as crop rotation, cover cropping, reduced tillage, and organic amendments that improve soil structure and foster beneficial microbial communities. In natural ecosystems, these properties contribute to biodiversity conservation, carbon sequestration, and water regulation, supporting overall ecosystem stability and function (Lal 2009, 2016). Understanding and measuring soil resistance and resilience are essential for developing effective soil management strategies to mitigate climate change impacts and other environmental stressors, promoting sustainable land use, and ensuring food security. This knowledge is crucial for policymakers, land managers, and farmers as they adapt to changing environmental conditions and maintain soil health and productivity in the long term.

This study aims to comprehensively investigate the multifaceted effects of drought stress on soil properties and their subsequent impact on plant growth dynamics. By adopting a focused approach on soil resistance and resilience, this research aims to gain insights into how various soil management practices can modulate these critical soil–plant interactions. The experimental design is meticulously crafted to assess not only the immediate repercussions of drought stress on soil health and plant vitality but also the intricate process of soil recovery post-stress alleviation. Through a combination of controlled experimental conditions and rigorous data collection protocols, this study aims to elucidate the underlying mechanisms governing soil–plant responses to drought stress and unveil potential strategies for enhancing soil resilience and mitigating the adverse impacts of drought on agricultural ecosystems. By bridging the gap between theoretical understanding and practical implications, this research endeavours to provide actionable insights that can inform sustainable soil management practices and contribute to the development of climate-resilient agricultural systems.

The pot experiment constitutes a methodically designed investigation wherein soil samples are subjected to controlled drought conditions, simulating real-world stress scenarios, and facilitating a precise examination of soil-plant responses. By concurrently comparing soils managed with distinct management practices, ranging from traditional to innovative soil conservation techniques, the research aims to differentiate the differential impacts of these practices on soil resistance and resilience. This comparative analysis serves as a pivotal tool in elucidating the efficacy of different management strategies in bolstering soil and plant recovery post-stress imposition. Furthermore, by extrapolating the findings from this experimental framework, the study aims to provide valuable insights and evidence-based recommendations for the development and implementation of sustainable soil management practices tailored to enhance soil and plant resilience in the face of impending drought scenarios. Ultimately, the main objective is to contribute to the advancement of climate-resilient agricultural systems, ensuring robust performance and productivity in the face of increasingly unpredictable climatic conditions.

Overall, this research addresses a critical need for understanding the complex interactions between drought stress, soil properties, and plant health. By exploring these interrelationships, the study provides valuable insights into how drought conditions alter soil chemistry, structure, and microbiota, which in turn affect plant physiology and growth. The findings will contribute to developing more resilient agricultural systems capable of withstanding and recovering from the increasing pressures of climate change.

5.3 Materials and Methods

5.3.1 Soil Sample Collection

Soil samples were collected from various agricultural sites with different management histories, as outlined in Table 5.1. At each site, paired locations were selected, consisting of conventionally managed areas and those using ecosystem-based management practices, to enable comparative soil analysis. Soil samples were processed by passing them through a 2 mm sieve before being used in the pot experiment.

Table 5.1. Sampling locations and management practices

Location	Site 1 (Conventional)	Site 2 (Ecosystem-based)
Soils for life	No cover crop	Cover crop trial
CWFS	No cover crop	Cover crop trial
Facey Group	No soil disturbance (Bednar)	Bednar treatment
HCPSL Pair 4	This block has been excessively tilled, no stubble conservation practices (aligns with a traditional sugarcane farming system	This block has been under controlled traffic, minimum tillage, a large mixed fallow cover crop during the fallow period and all cane stubbles retained.

Location	Site 1 (Conventional)	Site 2 (Ecosystem-based)
PIRSA Pair 2	No lime added	Lime treated

Soils for Life Trust - Canberra, ACT (SFL); Central West Farming Systems Inc. - Condobolin, NSW (CWFS); Herbert Cane Productivity Services Ltd. - Ingham, QLD (HCPSL); Facey Group Inc. - Wickepin WA (FGp); Department of Primary Industries and Regions in South Australia (PIRSA)

5.3.2 Experiment Design

The study is structured to include multiple treatment groups, each subjected to varying levels of water stress to simulate moderate, and severe drought conditions (Table 5.2). These groups will be paralleled by a control group, which will be maintained under optimal watering conditions throughout the duration of the experiment.

Table 2. Different levels of water stress treatments

Treatment	Drought Stress
T1	Control (55% WFPS)
T2	Moderate drought (30% WFPS)
T3	Severe drought (20% WFPS)

WFPS = Water fill pore space = (GWC × BD) / [1 - (BD / PD)]

Where GWC is gravimetric water content, BD is bulk density and PD is particle density.

These treatments were applied to pots containing soils from two management practices, conventional and ecosystem-based, to assess the influence of these practices on soil and plant responses to drought. This design was created as a 3 x 2 factorial arrangement, with each treatment and soil management practice combination replicated three times to ensure statistical robustness. The experiment included three critical sampling times to capture the dynamic responses of the soil: (1) shortly after seed germination and crop establishment (one month) to establish a baseline, (2) at the end of the resistance phase (two weeks after applying the stress) to evaluate the immediate effects of drought stress, and (3) at the end of the resilience phase to assess recovery (two weeks after releasing the stress). Unrestrictive soil sampling was performed at each sampling time using a small core sampler made from PVC tube. This temporal approach was designed to provide a comprehensive understanding of both the immediate and lasting impacts of drought stress under different management practices.

5.3.3 Initial Seed Sowing and Soil Moisture Management Before Drought Stress Treatments

Before the initiation of drought stress treatments, the experiment was begun with seed sowing to bring the plants to a suitable stage for drought exposure. Each pot, already filled with soil from either conventional or ecosystem-based management practices, was sown with wheat seeds. The sowing was done uniformly across all pots to ensure consistency in seed placement and depth, facilitating uniform germination and early growth across the treatment groups.

Immediately following the sowing, the initial weight of each pot was carefully recorded. This initial weight, representing the soil at field capacity, served as a baseline for maintaining consistent water levels in the early growth phase. All pots, regardless of their future treatment group, were watered regularly to maintain this baseline weight, ensuring optimal moisture conditions for seed germination and early plant development. Variability in plant development prior to the introduction of drought stress was minimised. The pots were watered as needed to compensate for any water loss, with the goal of maintaining the initial recorded weight. This consistent moisture level was sustained until the plants reached a predetermined developmental stage, suitable for the commencement of the drought stress treatments. Regular monitoring and recording of pot weights during this phase were essential to maintain the desired soil moisture levels, thereby ensuring that all plants were subjected to the stress treatments from a similar growth stage and health status. This approach in the early stages of the experiment was crucial for the accuracy and reliability of the results pertaining to plant and soil responses to drought stress under different soil management practices.

5.3.4 Application of Drought Stress Treatments

Once the plants in the experiment reached a growth stage suitable for drought stress exposure, the application of water stress treatments was commenced. These treatments were systematically implemented to create varying levels of drought conditions, classified as control (no stress), moderate stress, and severe stress based on Water Filled Pore Spaces (WFPS). Pot weights to be maintained under each treatment were calculated at the beginning. Pots were weighed regularly, and water was added or withheld to adjust the soil moisture to these specific levels. This weight monitoring accounted for water losses due to both soil evaporation and plant water uptake (Elsalahy et al. 2020). This process involved not just replenishing water lost to evaporation and plant uptake but also carefully controlling the addition to avoid exceeding the target WFPS for each group. By managing the water content in this manner, the desired levels of water stress were accurately imposed and maintained, allowing for a precise assessment of how different water availability levels affected both soil and plant parameters under controlled conditions.

5.3.5 Measuring Soil and Plant Parameters

5.3.5.1 Plant Parameters

Wheat plants were harvested at the end of the pot experiment, and shoots and roots were dried separately. Root and shoot dry matter were recorded after drying at 40°C until a constant weight was achieved. The total carbon and total nitrogen contents of soil samples were measured by dry combustion using a LECO CNS-2000 analyser (LECO Corporation, MI, USA).

5.3.5.2 Soil Parameters

Unrestrictive soil sampling was performed at the beginning, end of the stress period and end of the stress released period. Soil samples were analysed for chemical and biological properties. The concentration of hot water extractable organic carbon (HWEOC) and total nitrogen (HWETN) in the samples was determined following the method outlined by Chen et al. (2000). In summary, 4.0 g of fresh soil was combined with 40 mL of water in a capped falcon-tube and incubated at 70°C for 18 hours. The tubes underwent agitation on an end-over-end shaker for 15 minutes and were then filtered through a Whatman 42 filter paper (Whatman Ltd., Maidstone, UK). The filtrates were analysed for concentrations of extractable organic carbon and total nitrogen using a SHIMADZU TOC-VCPH (Shimazu, Kyoto, Japan) TOCN analyser. Soil mineral nitrogen was extracted with 2M KCl at a 1:4 ratio of soil to extractant using an end-over-end shaker, followed by filtration through a Whatman 42 filter paper (Rayment and Lyons 2011) after one hour of shaking. Concentrations of NH4+-N and NO3−-N were measured using a SEAL AA3 Continuous Segmented Flow Analyzer (SEAL Analytical Limited. USA). All results were expressed on an oven-dry basis.

Soil MBC and MBN contents were measured using the fumigation-extraction method with an Ec conversion factor of 2.64 (Vance et al. 1987) and an En conversion factor of 2.22 (Jenkinson 1988) as described by (Liu et al. 2018). Briefly, four replicates of 4 g of each treatment were fumigated with ethanol-free chloroform for 24 hours at 25 °C in a sealed desiccator in the dark at the beginning, and end of the resistance and resilience phases. The non-fumigated subsamples of 4 g of each soil were stored at 4°C during this period. The following day, after removing the beaker of chloroform, the desiccator was evacuated four times to remove the chloroform from the soils. Both fumigated and non-fumigated subsamples were extracted with freshly prepared 0.5 M K2SO4 at a 1:4 ratio and filtered. The filtrate was analysed using a Shimadzu TOC-VCSH/CSN total organic carbon and nitrogen analyser (Shimadzu Scientific Instruments, Japan) to calculate the concentrations of dissolved organic carbon and total nitrogen in fumigated and unfumigated samples. The MicroResp^{TM} technique was used to determine microbial respiration (Campbell et al. 2003).

To determine soil enzyme activity using fluorescence detection, substrates specific to β-glucosidase (BG), N-acetyl-β-glucosaminidase (NAG), and phosphatase (P) enzymes—4-Methylumbelliferyl β-D-glucopyranoside (MUB-β-D-glucopyranoside), 4-Methylumbelliferyl N-acetyl-β-D-glucosaminide (MUB-NAG), and 4-Methylumbelliferyl phosphate (MUB-P)—were used. Fluorescence intensity was measured using a microplate reader. Background fluorescence from control wells was subtracted from sample fluorescence to calculate enzyme activity in nanomoles of substrate hydrolysed per gram of soil per hour. This method provides insight into the functional capacity of soil microbial communities under different management practices (Black et al. 2011; German et al. 2011; Kuzyakov 2016).

5.3.6 Calculating Resistance and Resilience

The resistance (RS) and resilience (RL) indexes are calculated using the following formular (Orwin and Wardle 2004).

$$\text{Resistance (RS)} = 1 - [(2 \times |D_{rs}|) / (C_{rs} + |D_{rs}|)]$$

$$\text{Resilience (RL)} = [(2 \times |D_{rs}|) / (|D_{rs}| + |D_{rl}|)] - 1$$

Where, D_{rs} is the absolute difference between soil response (i.e., respiration) value in the control treatment (C_{rs}) and the stressed treatment at the end of the RS phase. D_{rl} is the difference between soil response (i.e., respiration) value in the control treatment and the stressed treatment at the end of the RL phase. The values of RS and RL indices range between -1 and +1. The value of +1 indicates the maximum RS (no impact of stress) or RL (complete recovery from the stress), and lower values indicate less RS or RL of soil microbial biomass and respiration to drought stress. Negative values of the RS index indicate a change greater than 100% in the response variable compared with that in the control condition. If the absolute value of D_{rl} becomes higher than the absolute value of D_{rs}, then the RL index will have a negative value. Mathematically, the RL index of control condition will be infinitely close to +1, while it has been considered as +1 in the RL equation.

5.3.7 Data Analysis

Differences in soil chemical and biological properties, as well as plant parameters (total carbon, nitrogen, and dry matter), between the drought stress treatments under conventional management practices and ecosystem-based management practices, were analysed using a two-way analysis of variance (ANOVA). This approach allowed for the evaluation of the main effects of management practice and drought stress level, as well as their interaction effects on the measured variables. Post hoc comparisons were performed using Tukey's Honest Significant Difference (HSD) test to identify specific differences between treatment means. Statistical significance was set at $p < 0.05$ for all tests. The analysis was conducted using the statistical software R (RCoreTeam, 2023). Results were reported as mean ± standard error (SE), and the statistical findings were visualised using appropriate graphical representations, such as bar plots or interaction plots, to illustrate the effects of drought stress and management practices on soil and plant parameters.

5.4 Results and Discussion

5.4.1 Soil Biological and Chemical Properties Just Before Exposing to the Stress Treatments

5.4.1.1 Microbial Biomass Carbon and Nitrogen Content in Soil

One month after seed planting and just before the stress treatments, MBC content showed a significant reduction in soil from the Facey Group (FGp) with a history of Bednar treatment compared to conventional management (Figure 5.1). However, in the Department of Primary Industries and Regions in South Australia (PIRSA), soil treated with lime showed an increase in MBC content compared to soil without lime (Figure 5.1). Additionally, there was no notable difference in MBC between soils with cover crop management history and those under conventional management in the Central West Farming System (CWFS) and Soils for Life (SFL). Similarly, there was no significant difference between soils with excessively tilled, no stubble conservation practices and those with minimum tillage and mixed fallow cover crop management in Herbert Cane Productivity Services Ltd. - Ingham, QLD (HCPSL) (Figure 5.1). MBN content was lower in the FGp soil under the Bednar treatment compared to conventional management. Conversely, in CWFS, cover crop management resulted in a notable increase in MBN content compared to conventional management (Figure 5.1). No significant differences in MBN content were observed between conventional management and the respective treated sites: lime treatment in the PIRSA, cover crop management in SFL, and minimum tillage with mixed fallow cover crop management in HCPSL (Figure 5.1).

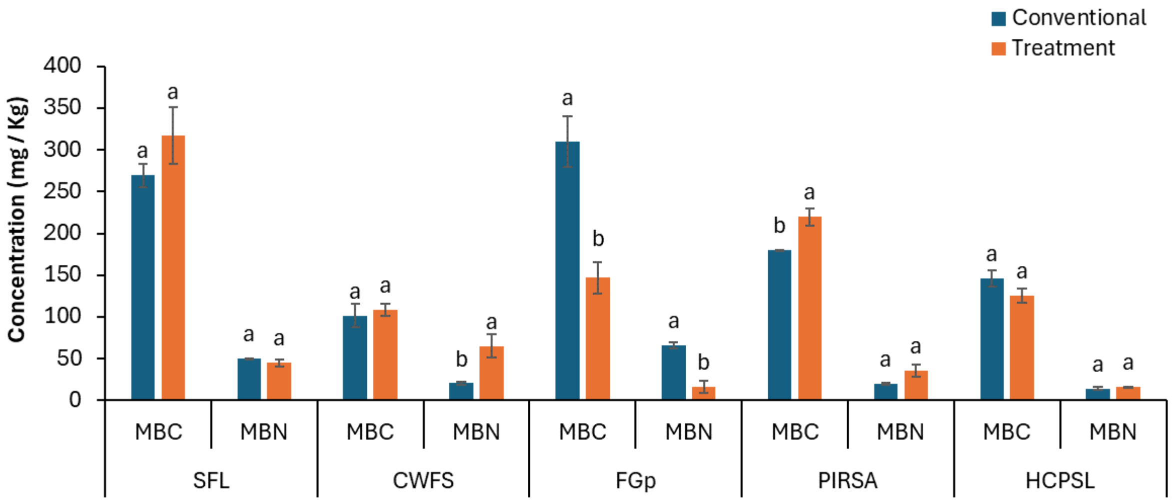

Figure 5.1. MBC and MBN content of soil across varied management histories. Significant differences in MBC and MBN content between two management practices at a 95% confidence level, indicated by different letters in each comparison. SFL - Soils for Life Trust - Canberra, ACT, CWFS - Central West Farming Systems Inc. - Condobolin, NSW, FGp - Facey Group Inc. - Wickepin WA, PIRSA - Department of Primary Industries and Regions in South Australia, HCPSL - Herbert Cane Productivity Services Ltd. - Ingham, QLD.

The observed changes in MBC and MBN content can be attributed to the historical soil management practices applied in the fields from which the soil was collected and the influence of crop growth. The significant reduction in MBC and MBN in the FGp soil under the Bednar treatment compared to conventional management is likely due to past soil disturbance, chemical inputs that disrupt microbial habitats, and the potential impact of crop growth, which can reduce nutrient availability for microbes through uptake and competition. In contrast, the increase in MBC in lime-treated soil in the PIRSA can be explained by improved soil pH, nutrient availability, and more favourable conditions for microbial activity. The notable increase in MBN under cover crop management in the CWFS compared to conventional management suggests that cover crops enhance nitrogen availability and microbial growth. The lack of significant differences in MBC and MBN between conventional management and treated sites in PIRSA, SFL, and HCPSL indicates that similar organic matter inputs and microbial resilience to historical management practices may lead to stable microbial biomass levels across these different systems. Additionally, since moisture levels were maintained consistently across all sites, the differences in MBC and MBN can be more directly attributed to the soil management history and its impact on microbial communities.

5.4.1.2 Microbial Respiration and Metabolic Quotient

Microbial respiration was significantly higher under cover crop management in SFL and CWFS one month after seed planting in the pots (Figure 5.2A). There was no significant difference in microbial respiration between the Bednar treatment history and its control in FGp. Conversely, microbial respiration was significantly lower under lime treatment in PIRSA and under minimum tillage with mixed fallow cover crop management in HCPSL compared to their controls (no lime treatment and excessively tilled, no stubble conservation practices, respectively) (Figure 5.2.A). The microbial qCO_2 showed a notable decrease under cover crop management in SFL and the Bednar treatment in FGp. We did not observe a significant difference in qCO_2 between conventional management history and their respective treatments in the other sites (Figure 5.2 B).

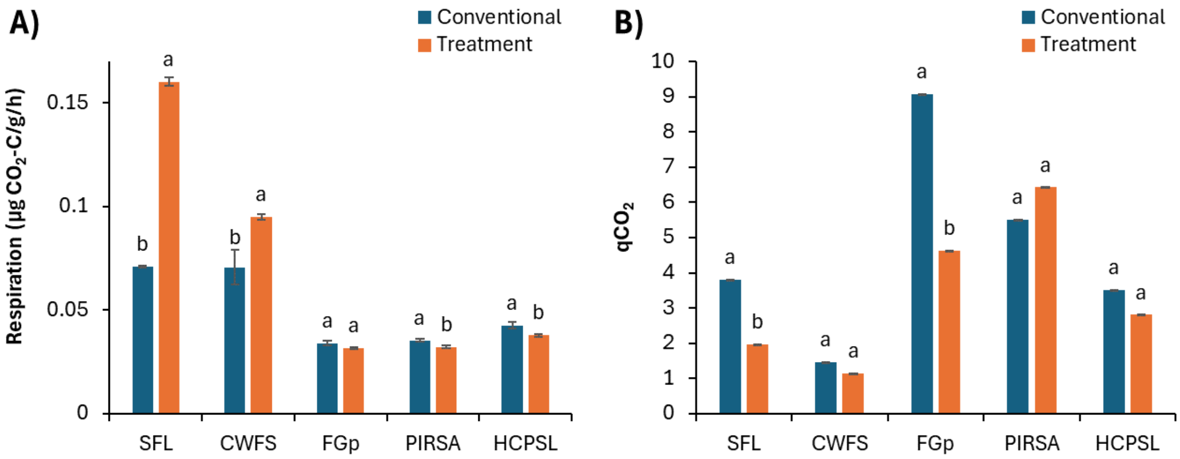

Figure 5.2. Microbial respiration (A) and qCO_2 (B) in soil across varied management histories. Significant differences in MBC and MBN content between two management practices at a 95% confidence level, indicated by different letters in each comparison. SFL - Soils for Life Trust - Canberra, ACT, CWFS - Central West Farming Systems Inc. - Condobolin, NSW, FGp - Facey

Group Inc. - Wickepin WA, PIRSA - Department of Primary Industries and Regions in South Australia, HCPSL - Herbert Cane Productivity Services Ltd. - Ingham, QLD.

The observed differences in microbial respiration and qCO_2 in the pot experiment, where plants were grown for one month, can be attributed to the long-term soil management histories of the collected soils and their influence on microbial activity and efficiency. The higher microbial respiration under cover crop management in SFL and CWFS suggests that long-term cover cropping enhances soil organic matter, leading to increased microbial activity even in the confined pot environment. The lack of difference in microbial respiration between the Bednar treatment and its control indicates that the historical disturbance from the Bednar treatment might not significantly affect microbial activity compared to conventional management within the short experimental timeframe. The lower microbial respiration under lime treatment in the PIRSA could be due to the long-term stabilising effect of lime on soil pH, reducing microbial stress and respiration rates in the pots. Similarly, the lower respiration under minimum tillage with mixed fallow cover crop management in HCPSL compared to excessively tilled soil could result from improved soil structure and reduced microbial disturbance over time. The notable decrease in qCO2 under cover crop management in SFL and Bednar treatment in FGp suggests increased microbial efficiency and a shift towards a more stable and less stressed microbial community within the one-month period. The lack of significant difference in qCO_2 between conventional management and other treatments at the remaining sites suggests that microbial efficiency remains relatively stable across these long-term management practices within the experimental timeframe.

5.4.1.3 Soil Enzyme Activity

Soil enzyme activity analysis revealed no significant differences in β-glucosidase (BG), N-acetyl-β-glucosaminidase (NAG), and phosphatase (P) activities between cover crop management and conventional management histories in SFL (Figure 5.3). Similarly, there were no significant differences in enzyme activities between soil management histories with and without lime application in PIRSA. In the CWFS, β-glucosidase (BG) activity was higher under cover crop management compared to conventional management, while NAG and P activities showed no significant differences. In the FGp, soils under Bednar treatment exhibited lower BG activity compared to conventional management, with no significant differences observed in NAG and P activities. Conversely, in HCPSL, soils under minimum tillage with mixed fallow cover crop management showed higher NAG and P activities compared to excessively tilled soils, with no significant difference in BG activity between the two management practices (Figure 5.3).

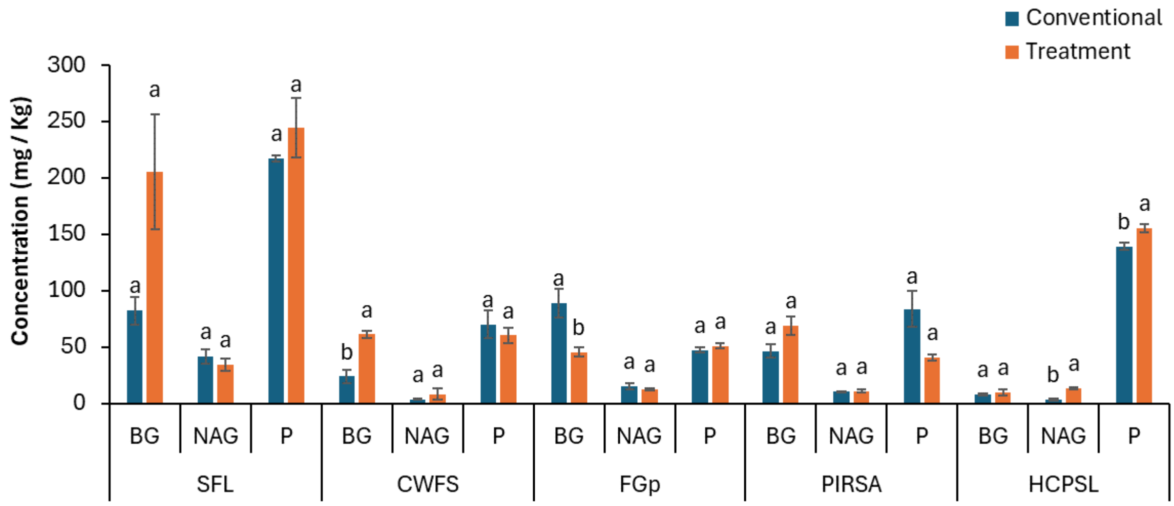

Figure 5.3. Enzyme activity in soil across varied management histories. Significant differences in MBC and MBN content between two management practices at a 95% confidence level, indicated by different letters in each comparison. SFL - Soils for Life Trust - Canberra, ACT, CWFS - Central West Farming Systems Inc. - Condobolin, NSW, FGp - Facey Group Inc. - Wickepin WA, PIRSA - Department of Primary Industries and Regions in South Australia, HCPSL - Herbert Cane Productivity Services Ltd. - Ingham, QLD. BG - β-glucosidase, NAG - N-acetyl-β-glucosaminidase, and P - phosphatase

The observed patterns in soil enzyme activities reflect the complex interactions between long-term soil management practices and microbial communities. The lack of significant differences in β-glucosidase (BG), N-acetyl-β-glucosaminidase (NAG), and phosphatase (P) activities between cover crop and conventional management histories in SFL suggest that both management approaches may similarly support enzyme-mediated nutrient cycling in soil. Similarly, the comparable enzyme activities between lime-applied and non-lime-applied soils in PIRSA indicate that other factors besides soil pH may predominantly influence enzyme dynamics in these soils. In the CWFS, higher BG activity under cover crop management may be attributed to increased organic inputs promoting enzymatic degradation, while consistent NAG and P activities across management types suggest stable microbial processes. Lower BG activity under Bednar treatment in the FGp could be due to previous disturbances affecting substrate availability, whereas higher NAG and P activities under minimum tillage with mixed fallow cover crop management in HCPSL likely result from enhanced soil structure and organic matter retention fostering enzyme production and activity. These findings underscore the nuanced impacts of diverse management practices on soil microbial enzyme dynamics critical for nutrient cycling in agricultural soils.

5.4.1.4 Hot Water Extractable Organic Carbon and Nitrogen

In the pot trial one month after seed planting, HWEOC analysis revealed lower values in soils under cover crop management compared to conventional management in SFL. Conversely, soils under minimum tillage with mixed fallow cover crop management in HCPSL exhibited higher HWEOC values compared to excessively tilled soils. No significant differences in HWEOC were observed in soils under cover crop management in the CWFS, Bednar treatment in the FGp, or lime treatment history in PIRSA (Figure 5.4).

Regarding HWETN, no significant differences were found between soils under cover crop management and conventional management in SFL and CWFS. Similarly, there were no differences between soils with and without lime treatment in PIRSA. Lower HWETN values were observed under Bednar treatment compared to conventional management in FGp. In HCPSL, soils under minimum tillage with mixed fallow cover crop management showed higher HWETN values compared to excessively tilled soils (Figure 5.4).

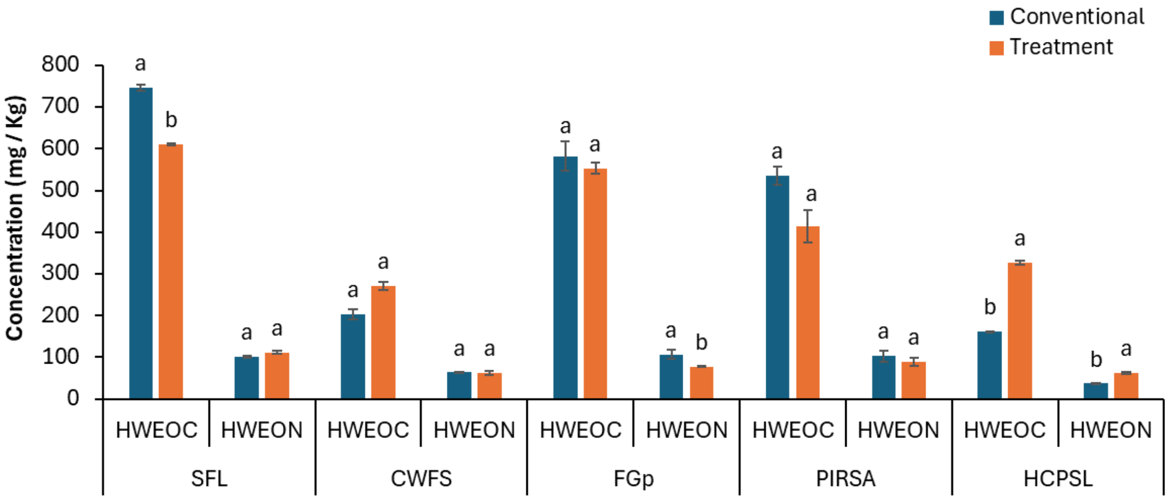

Figure 5.4. HWEOC and HWETN contents in soil across varied management histories. Significant differences in MBC and MBN content between two management practices at a 95% confidence level, indicated by different letters in each comparison. SFL - Soils for Life Trust - Canberra, ACT, CWFS - Central West Farming Systems Inc. - Condobolin, NSW, FGp - Facey Group Inc. - Wickepin WA, PIRSA - Department of Primary Industries and Regions in South Australia, HCPSL - Herbert Cane Productivity Services Ltd. - Ingham, QLD.

The observed differences in HWEOC and HWETN content among different soil management histories in the pot trial reflect the varying impacts of long-term agricultural practices on soil organic matter dynamics. The lower HWEOC values under cover crop management in SFL may be attributed to increased organic matter decomposition and nutrient uptake by cover crops, leading to reduced carbon availability in the soil. Conversely, higher HWEOC values under minimum tillage with mixed fallow cover crop management in HCPSL suggest enhanced carbon preservation and accumulation due to reduced soil disturbance and improved organic matter retention. The lack of significant differences in HWEOC among soils under cover crop management in the CWFS, Bednar treatment in the FGp, and lime treatment history in PIRSA indicates similar levels of organic carbon across these management histories. Regarding HWETN, similar nitrogen availability between cover crop and conventional management in SFL and CWFS suggests comparable nitrogen cycling rates under these practices, while higher HWETN values under minimum tillage with mixed fallow cover crop management in HCPSL may result from enhanced nitrogen retention and reduced leaching compared to excessively tilled soils. These findings underscore how different agricultural practices can influence soil carbon and nitrogen dynamics, crucial for soil fertility and ecosystem functioning.

5.4.1.5 Soil Mineral Nitrogen

Soil mineral nitrogen dynamics one month after wheat seed planting in pots varied significantly across soil collected from different long-term management histories. In SFL, soils under cover crop management exhibited lower NH_4^+ levels, higher NO_3^- concentrations, and overall higher total mineral nitrogen content compared to conventional management (Figure 5.5). In the CWFS, cover crop management showed no significant difference in NH_4^+ and total mineral nitrogen content but lower NO_3^- concentrations compared to conventional management. The FGp soils treated with Bednar methods displayed lower NH_4^+, NO_3^-, and total mineral nitrogen compared to conventionally managed soils (Figure 5.5). Conversely, in PIRSA, soils treated with lime showed no significant differences in NH_4^+, NO_3^-, or total mineral nitrogen content compared to soils without lime treatment (Figure 5.5). In HCPSL, soils under minimum tillage with mixed fallow cover crop management exhibited higher NH_4^+ and total mineral nitrogen content but showed no difference in NO_3^- compared to soils under excessively tilled conditions (Figure 5.5).

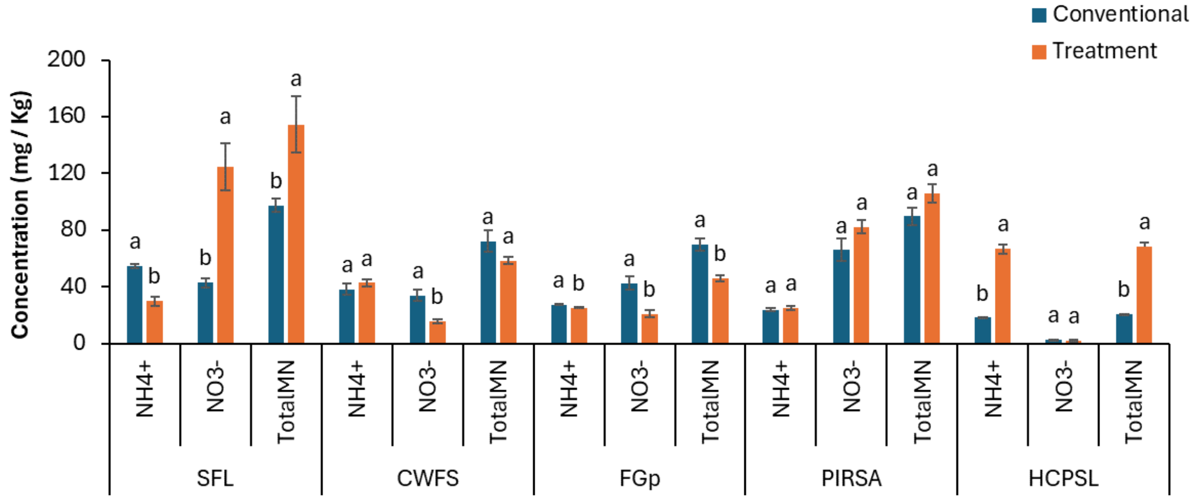

Figure 5.5. Mineral nitrogen content (NH_4^+, NO_3^- and total mineral nitrogen (MN)) in soil across varied management histories. Significant differences in MBC and MBN content between two management practices at a 95% confidence level, indicated by different letters in each comparison. SFL - Soils for Life Trust - Canberra, ACT, CWFS - Central West Farming Systems Inc. - Condobolin, NSW, FGp - Facey Group Inc. - Wickepin WA, PIRSA - Department of Primary Industries and Regions in South Australia, HCPSL - Herbert Cane Productivity Services Ltd. - Ingham, QLD.

The observed variations in soil mineral nitrogen dynamics across different management histories can be explained by various agricultural practices influencing nitrogen availability and transformations in soil. In SFL, the lower NH_4^+ levels under cover crop management may stem from sustained microbial activity and nutrient uptake by cover crops over time, enhancing nitrogen transformation dynamics favouring NO_3^- accumulation. Similarly, in the CWFS, prolonged cover crop management likely promoted nitrogen immobilisation processes, contributing to lower NO_3^- levels compared to conventional management, which may have higher rates of nitrogen mineralisation. In the FGp, the persistent application of Bednar treatments may have altered soil nitrogen cycling dynamics, leading to reduced NH_4^+, NO_3^-, and total mineral nitrogen content. Lime treatment in PIRSA, applied over an extended period, might have stabilised soil pH, thereby maintaining consistent NH_4^+ and NO_3^- levels despite treatment differences. In HCPSL, the long-term adoption of minimum tillage with mixed fallow cover crop management likely enhanced soil nitrogen retention and availability, explaining the higher NH_4^+ and total mineral nitrogen content observed compared to excessively tilled soils, while NO_3^- levels remained similar. These insights underscore how prolonged management practices shape soil nitrogen dynamics and nutrient availability crucial for crop productivity in agricultural systems.

5.4.2 The Effect of Moisture Stress on Soil Biological Properties

5.4.2.1 Soil Microbial Biomass Carbon Content

One-month-old wheat plants in pots were exposed to different water stress treatments (no stress, moderate stress, and severe stress) for 14 days. At the end of the 14-day period, MBC content in soil varied across different management practices. In soils from SFL, there were no significant differences in MBC between stress treatments and the control in both cover crop and conventional management histories (Figure 5.6A). In the CWFS, moderate stress under cover crop management resulted in significantly lower MBC compared to no stress, though there was no difference with severe stress; severe stress did not differ significantly from no stress. Under conventional management in CWFS, moderate stress led to higher MBC compared to both no stress and severe stress treatments (Figure 5.6B). For the FGp, there were no significant differences in MBC between stress treatments and the control in either Bednar treatment or conventional management histories (Figure 5.6C). Similarly, in PIRSA, no significant differences in MBC were observed between stress treatments and the control for both lime-treated and non-lime-treated soils (Figure 5.6D). However, in HCPSL, while excessively tilled soils showed no significant differences in MBC between stress treatments, soils under minimum tillage with mixed fallow cover crop management exhibited higher MBC under severe stress compared to both no stress and moderate stress treatments, with no difference between the latter two (Figure 5.6E).

After wheat plants were exposed to water stress for 14 days, followed by a stress release period where water was added, allowing the plants to remain under no stress conditions for another 14 days, the MBC content in soil varied across different management practices. In soils from SFL, there were no significant differences in MBC between stress treatments and the control in either cover crop or conventional management histories (Figure 5.6.A). In the CWFS, both moderate and severe stress treatments under cover crop management resulted in lower MBC compared to no stress, with severe stress showing the lowest MBC content. There were no significant differences in MBC between stress treatments and the no stress treatment under conventional management; however, MBC values under conventional management were lower than those with moderate and no stress conditions under cover crop management (Figure 5.6B). In the FGp, there were no significant differences in MBC between stress treatments and the control in either Bednar treatment or conventional management histories, though overall, Bednar treatment showed lower MBC values (Figure 5.6.C). In PIRSA, there were no significant differences in MBC between stress treatments and the control in soils with no lime treatment history. Under lime treatment, severe stress resulted in lower MBC compared to the control, but moderate stress did not show a difference compared to no stress or severe stress treatments (Figure 5.6D). In HCPSL, moderate stress treatment resulted in higher MBC compared to both no stress and severe stress treatments under excessively tilled history, while soils under minimum tillage with mixed fallow cover crop management showed no significant differences in MBC between stress treatments and the no stress treatment (Figure 5.6E).

In SFL, the severe stress treatment under conventional management history exhibited the lowest resistance index compared to moderate stress under cover crop management history (Figure 5.7A). In contrast, in the CWFS, moderate stress under conventional management history showed the lowest resistance index compared to moderate and severe stress under cover crop management history, as well as severe stress under conventional management history (Figure 5.7B). No significant differences were observed in resilience indices between stress treatments under conventional and cover crop management histories in either SFL or CWFS (Figure 5.7 A and B). The resistance and resilience indices of MBC for the moderate and severe stress treatments under conventional and different management approaches in the FGp (FGp, Bednar treatment), PIRSA (PIRSA, lime treatment), and HCPSL (HCPSL, minimum tillage with mixed fallow cover crop management) did not show notable differences (Figure 5.7 C, D and E).

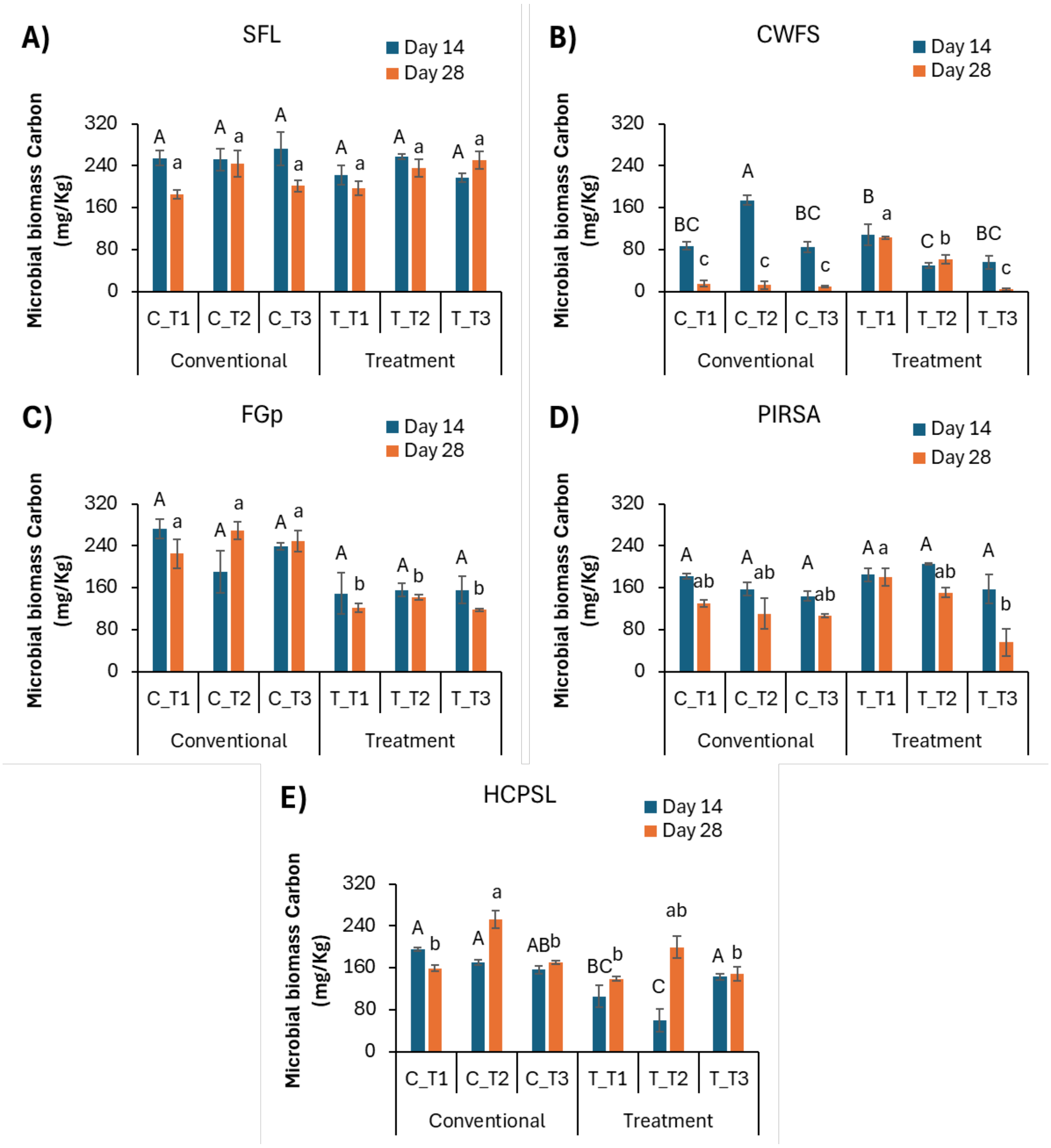

Figure 5.6. Microbial biomass carbon content in soil across varied management histories under water stress treatments: (A) SFL (B) CWFS (C) FGp (D) PIRSA (E) HCPSL. C = conventionally managed soil, T = Cover crop management in SFL and CWFS, Bednar treatment in FGp, Lime application in PIRSA, and minimum tillage with mixed fallow cover crop management in HCPSL. T1 = no stress (50% WFPS), T2 = moderate stress (30% WFPS), T3 = severe stress (20% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

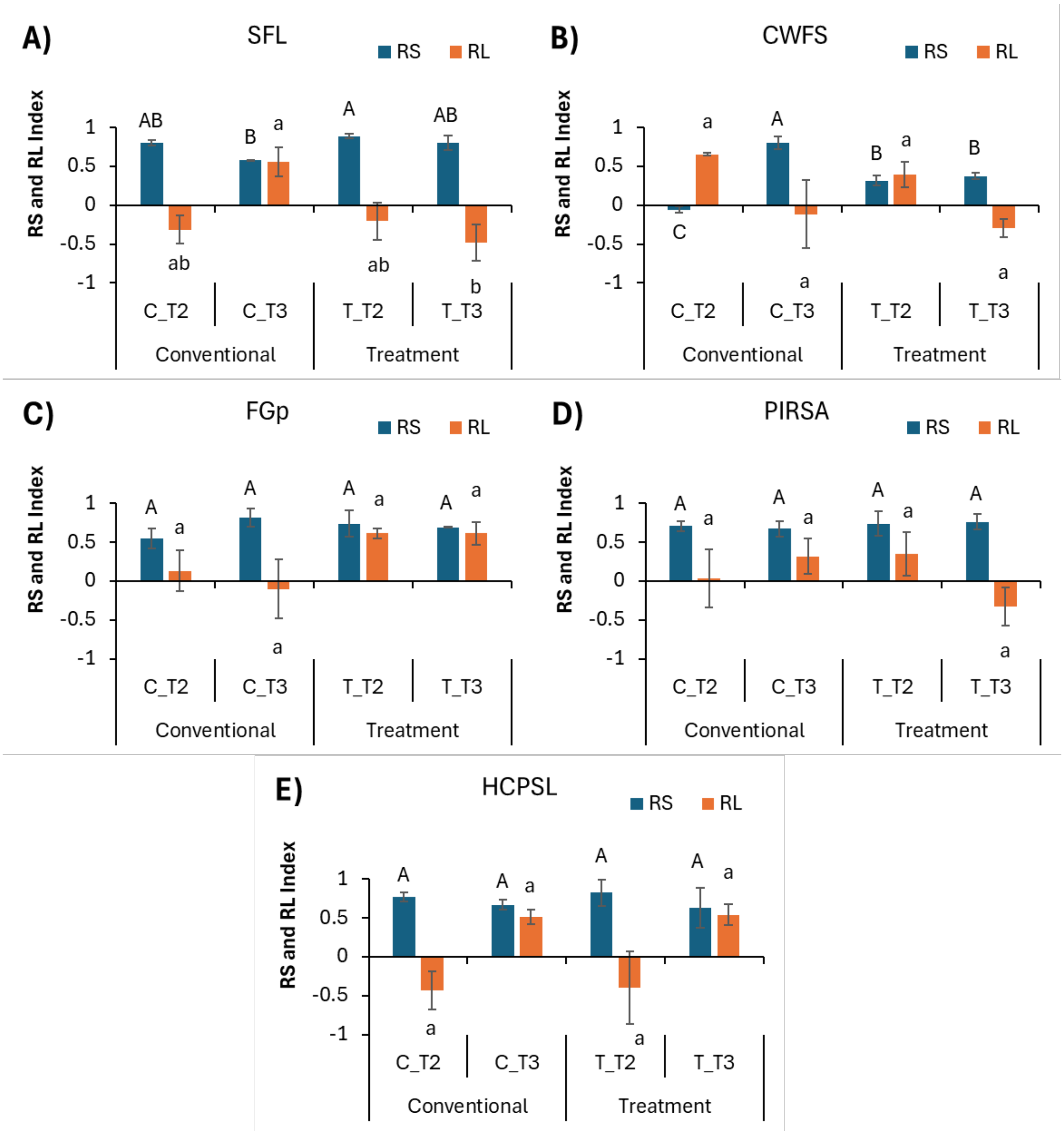

Figure 5.7. Resistance and Resilience Index of Microbial biomass carbon content in soil across varied management histories under water stress treatments: (A) SFL (B) CWFS (C) FGp (D) PIRSA (E) HCPSL. C = conventionally managed soil, T = Cover crop management in SFL and CWFS, Bednar treatment in FGp, Lime application in PIRSA, and minimum tillage with mixed fallow cover crop management in HCPSL. T1 = no stress (50% WFPS), T2 = moderate stress (30% WFPS), T3 = severe stress (20% WFPS). Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

The study highlights how different soil management practices influence the resilience and response of MBC to water stress. Soils managed with cover crops generally showed more sensitivity to moderate stress, suggesting that these systems might have a threshold level of stress beyond which microbial activity is significantly impacted. Conversely, conventional management systems sometimes showed increased MBC under moderate stress, possibly due to a stress-induced stimulation of microbial activity, indicating a potential adaptive response. The stability of MBC in the FGp and PIRSA soils suggests that these management practices create resilient microbial communities that are less affected by water stress. However, lime treatment in PIRSA soils did not provide additional resilience under severe stress. The HCPSL results highlight that minimum tillage with mixed fallow cover crop management can enhance microbial resilience to severe stress, whereas excessive tillage may exacerbate stress impacts. Overall, the findings underscore the importance of tailored soil management practices to maintain or enhance soil microbial health and resilience under varying stress conditions, demonstrating that certain practices, like cover cropping and minimum tillage, can foster more stable and resilient soil microbial communities.

5.4.2.2 Soil Microbial Biomass Nitrogen Content

At the end of the 14 days of stress this period, MBN content in the soil varied across various management practices. In SFL, there was no significant difference in MBN between stress treatments and the control under conventional management history; however, under cover crop management history, severe stress resulted in lower MBN compared to moderate and no stress treatments (Figure 5.8A). In the CWFS, no differences in MBN were observed between stress treatments and the control for either cover crop or conventional management histories (Figure 5.8B). Similarly, in the FGp, no significant differences in MBN were found between stress treatments and the control in both Bednar treatment and conventional management histories (Figure 5.8C). In PIRSA, no differences in MBN were observed between stress treatments and the control under lime treatment history. Conversely, under conventional management history (no lime treatment), both moderate and severe stress treatments showed lower MBN content than the no stress treatment (Figure 5.8D). In HCPSL, no significant differences in MBN were found between stress treatments and the no stress treatment under minimum tillage with mixed fallow cover crop management history. However, soils under excessively tilled history showed lower MBN content in the severe stress treatment compared to the no stress and moderate stress treatments (Figure 5.8E).

After a stress release period where water was added and plants were maintained under no stress conditions for another 14 days, the MBN content in soil at the end of the 28-day period showed the following variations across different management practices. In SFL, there was no significant difference in MBN between stress treatments and the control in either cover crop or conventional management histories (Figure 5.8A). In CWFS, no differences in MBN were observed between stress treatments and the control under cover crop management history; however, the severe stress treatment under conventional management history showed higher MBN content compared to the no stress and moderate stress treatments (Figure 5.8B). In FGp, there were no significant differences in MBN between stress treatments and the control in both Bednar treatment and conventional management histories (Figure 5.8C). In PIRSA, no significant differences in MBN were observed between stress treatments and the control under either lime treatment or conventional management histories, although the lime treatment history showed higher MBN content under no stress treatment compared to all treatments under conventional management history (Figure 5.8D). In HCPSL, no significant differences in MBN were found between stress treatments and the control under either excessively tilled history or minimum tillage with mixed fallow cover crop management history (Figure 5.8E).

No significant differences were observed in resistance and resilience indices for MBN between stress treatments under conventional and cover crop management histories in either SFL (Figure 5.9A) or CWFS (Figure 5.9B), and between excessive tillage and minimum tillage management histories in HCPSL (Figure 5.9E). In FGp, severe water stress under the Bednar approach resulted in a lower resistance index for MBN compared to moderate and severe stress under conventional management. However, no differences were found in the resilience index for the stress treatments between the two management approaches (Figure 5.9C). In PIRSA, soils with lime management history showed the highest resistance index under moderate stress, compared to moderate and severe stress treatments under no lime management history (Figure 5.9D).

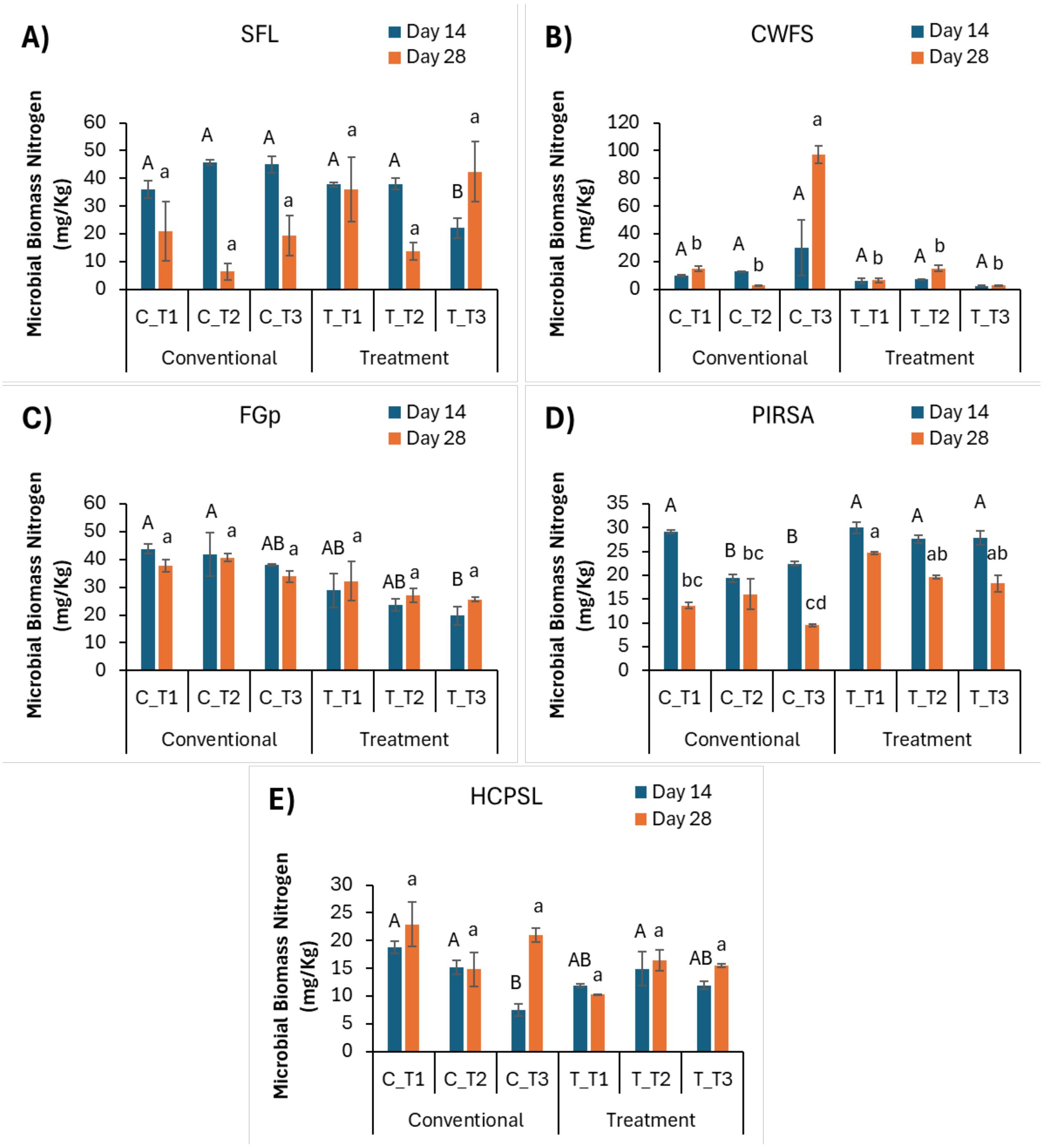

Figure 5.8. Microbial biomass nitrogen content in soil across varied management histories: (A) SFL (B) CWFS (C) FGp (D) PIRSA (E) HCPSL. C = conventionally managed soil, T = Cover crop management in SFL and CWFS, Bednar treatment in FGp, Lime application in PIRSA, and minimum tillage with mixed fallow cover crop management in HCPSL. T1 = no stress (50% WFPS), T2 = moderate stress (30% WFPS), T3 = severe stress (20% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

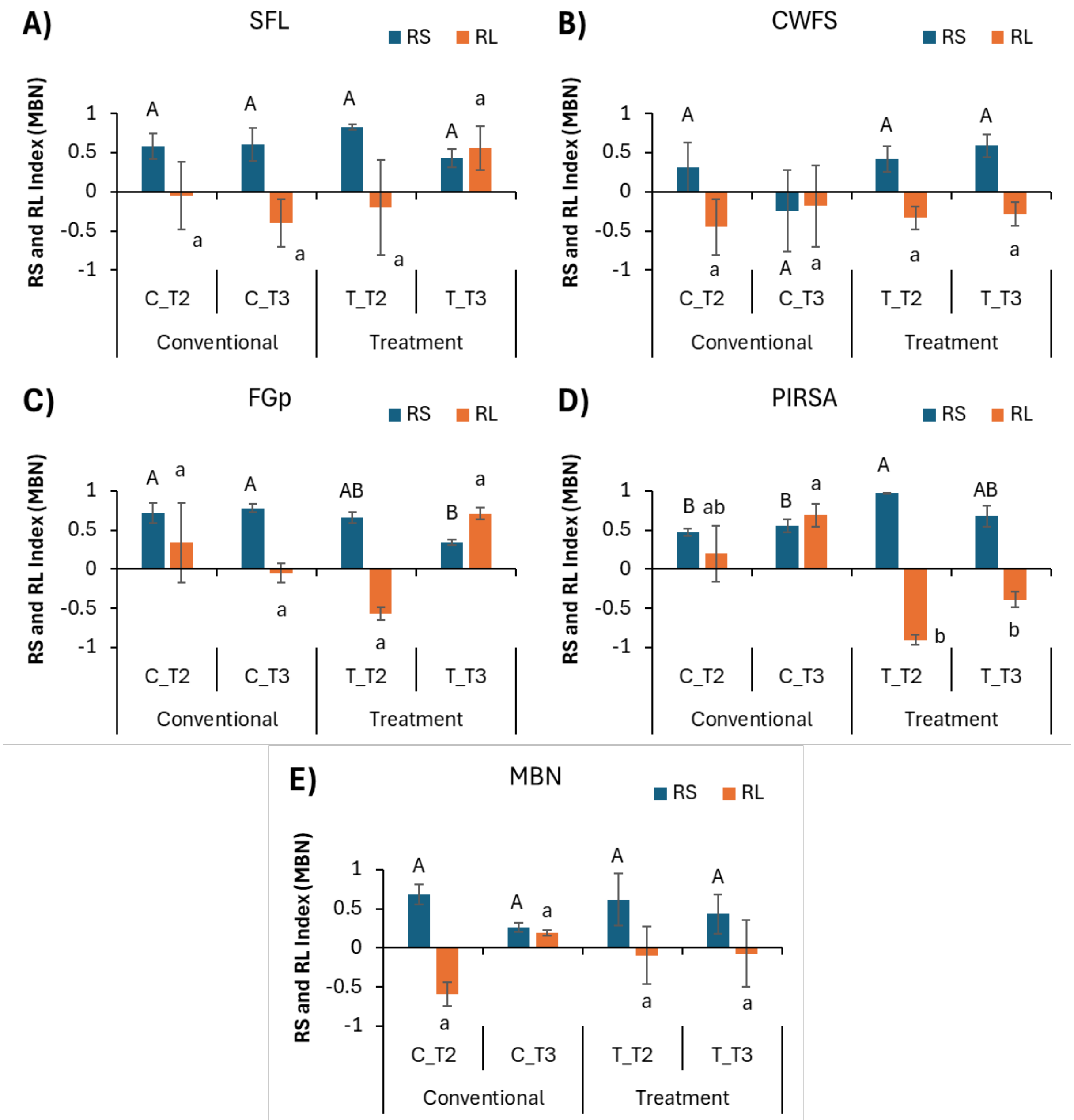

Figure 5.9. Resistance and resilience index of MBN content in soil across varied management histories: (A) SFL (B) CWFS (C) FGp (D) PIRSA (E) HCPSL. C = conventionally managed soil, T = Cover crop management in SFL and CWFS, Bednar treatment in FGp, Lime application in PIRSA, and minimum tillage with mixed fallow cover crop management in HCPSL. T1 = no stress (50% WFPS), T2 = moderate stress (30% WFPS), T3 = severe stress (20% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

The varying responses of MBN to water stress across different management practices can be influenced by several factors, including plant nutrient uptake. In SFL, the stability of MBN under conventional management suggests a robust microbial community, while the sensitivity under cover crop management indicates that severe stress can disrupt microbial processes more easily in these systems, potentially due to increased plant nutrient uptake under stress. In CWFS, the resilience of MBN across treatments points to an adaptive microbial community, with increased MBN after severe stress under conventional management possibly due to microbial adaptations or nutrient release upon rewetting, and the role of wheat plants in moderating nutrient cycling. The consistency of MBN in FGp under both management practices indicates strong microbial resilience, with the plants likely maintaining a stable nutrient uptake despite stress levels. In PIRSA, lime treatment likely enhances microbial stability, as evidenced by the lack of MBN changes under stress, whereas conventional management without lime shows vulnerability, with reduced MBN under stress potentially due to increased plant nutrient uptake in response to stress. In HCPSL, minimum tillage with mixed fallow cover crop management appears to foster a resilient microbial community, with wheat plants possibly stabilising nutrient cycling, while excessive tillage exacerbates the negative impacts of severe stress, leading to lower MBN, possibly due to higher nutrient demand by plants under stress. The lack of significant differences in resistance and resilience indices in SFL, CWFS, and HCPSL suggests overall microbial stability, but the reduced resistance in FGp under severe stress with the Bednar approach and higher resistance with lime treatment in PIRSA highlight the importance of specific management practices and plant interactions in mitigating the effects of stress on soil microbial communities.

5.4.2.3 Soil Respiration

At the end of the stress period (Day 14), microbial respiration in the soil varied across various management practices. In SFL, the no stress treatment under both cover crop and conventional management histories showed higher respiration rates compared with moderate and severe stress treatments. Additionally, no stress and moderate stress treatments under cover crop management exhibited higher respiration compared with the respective treatments under conventional management history (Figure 5.10A). Similarly, in CWFS, the no stress treatment under both cover crop and conventional management histories showed higher respiration rates compared with the severe stress treatment, and all treatments under cover crop management showed higher respiration compared with the respective treatments under conventional management history (Figure 5.10B). In contrast, FGp exhibited no significant difference in respiration between stress treatments and the control in either Bednar treatment history or conventional management history (Figure 5.10C). PIRSA showed no significant difference in respiration between stress treatments and the control in either lime treatment history or conventional management history; however, a higher respiration rate was observed when comparing the no stress treatment under lime management history with the severe stress treatment under conventional management history (Figure 5.10D). For HCPSL, there was no significant difference in respiration between stress treatments and the no stress treatment under minimum tillage with mixed fallow cover crop management history, but soils under excessively tilled history showed higher respiration in the moderate stress treatment compared with the no stress treatment (Figure 5.10E).

After releasing the water stress, at the end of the 28-day period, microbial respiration in soil varied across various management practices. In SFL, the no stress treatment under cover crop management showed the highest respiration compared to moderate and severe stress treatments, and all stress treatments in soil under conventional management history (Figure 5.10A). Similarly, in CWFS, the no stress treatment under cover crop management showed the highest respiration compared to moderate and severe stress treatments, and all stress treatments in soil under conventional management history (Figure 5.10B). In FGp, the no stress treatment under Bednar treatment showed the highest respiration compared to moderate and severe stress treatments, and all stress treatments in soil under conventional management history (Figure 5.10C). In PIRSA, there was no significant difference in respiration between the severe stress treatment and the control in lime treatment history; however, the moderate stress treatment showed a lower respiration rate compared to the no stress treatment. Under conventional management history (no lime treatment), all stress treatments showed lower respiration rates compared to the respiration in water stress treatments under lime treatment history (Figure 5.10D). In HCPSL, there was no significant difference in respiration between stress treatments and the control under either excessively tilled history or minimum tillage with mixed fallow cover crop management history (Figure 5.10E).

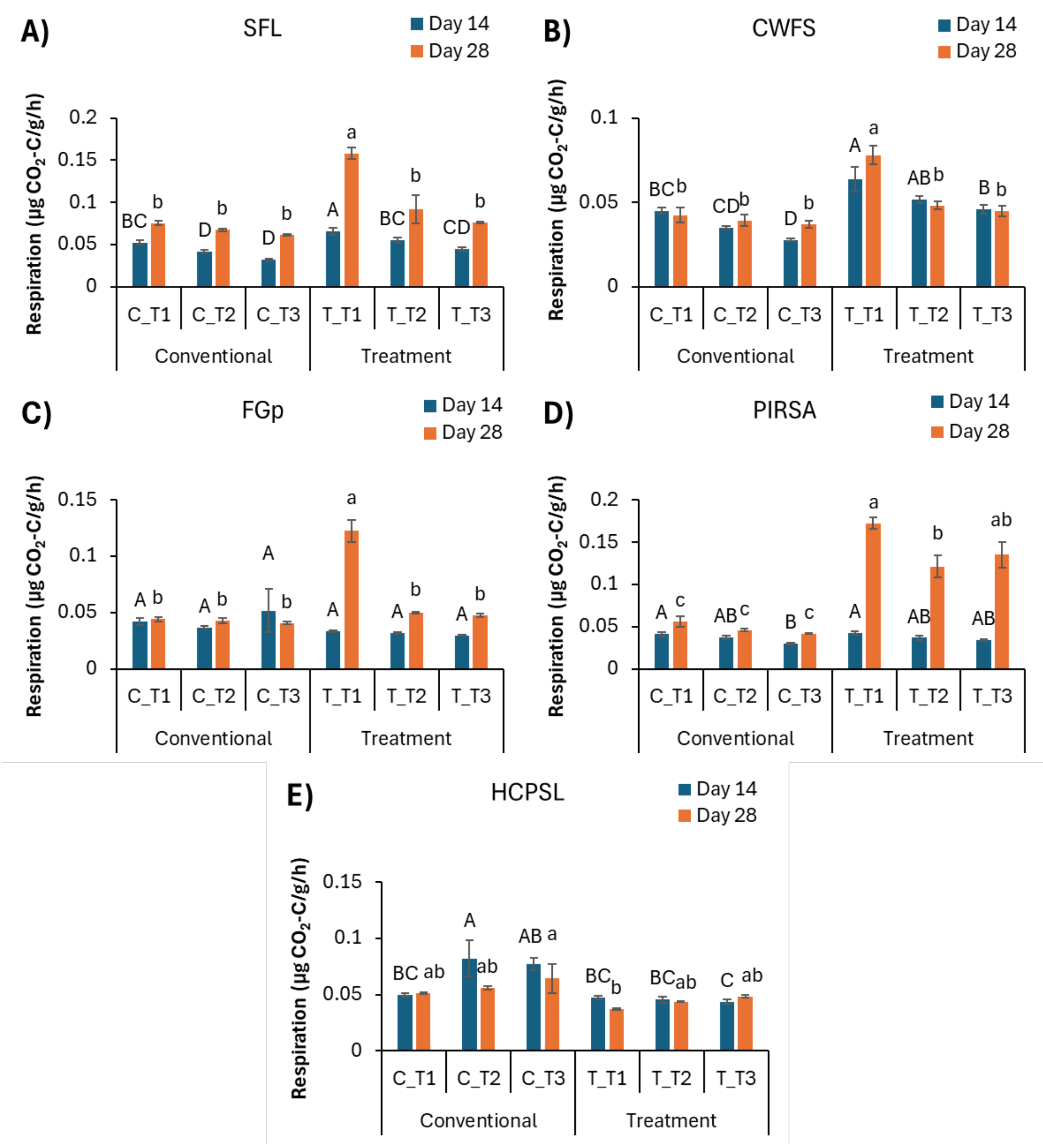

Figure 5.10. Microbial respiration in soil across varied management histories under water stress treatments: (A) SFL (B) CWFS (C) FGp (D) PIRSA (E) HCPSL. C = conventionally managed soil, T = Cover crop management in SFL and CWFS, Bednar treatment in FGp, Lime application in PIRSA, and minimum tillage with mixed fallow cover crop management in HCPSL. T1 = no stress (50% WFPS), T2 = moderate stress (30% WFPS), T3 = severe stress (20% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

The resistance index for soil respiration under moderate water stress treatment was higher than that under severe water stress treatment in both conventional and cover crop management histories in SFL (Figure 5.11A). However, in CWFS, moderate water stress treatment under cover crop management (Figure 5.11B), lime-treated soil in PIRSA (Figure 5.11D), and minimum tillage with fallow crop management in HCPSL (Figure 5.11E) showed higher resistance indices than severe stress treatments under their respective conventional management histories. Furthermore, both moderate and severe water stress treatments under Bednar treatment in FGp exhibited higher resistance indices for respiration compared to severe stress treatment under conventional management history (Figure 5.11C). No notable differences in the resilience index were observed between the two management approaches for the water stress treatments in SFL (Figure 5.11A), CWFS (Figure 5.11B), PIRSA (Figure 5.11D), or HCPSL (Figure 5.11E). However, in FGp, the resilience index for the water stress treatments under conventional management was higher than under Bednar treatment (Figure 5.11C).

The variations in microbial respiration observed in the study can be explained by the differing impacts of soil management practices on soil health and microbial activity. Ecosystem-based management practices, such as cover cropping and minimum tillage, typically enhance soil organic matter content, improve soil structure, and support more diverse and resilient microbial communities. These practices provide a steady supply of organic carbon and nutrients, which promote higher microbial respiration rates, even under moderate water stress conditions. In contrast, conventionally managed soils, which often lack these organic inputs and may suffer from poorer soil structure and reduced microbial diversity, tend to exhibit lower microbial activity and respiration rates, especially under stress. The higher resistance indices for soils under ecosystem-based management indicate that these soils can better withstand stress without significant drops in microbial activity, while their higher resilience indices suggest a greater ability to recover microbial activity after stress is alleviated. These findings underscore the importance of sustainable agricultural practices in maintaining soil health and microbial functionality, which are critical for soil fertility and crop productivity.

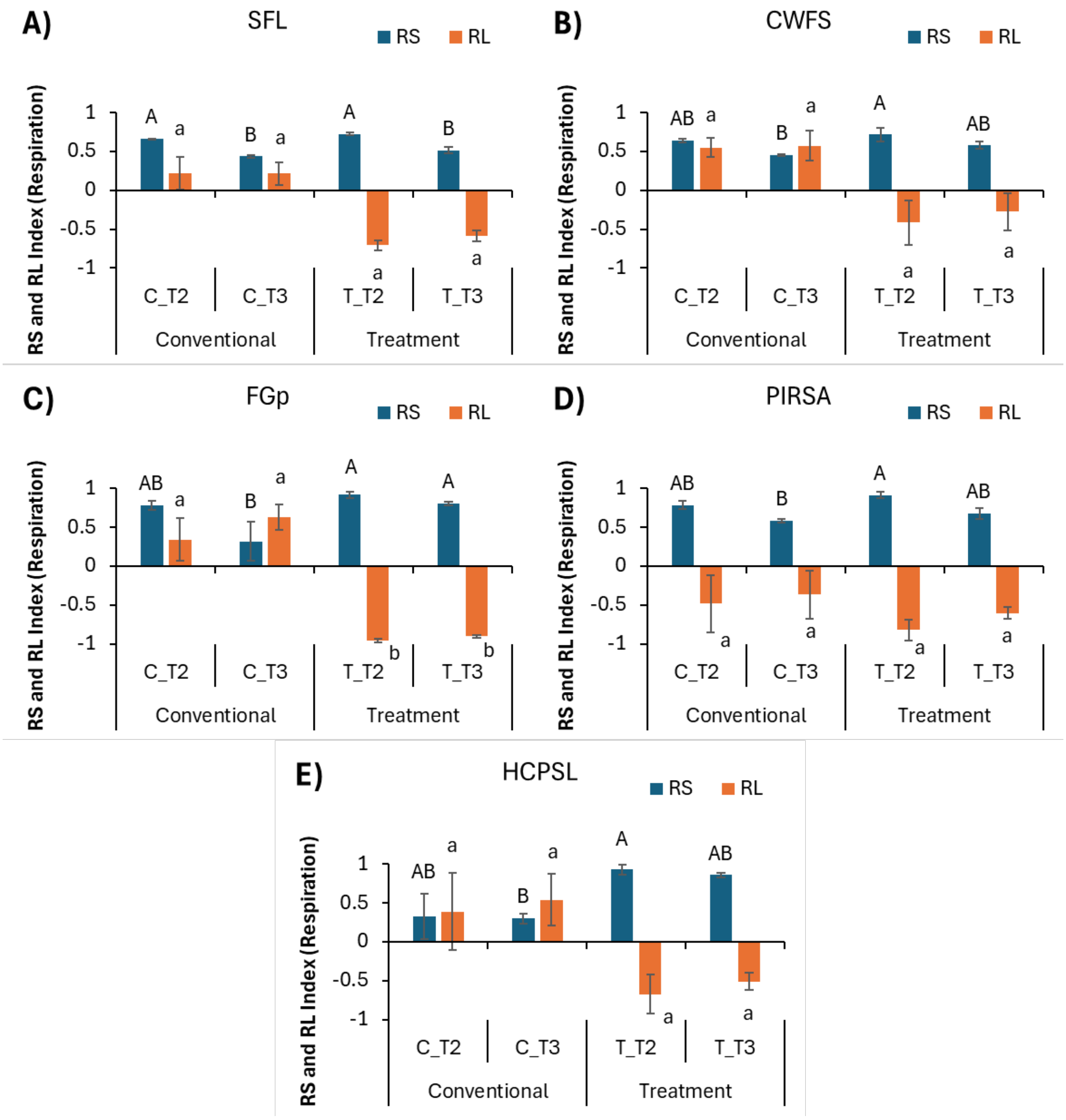

Figure 5.11. Resistance (RS) and Resilience (RL) Index of microbial respiration in soil across varied management histories under water stress treatments: (A) SFL (B) CWFS (C) FGp (D) PIRSA (E) HCPSL. C = conventionally managed soil, T = Cover crop management in SFL and CWFS, Bednar treatment in FGp, Lime application in PIRSA, and minimum tillage with mixed fallow cover crop management in HCPSL. T1 = no stress (50% WFPS), T2 = moderate stress (30% WFPS), T3 = severe stress (20% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

5.4.2.4 Microbial Metabolic Quotient

At the end of the stress period, the microbial qCO_2 in soils from various management practices was assessed. In SFL, the severe water stress treatment under conventional management history exhibited a higher qCO_2 compared to the no stress treatment and all stress treatments under cover crop management history. Additionally, under cover crop management, there were no significant differences in qCO_2 between the no stress and stress treatments (Figure 5.12A). Similarly, in CWFS, the moderate water stress treatment under conventional management history showed a higher qCO_2 compared to both the no stress and severe stress treatments, as well as all stress treatments under cover crop management history. However, qCO_2 did not differ significantly between no stress and stress treatments under cover crop management in CWFS (Figure 5.12B). Conversely, in FGp, there were no significant differences in qCO_2 between stress treatments and the control in either the Bednar treatment history or conventional management history (Figure 5.12C). PIRSA also showed no significant differences in qCO_2 between stress treatments and the control in either lime treatment history or conventional management history (Figure 5.12D). In HCPSL, under minimum tillage with mixed fallow cover crop management history, there was no significant difference in qCO_2 between the moderate stress and no stress treatments; however, the severe water stress treatment showed a higher qCO_2 compared to the moderate and no stress treatments. In soils under excessively tilled history, the no stress treatment exhibited a higher qCO_2 compared to the moderate and severe water stress treatments (Figure 5.12E).

Following the release of water stress after 14 days, plants were grown under no stress conditions, resulting in varied qCO_2 levels in soils from different management practices by the end of the 28-day period. In SFL, the no stress treatment under cover crop management showed the lowest qCO_2 compared to the severe stress treatment and both moderate and severe stress treatments under conventional management history (Figure 5.12A). Similarly, in CWFS, the no stress and moderate stress treatments under cover crop management showed the highest qCO_2 compared to the severe stress treatment and all stress treatments under conventional management history (Figure 5.12B). In FGp, all treatments under the Bednar approach exhibited the lowest qCO_2 compared to all stress treatments under conventional management history (Figure 5.12C). PIRSA continued to show no significant differences in qCO_2 between stress treatments and the control in either lime treatment history or conventional management history (Figure 5.12D). Lastly, in HCPSL, under excessively tilled history, the moderate stress treatment showed a higher qCO_2 compared to the no stress and severe stress treatments, while in soils under minimum tillage with mixed fallow cover crop management history, the severe water stress treatment exhibited the lowest qCO_2 compared to the no stress and moderate stress treatments (Figure 5.12E).

The resistance indices of the microbial qCO_2 for the stress treatment did not show a notable difference between the two management approaches in SFL (conventional vs. cover crop management), FGp (conventional vs. Bednar treatment), PIRSA (conventional vs. lime application), or HCPSL (excessive tillage vs. minimum tillage) (Figure 5.13A, C, D and E). However, under conventional management in CWFS, moderate water stress resulted in the lowest resistance index for qCO_2 compared to severe stress and both moderate and severe stress treatments under cover crop management. Additionally, there was no notable difference in the resistance index of qCO_2 between moderate and severe water stress treatments under cover crop management (Figure 5.13B). The resilience index of qCO_2 also showed no difference between stress treatments under the two management approaches at any sites (Figure 5.13A, B, C, D and E).

The observed variations in qCO_2 levels and resistance indices can be attributed to differences in soil microbial community composition, soil structure, nutrient availability, and stress response mechanisms influenced by the specific management practices. Soils under conventional management often have less diverse microbial communities that become more metabolically active under severe stress, leading to higher qCO_2. In contrast, cover crop management promotes microbial diversity and resilience, resulting in stable qCO_2 levels under stress. Enhanced soil structure and organic matter from cover crops buffer against stress, maintaining microbial metabolic stability. Tillage practices also play a role, with excessively tilled soils showing higher qCO_2 under no stress due to disrupted soil structure and less efficient microbial communities. The resistance and resilience indices indicate that microbial communities' capacity to withstand and recover from stress is influenced by management practices, with cover crop management generally providing better stress resilience.

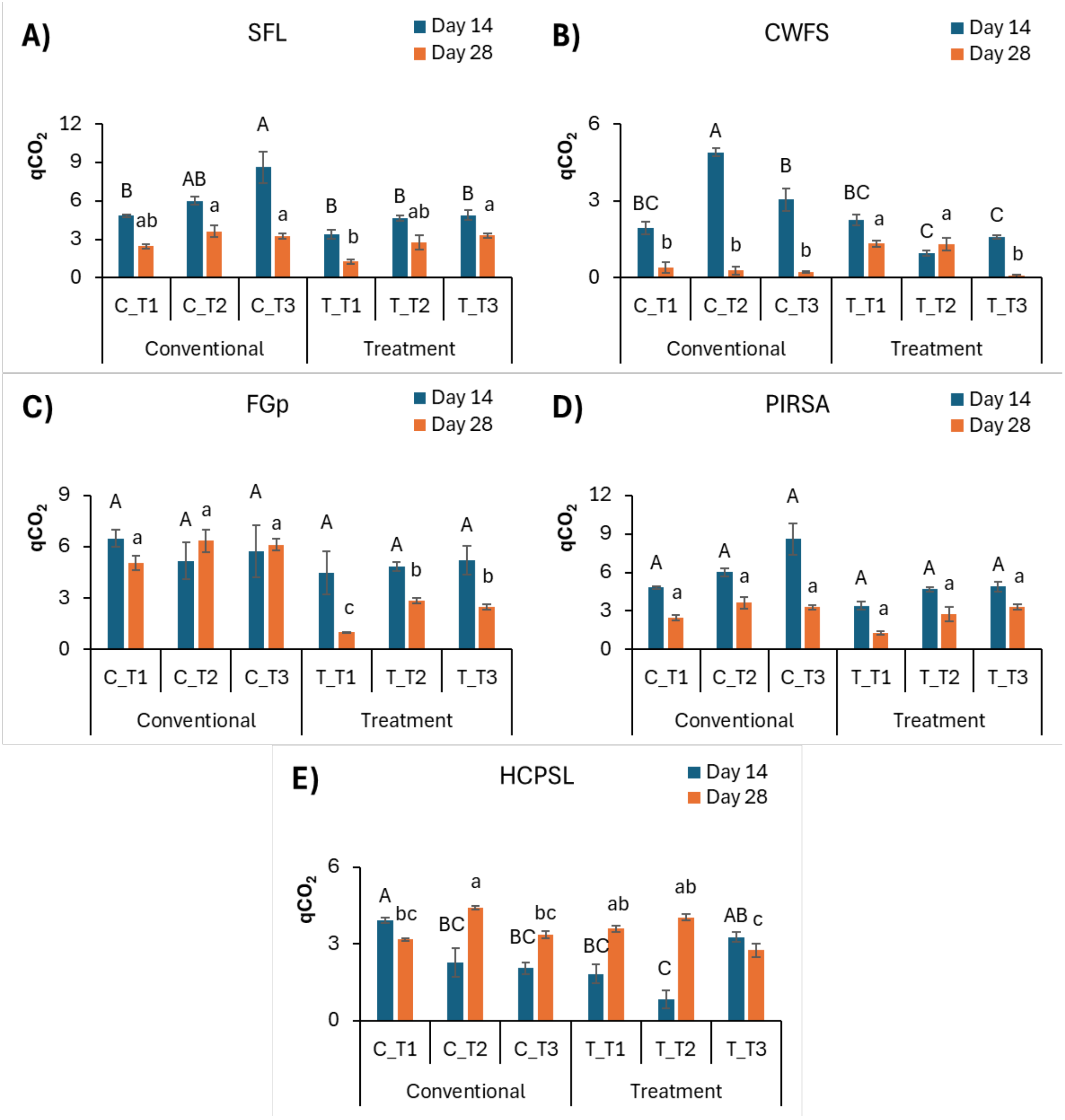

Figure 5.12. Microbial qCO_2 in soil across varied management histories under water stress treatments: (A) SFL (B) CWFS (C) FGp (D) PIRSA (E) HCPSL. C = conventionally managed soil, T = Cover crop management in SFL and CWFS, Bednar treatment in FGp, Lime application in PIRSA, and minimum tillage with mixed fallow cover crop management in HCPSL. T1 = no stress (50% WFPS), T2 = moderate stress (30% WFPS), T3 = severe stress (20% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

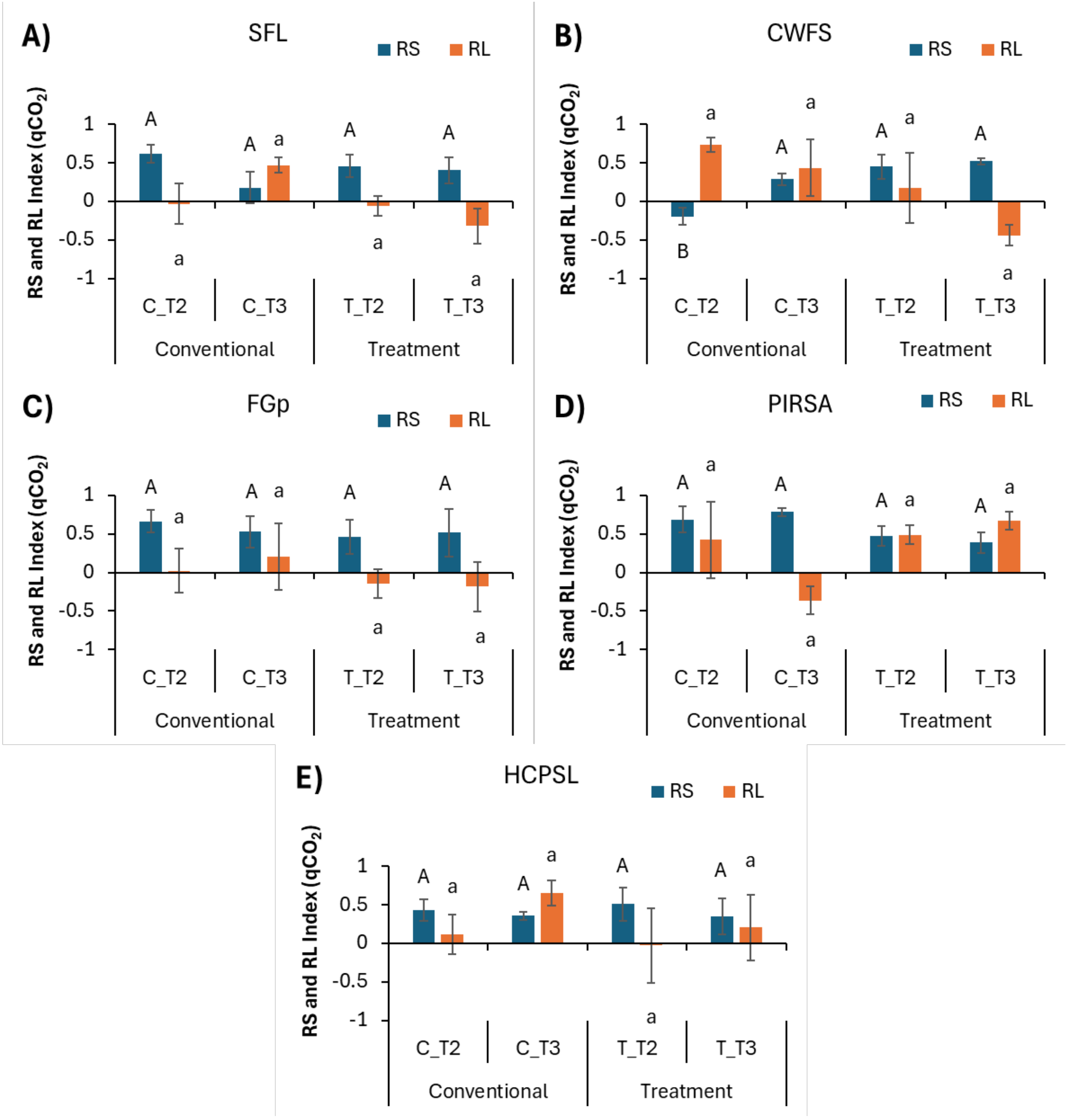

Figure 5.13. Resistance and resilience index of microbial qCO_2 in soil across varied management histories under water stress treatments: (A) SFL (B) CWFS (C) FGp (D) PIRSA (E) HCPSL. C = conventionally managed soil, T = Cover crop management in SFL and CWFS, Bednar treatment in FGp, Lime application in PIRSA, and minimum tillage with mixed fallow cover crop management in HCPSL. T1 = no stress (50% WFPS), T2 = moderate stress (30% WFPS), T3 = severe stress (20% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

5.4.2.5 Soil Enzyme Activity

At the end of the 14-day period, enzyme activity (β-glucosidase (BG), N-acetyl-β-D-glucosaminidase (NAG), and phosphatase (P)) in soil across various management practices showed distinct patterns (Table 5.3). In SFL, there was no difference in BG, NAG, or P activity between stress treatments and the control under either cover crop management or conventional management history. In CWFS, BG activity did not differ between stress treatments and the control under both management histories. However, the no stress treatment under cover crop management exhibited higher NAG activity compared to moderate and severe stress treatments under conventional management, and higher P activity was observed under no stress in cover crop management compared to moderate and severe stress treatments under both management histories. In FGp, BG activity was higher in the no stress treatment compared to moderate and severe stress treatments under conventional management. Under Bednar treatment history, moderate water stress did not differ significantly from the no stress treatment, while severe stress showed the lowest BG activity. NAG activity was significantly higher under no stress in Bednar management compared to moderate and severe stress under both management approaches, while P activity showed no difference between stress treatments and the control in either management history. In PIRSA, BG activity showed no difference between stress treatments and the control under conventional management, whereas under lime treatment history, moderate stress had the lowest BG activity compared to no stress and severe stress treatments. Overall, BG activity under conventional management was lower than in no stress and severe stress treatments under lime treatment. NAG activity did not differ between stress treatments and the control under either management history, whereas P activity was significantly higher in stress treatments under conventional management compared to lime treatment history. In HCPSL, BG activity did not differ between moderate stress and no stress under excessive tillage, but both were significantly lower than BG activity under no stress and moderate stress in minimum tillage with mixed fallow cover crop management. NAG and P activities were higher in no stress compared to moderate and severe stress under minimum tillage with mixed fallow cover crop management, and all treatments under excessive tillage management history.

Table 5.3. Enzyme activity in soil across varied management histories under water stress treatments at the end of the stress period (Day 14)

Site	Treatment	Enzyme Activity (nmol h^{-1} g^{-1})

		β-glucosidase (BG)	N-acetyl-β-D-glucosaminidase (NAG)	phosphatase (P)
SFL	C_T1	73.35 ± 3.95[a]	32.2 ± 1.7[a]	168.24 ± 12.81[a]
	C_T2	80.45 ± 3.74[a]	37.85 ± 7.25[a]	201.58 ± 22.19[a]
	C_T3	67.48 ± 2.3[a]	27.32 ± 5.74[a]	145.83 ± 14.58[a]
	T_T1	123.74 ± 17.32[a]	30.54 ± 8.49[a]	166.09 ± 9.05[a]
	T_T2	131.52 ± 25.91[a]	27.42 ± 4.57[a]	155.23 ± 22.38[a]
	T_T3	112.1 ± 21.09[a]	21.75 ± 6.63[a]	116.31 ± 21.83[a]
CWFS	C_T1	41.83 ± 3.62[a]	3.75 ± 1.02[ab]	69.3 ± 4.15[ab]
	C_T2	48.59 ± 13.39[a]	1.44 ± 0.92[b]	34.89 ± 6.43[c]
	C_T3	27.3 ± 6.05[a]	1.38 ± 0.4[b]	53.9 ± 7.96[bc]
	T_T1	56.24 ± 1.78[a]	6.88 ± 0.9[a]	87.1 ± 9.5[a]
	T_T2	32.28 ± 1.49[a]	1.93 ± 1.35[ab]	43.89 ± 4.44[bc]
	T_T3	32.64 ± 6.46[a]	2.63 ± 0.38[ab]	41.48 ± 6.77[bc]
FGp	C_T1	133.22 ± 1.32[a]	16.96 ± 1.78[ab]	56.62 ± 7.58[ab]
	C_T2	64.47 ± 6.42[b]	13.86 ± 0.4[bc]	68.94 ± 14.56[a]
	C_T3	59.75 ± 5.85[b]	10.9 ± 0.37[cd]	37.37 ± 1.46[ab]
	T_T1	49.3 ± 3.29[b]	19.56 ± 1.5[a]	54.91 ± 1.42[ab]
	T_T2	56.85 ± 14.14[b]	10.04 ± 0.44[cd]	44.57 ± 1.4[ab]
	T_T3	30.36 ± 4.45[c]	8.09 ± 0.79[d]	28.66 ± 0.96[b]
PIRSA	C_T1	77.07 ± 5.65[b]	18.35 ± 1.14[a]	115.66 ± 2.37[a]
	C_T2	76.72 ± 3.61[b]	11.72 ± 0.49[a]	86.25 ± 0.43[ab]
	C_T3	80.66 ± 2.12[b]	11.01 ± 1.9[a]	81.62 ± 0.69[b]
	T_T1	133.72 ± 11.57[a]	16.49 ± 3.09[a]	48.8 ± 1.33[c]
	T_T2	70.97 ± 1.01[b]	10.63 ± 0.84[a]	33.33 ± 1.81[e]
	T_T3	110.93 ± 3.51[a]	10.67 ± 1.35[a]	41.22 ± 1.85[d]
HCPSL	C_T1	5.69 ± 1.11[b]	2.55 ± 0.5[b]	168.71 ± 3.34[b]
	C_T2	6.42 ± 0.66[b]	3.29 ± 1.72[b]	120.65 ± 2.93[c]
	C_T3	8.46 ± 1.29[b]	3.04 ± 3.02[b]	83.54 ± 2.17[cd]
	T_T1	44.3 ± 8.53[a]	25.08 ± 2.02[a]	238.04 ± 22.1[a]
	T_T2	49.43 ± 9.72[a]	5.68 ± 1.06[b]	100.71 ± 1.47[c]
	T_T3	14.32 ± 0.52[b]	5.93 ± 0.55[b]	109.1 ± 2.89[c]

SFL - Soils for Life Trust - Canberra, ACT, CWFS - Central West Farming Systems Inc. - Condobolin, NSW, FGp - Facey Group Inc. - Wickepin WA, PIRSA - Department of Primary Industries and Regions in South Australia, HCPSL - Herbert Cane Productivity Services Ltd. - Ingham, QLD. C = conventionally managed soil, T = Cover crop management in SFL and CWFS, Bednar treatment in FGp, Lime application in PIRSA, and minimum tillage with mixed fallow cover crop management in HCPSL. T1 = no stress (50% WFPS), T2 = moderate stress (30% WFPS), T3 = severe stress (20% WFPS). Means of the treatments the same letter is not different at the 5% level of significance for the resistance in each comparison.

The resistance indices of the stress treatments for soil enzyme activity exhibited specific trends (Table 5.4). In SFL, there was no difference in the resistance index for β-glucosidase (BG), N-acetyl-β-D-glucosaminidase (NAG), or phosphatase (P) activity between stress treatments under either cover crop management history or conventional management history. Similarly, in CWFS, no difference was observed in the resistance index for BG or P activity between stress treatments under either management history. However, the resistance index of NAG activity in moderate stress treatment under cover crop management was significantly higher compared to both moderate and severe water stress treatments under conventional management. In FGp, there was no difference in the resistance index for BG or P activity between stress treatments under either Bednar management history or conventional management history. The resistance index for NAG activity in water stress treatments under Bednar management was lower than that in moderate stress treatment under conventional management. In PIRSA, there was no difference in the resistance index for BG or NAG activity between stress treatments under either lime management history or conventional management history, although severe stress treatment under lime management showed a higher resistance index compared to moderate water stress treatment. In HCPSL, no difference was observed in the resistance index for BG or NAG activity between stress treatments under either excessive tillage management history or minimum tillage with mixed fallow cover crop management history. However, moderate water stress treatment showed the highest resistance index for P activity under excessive tillage management history.

Table 5.4. Resistance index of enzyme activity in soil across varied management histories under water stress treatments

Site	**Treatment**	**Resistance Index of Enzyme Activity (RS)**		
		β-glucosidase (BG)	**N-acetyl-β-D-glucosaminidase (NAG)**	**phosphatase (P)**
SFL	C_T2	0.67 ± 0.06[a]	0.43 ± 0.07[a]	0.68 ± 0.24[a]
	C_T3	0.74 ± 0.04[a]	0.53 ± 0.1[a]	0.68 ± 0.08[a]
	T_T2	0.74 ± 0.02[a]	0.34 ± 0.11[a]	0.76 ± 0.1[a]
	T_T3	0.84 ± 0.03[a]	0.54 ± 0.03[a]	0.67 ± 0.25[a]
CWFS	C_T2	0.68 ± 0.07[a]	0.1 ± 0.03[b]	0.34 ± 0.06[a]
	C_T3	0.57 ± 0.07[a]	0.17 ± 0.1[b]	0.65 ± 0.11[a]
	T_T2	0.4 ± 0.02[a]	0.79 ± 0.16[a]	0.37 ± 0.09[a]
	T_T3	0.53 ± 0.16[a]	0.25 ± 0.08[ab]	0.32 ± 0.05[a]
FGp	C_T2	0.32 ± 0.05[a]	0.72 ± 0.1[a]	0.37 ± 0.2[a]
	C_T3	0.29 ± 0.03[a]	0.5 ± 0.08[ab]	0.52 ± 0.07[a]
	T_T2	0.75 ± 0.25[a]	0.35 ± 0.02[b]	0.69 ± 0.05[a]

	T_T3	0.46 ± 0.09[a]	0.31 ± 0.01[b]	0.35 ± 0.01[a]
PIRSA	C_T2	0.81 ± 0.04[a]	0.47 ± 0.04[a]	0.6 ± 0.02[ab]
	C_T3	0.7 ± 0.09[a]	0.31 ± 0.01[a]	0.55 ± 0.01[ab]
	T_T2	0.47 ± 0.11[a]	0.56 ± 0.19[a]	0.53 ± 0.06[b]
	T_T3	0.55 ± 0.03[a]	0.4 ± 0.09[a]	0.74 ± 0.06[a]
HCPSL	C_T2	0.29 ± 0.35[a]	-0.03 ± 0.18[a]	0.56 ± 0.03[a]
	C_T3	0.02 ± 0.5[a]	0.01 ± 0[a]	0.33 ± 0.01[b]
	T_T2	0.18 ± 0.44[a]	0.16 ± 0.05[a]	0.28 ± 0.04[b]
	T_T3	0.17 ± 0.07[a]	0.15 ± 0.02[a]	0.3 ± 0.03[b]

SFL - Soils for Life Trust - Canberra, ACT, CWFS - Central West Farming Systems Inc. - Condobolin, NSW, FGp - Facey Group Inc. - Wickepin WA, PIRSA - Department of Primary Industries and Regions in South Australia, HCPSL - Herbert Cane Productivity Services Ltd. - Ingham, QLD. C = conventionally managed soil, T = Cover crop management in SFL and CWFS, Bednar treatment in FGp, Lime application in PIRSA, and minimum tillage with mixed fallow cover crop management in HCPSL. T2 = moderate stress (30% WFPS), T3 = severe stress (20% WFPS). Means of the treatments the same letter is not different at the 5% level of significance for the resistance in each comparison.

At the end of the 28-day period (After stress was released by adding water, and the plants were left under no stress conditions for another 14 days), enzyme activity in soil across various management practices showed distinct patterns (Table 5.5). In SFL, there was no difference in β-glucosidase (BG) and phosphatase (P) activity between stress treatments and the control under either cover crop management or conventional management history. However, moderate water stress under cover crop management showed higher BG activity compared to no stress and severe water stress under conventional management. N-acetyl-β-D-glucosaminidase (NAG) activity did not differ between water stress treatments and no stress treatment under cover crop management, but moderate water stress under conventional management showed higher NAG activity compared to severe stress and no stress treatments. In CWFS, there was no difference in BG and P activity between stress treatments and the control under either cover crop management or conventional management history. Comparing the two management approaches, all water stress treatments under cover crop management exhibited higher P activity compared to water stress treatments under conventional management. Severe water stress under conventional management showed higher NAG activity compared to no stress and moderate stress treatments, while there was no difference in NAG activity between stress treatments and the control under cover crop management. In FGp, there was no difference in

BG and P activity between stress treatments and the control under either Bednar approach or conventional management history. NAG activity was significantly lower under severe water stress compared to no stress and moderate water stress treatments under the Bednar approach, with no difference in NAG activity between stress treatments and the control under conventional management.

In PIRSA, there was no difference in BG activity between moderate water stress and no stress under conventional management, but severe stress showed lower BG activity compared to no stress. Under lime management, both moderate and severe stress treatments did not differ significantly in BG activity compared to no stress. NAG activity did not differ between water stress treatments and no stress under lime management, while under conventional management, both moderate and severe water stress treatments showed lower NAG activity compared to no stress. P activity in the no stress treatment was significantly higher compared to water stress treatments under both conventional and lime management. In HCPSL, severe water stress showed lower BG activity compared to moderate stress under excessive tillage management. There was no difference in BG activity between moderate stress and no stress under minimum tillage with mixed fallow cover crop management. NAG activity did not differ between stress treatments and the control under either excessive tillage or minimum tillage with mixed fallow cover crop management. P activity was significantly lower in severe stress treatment compared to no stress under minimum tillage with mixed fallow cover crop management. Under excessive tillage management, P activity severe stress treatment was lower compared to both no stress and moderate stress treatments.

Table 5.5. Enzyme activity in soil across varied management histories under water stress treatments at the end of the stress release period (Day 28)

Site	Treatment	Enzyme Activity (nmol h^{-1} g^{-1})		
		β-glucosidase (BG)	N-acetyl-β-D-glucosaminidase (NAG)	phosphatase (P)
SFL	C_T1	40.76 ± 6.9^{b}	22.26 ± 2.9^{b}	164.83 ± 28.39^{a}
	C_T2	68.67 ± 3.32^{ab}	46.42 ± 4.86^{a}	235.72 ± 30.96^{a}
	C_T3	48.03 ± 5.46^{b}	25.94 ± 1.7^{b}	228.77 ± 16.5^{a}
	T_T1	90.55 ± 3.38^{ab}	26.2 ± 1.65^{b}	195.21 ± 22.25^{a}
	T_T2	127.15 ± 21.54^{a}	36.1 ± 7.05^{ab}	207.84 ± 19.3^{a}
	T_T3	81.14 ± 22.41^{ab}	28.5 ± 4.11^{ab}	190.2 ± 18.98^{a}
CWFS	C_T1	47.19 ± 4.58^{a}	2.11 ± 0.52^{b}	69.76 ± 1.95^{b}
	C_T2	26.3 ± 5.92^{a}	1.64 ± 0.85^{b}	58.74 ± 2.74^{b}
	C_T3	52.82 ± 23.13^{a}	28.14 ± 14.04^{a}	60.24 ± 8.07^{b}
	T_T1	47.85 ± 13.98^{a}	3.58 ± 0.67^{b}	134.47 ± 1.57^{a}
	T_T2	58.11 ± 13.88^{a}	6.56 ± 0.24^{ab}	117.78 ± 8.14^{a}
	T_T3	49.7 ± 5.55^{a}	7.66 ± 0.83^{ab}	121.45 ± 8.91^{a}
FGp	C_T1	65.38 ± 12.18^{a}	12.57 ± 1.15^{ab}	55.48 ± 5.86^{a}
	C_T2	55.52 ± 1.38^{a}	13.06 ± 0.73^{ab}	43.03 ± 2.84^{a}
	C_T3	50.83 ± 4.61^{a}	9.03 ± 1.04^{b}	42.56 ± 1.69^{a}
	T_T1	53.84 ± 5.47^{a}	15.94 ± 1.38^{a}	51.15 ± 2.21^{a}
	T_T2	46.93 ± 6.43^{a}	14.42 ± 0.82^{a}	56.45 ± 3.19^{a}
	T_T3	66.3 ± 27.87^{a}	9.63 ± 0.43^{b}	52.16 ± 5.79^{a}
PIRSA	C_T1	73.04 ± 1.27^{a}	19.41 ± 1.81^{a}	123.75 ± 0.5^{a}
	C_T2	54.6 ± 2.42^{ab}	8.62 ± 1.11^{b}	79.75 ± 4.53^{b}
	C_T3	32.98 ± 3.55^{b}	11.27 ± 2.47^{ab}	81.8 ± 1.31^{b}
	T_T1	55.42 ± 9.68^{ab}	12.03 ± 2.67^{ab}	53.99 ± 0.88^{c}
	T_T2	58.01 ± 5.7^{a}	9.6 ± 0.98^{ab}	31.12 ± 0.84^{d}
	T_T3	43.48 ± 1.06^{b}	15.81 ± 2.28^{ab}	22.65 ± 0.79^{d}
HCPSL	C_T1	5.16 ± 1.03^{a}	7.01 ± 5.99^{a}	147.58 ± 2.92^{c}
	C_T2	14.26 ± 2.95^{a}	5.13 ± 1.81^{a}	157.4 ± 5.6^{bc}
	C_T3	2.09 ± 1.73^{b}	1.48 ± 0.33^{a}	47.11 ± 1.31^{d}
	T_T1	7.36 ± 1.22^{ab}	9.06 ± 0.47^{a}	186.17 ± 2.48^{a}
	T_T2	13.43 ± 0.42^{ab}	5.63 ± 1.16^{a}	178.84 ± 4.17^{ab}
	T_T3	11.79 ± 0.54^{ab}	7.27 ± 0.53^{a}	161.26 ± 9.01^{bc}

SFL - Soils for Life Trust - Canberra, ACT, CWFS - Central West Farming Systems Inc. - Condobolin, NSW, FGp - Facey Group Inc. - Wickepin WA, PIRSA - Department of Primary Industries and Regions in South Australia, HCPSL - Herbert Cane Productivity Services Ltd. - Ingham, QLD. C = conventionally managed soil, T = Cover crop management in SFL and CWFS, Bednar treatment in FGp, Lime application in PIRSA, and minimum tillage with mixed fallow cover crop management in HCPSL. T1 = no stress (50% WFPS), T2 = moderate stress

(30% WFPS), T3 = severe stress (20% WFPS). Means of the treatments the same letter is not different at the 5% level of significance for the resistance in each comparison.

The resilience indices of the stress treatments for soil enzyme activity also showed notable patterns (Table 5.6). In SFL, there was no difference in the resilience index for BG or P activity between stress treatments under either cover crop management history or conventional management history. However, the resilience index for NAG activity was significantly higher in severe stress treatment under cover crop management compared to moderate stress treatment and both stress treatments under conventional management. In CWFS, no difference was observed in the resilience index for BG activity between stress treatments under either cover crop management or conventional management. The resilience index for NAG activity was significantly higher in moderate stress treatment under conventional management compared to all other stress treatments. No difference in the resilience index for P activity was noted between stress treatments under cover crop management, while under conventional management, the resilience index for P activity was lower in severe stress treatment compared to moderate stress treatment. In FGp, there was no difference in the resilience index for BG, NAG, or P activity between stress treatments under either Bednar management history or conventional management history. In PIRSA, the resilience index for BG activity in both water stress treatments under lime management was significantly higher than in water stress treatments under conventional management. There was no difference in the resilience index for NAG activity between stress treatments under either lime management or conventional management. However, severe water stress treatment showed the lowest resilience index for P activity under lime management. In HCPSL, no difference was observed in the resilience index for BG or NAG activity between stress treatments under either excessive tillage management history or minimum tillage with mixed fallow cover crop management history. Severe water stress treatment showed the lowest resilience index for P activity under excessive tillage management history.

Table 5.6. Resilience index of enzyme activity in soil across varied management histories under water stress treatments

Site	Treatment	Resilience Index of Enzyme Activity (RL)		
		β-glucosidase (BG)	**N-acetyl-β-D-glucosaminidase (NAG)**	**phosphatase (P)**
SFL	C_T2	-0.39 ± 0.16[a]	-0.3 ± 0.1[c]	-0.48 ± 0.33[a]
	C_T3	0.13 ± 0.32[a]	0.24 ± 0.06[b]	0 ± 0.37[a]
	T_T2	-0.08 ± 0.31[a]	0.46 ± 0.12[b]	-0.39 ± 0.29[a]
	T_T3	-0.47 ± 0.05[a]	0.93 ± 0.06[a]	0.48 ± 0.06[a]
CWFS	C_T2	0.15 ± 0.2[a]	0.69 ± 0.07[a]	0.32 ± 0.22[a]
	C_T3	-0.3 ± 0.26[a]	-0.73 ± 0.04[b]	-0.63 ± 0.1[b]
	T_T2	0.54 ± 0.06[a]	-0.81 ± 0.17[b]	0.23 ± 0.14[a]
	T_T3	-0.09 ± 0.38[a]	-0.51 ± 0[b]	0.27 ± 0.24[a]
FGp	C_T2	0.67 ± 0.2[ab]	0.4 ± 0.26[a]	0.29 ± 0.28[a]
	C_T3	0.73 ± 0.2[a]	0.36 ± 0.29[a]	0.16 ± 0.26[a]
	T_T2	-0.55 ± 0.44[b]	0.86 ± 0.03[a]	0.31 ± 0.12[a]
	T_T3	0.42 ± 0.11[ab]	0.28 ± 0.1[a]	0.46 ± 0.14[a]
PIRSA	C_T2	-0.5 ± 0.07[b]	-0.2 ± 0.18[a]	-0.19 ± 0.03[a]
	C_T3	-0.58 ± 0.14[b]	-0.21 ± 0.47[a]	-0.1 ± 0.03[a]
	T_T2	0.8 ± 0.05[a]	-0.32 ± 0.5[a]	-0.2 ± 0.1[a]
	T_T3	0.66 ± 0.16[a]	0.28 ± 0.16[a]	-0.62 ± 0.09[b]
HCPSL	C_T2	-0.31 ± 0.34[a]	-0.27 ± 0.17[a]	0.7 ± 0.15[a]
	C_T3	0.37 ± 0.29[a]	0.29 ± 0.5[a]	-0.08 ± 0.02[b]
	T_T2	0.33 ± 0.27[a]	0.7 ± 0.07[a]	0.88 ± 0.11[a]
	T_T3	0.46 ± 0.18[a]	0.81 ± 0.02[a]	0.66 ± 0.15[a]

SFL - Soils for Life Trust - Canberra, ACT, CWFS - Central West Farming Systems Inc. - Condobolin, NSW, FGp - Facey Group Inc. - Wickepin WA, PIRSA - Department of Primary Industries and Regions in South Australia, HCPSL - Herbert Cane Productivity Services Ltd. - Ingham, QLD. C = conventionally managed soil, T = Cover crop management in SFL and CWFS, Bednar treatment in FGp, Lime application in PIRSA, and minimum tillage with mixed fallow cover crop management in HCPSL. T2 = moderate stress (30% WFPS), T3 = severe stress (20% WFPS). Means of the treatments the same letter is not different at the 5% level of significance for the resistance in each comparison.

The observed patterns in enzyme activity and their resistance and resilience indices across different soil management practices can be attributed to several factors. After 14 days of water stress followed by 14 days of stress release, enzyme activities (β-glucosidase (BG), N-acetyl-β-D-glucosaminidase (NAG), and phosphatase (P)) displayed distinct variations depending on the management history. In SFL, similar enzyme activities between stress treatments and control under both cover crop and conventional management histories suggest a stable microbial community less affected by water stress. In contrast, CWFS showed higher NAG and P activities under no stress and cover crop management, indicating a more robust microbial response to cover crop benefits. FGp results highlighted that conventional management leads to lower BG and NAG activities under stress, whereas the Bednar treatment showed resilience except under severe stress. PIRSA's higher enzyme activities under lime management indicate that lime amendments might buffer the soil against stress impacts, unlike conventional practices. HCPSL's enzyme activities were significantly influenced by tillage intensity, with minimum tillage showing higher resilience and activity than excessive tillage, suggesting that reduced disturbance promotes a more resilient microbial community. These differences can be explained by the varying capacities of microbial communities to adapt to stress, influenced by soil amendments, organic matter input from cover crops, and the degree of physical disturbance from tillage practices. The resistance and resilience indices further emphasise that management practices enhancing soil organic matter and reducing disturbance (like cover crops and minimum tillage) contribute to more stable enzyme activities, indicating a more resilient soil microbial community capable of maintaining function under stress conditions.

5.4.3 The Effect of Moisture Stress on Soil Chemical Properties

5.4.3.1 Hot Water Extractable Organic Carbon Content

At the end of the 14-day period, HWEOC content in soil across various management practices exhibited distinct patterns. In SFL, there was no difference in HWEOC content between stress treatments and the control under either cover crop or conventional management history. However, severe water stress under cover crop management showed a lower HWEOC content compared to no stress and moderate stress treatments under conventional management (Figure 5.14A). In CWFS, HWEOC content did not differ between stress treatments and the control under conventional management, but severe water stress under cover crop management exhibited lower HWEOC content compared to no stress. Additionally, moderate and severe water stress under conventional management had lower HWEOC content compared to no stress and moderate stress under cover crop management (Figure 5.14B). In FGp, there was no difference in HWEOC content between stress treatments and the control under either Bednar or conventional management history, although severe water stress under Bednar management had lower HWEOC content compared to moderate stress under conventional management (Figure 5.14C). In PIRSA, HWEOC content did not differ between stress treatments and the control under either lime or conventional management history, but no stress treatment under lime management showed lower HWEOC content compared to moderate stress under conventional management (Figure 5.14D). In HCPSL, there was no difference in HWEOC content between stress treatments and the control under either excessive tillage or minimum tillage with mixed fallow cover crop management. However, moderate and severe water stress under minimum tillage with mixed fallow cover crop management showed higher HWEOC content compared to all treatments under excessive tillage (Figure 5.14E).

After 14 days of water stress followed by 14 days of stress release (Day 28), the HWEOC content in soil displayed various trends. In SFL, there was no difference in HWEOC content between stress treatments and the control under conventional management. Under cover crop management, severe water stress had lower HWEOC content than no stress, while moderate stress did not differ significantly from no stress. Comparatively, all treatments under cover crop management showed lower HWEOC content than those under conventional management (Figure 5.14A). In CWFS, there was no difference in HWEOC content between stress treatments and the control under conventional management, but severe water stress under cover crop management showed lower HWEOC content than no stress and moderate stress. Comparatively, all treatments under cover crop management exhibited higher HWEOC content compared to no stress and moderate stress under conventional management (Figure 5.14B). In FGp, there was no difference in HWEOC content between stress treatments and the control under Bednar management, but severe water stress under conventional management had lower HWEOC content than no stress (Figure 5.14C). In PIRSA, HWEOC content did not differ between stress treatments and the control under either lime or conventional management history (Figure 5.14D). In HCPSL, there was no difference in HWEOC content between stress treatments and the control under excessive tillage management, but severe water stress under minimum tillage with mixed fallow cover crop management showed higher HWEOC content compared to no stress and moderate stress treatments (Figure 5.14E).

No notable difference was observed in the resistance index of the HWEOC in stress treatments between the two soil management approaches at any of the tested sites (i.e., SFL, CWFS, FGp, PIRSA, and HCPSL) (Figure 5.15). The resilience index also did not show a significant difference in water stress treatments between the management practices at any site, except for SFL, where the severe stress treatment under cover crop management exhibited the lowest resilience index (Figure 5.15).

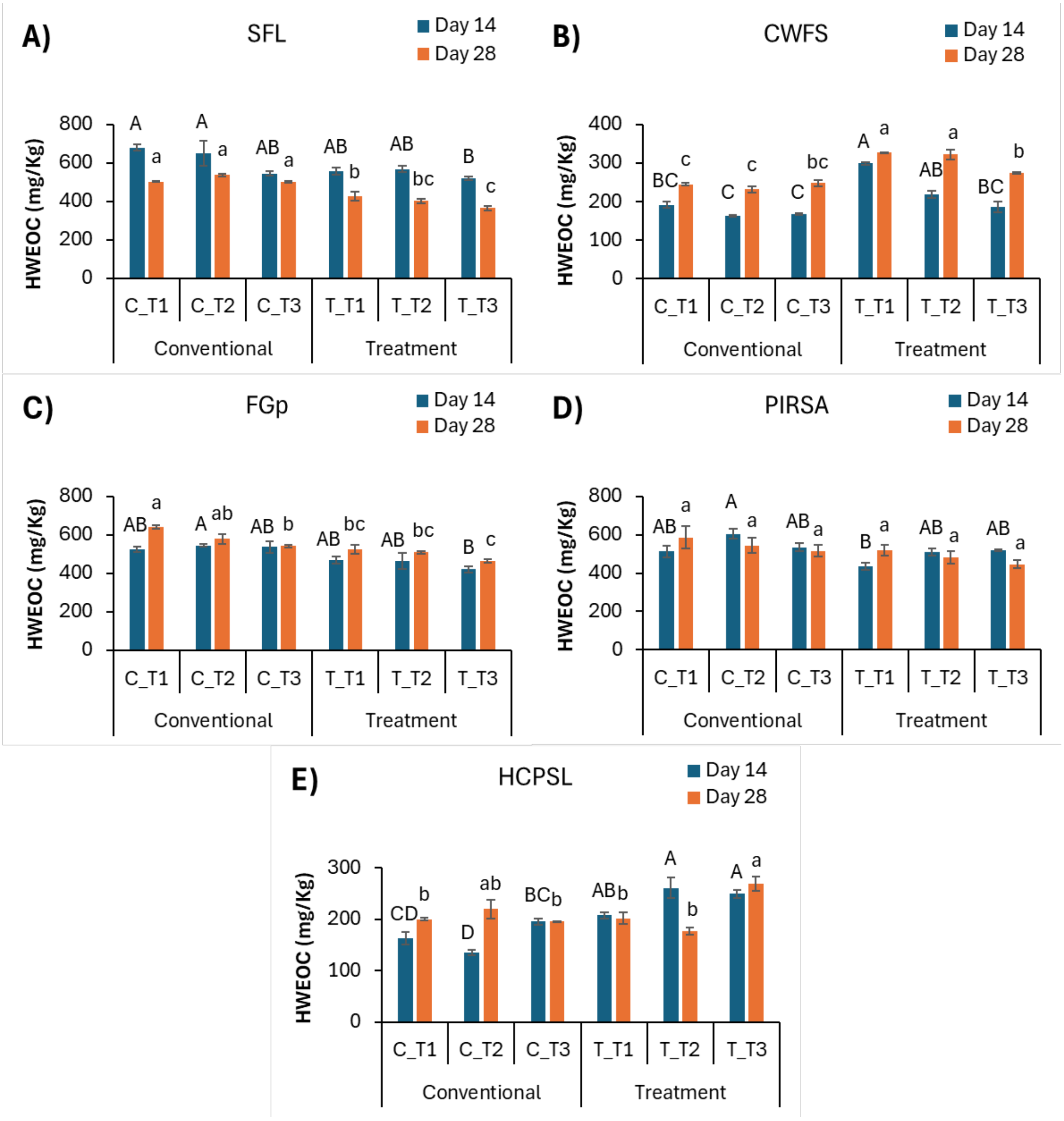

Figure 5.14. HWEOC in soil across varied management histories under water stress treatments: (A) SFL (B) CWFS (C) FGp (D) PIRSA (E) HCPSL. C = conventionally managed soil, T = Cover crop management in SFL and CWFS, Bednar treatment in FGp, Lime application in PIRSA, and minimum tillage with mixed fallow cover crop management in HCPSL. T1 = no stress (50% WFPS), T2 = moderate stress (30% WFPS), T3 = severe stress (20% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

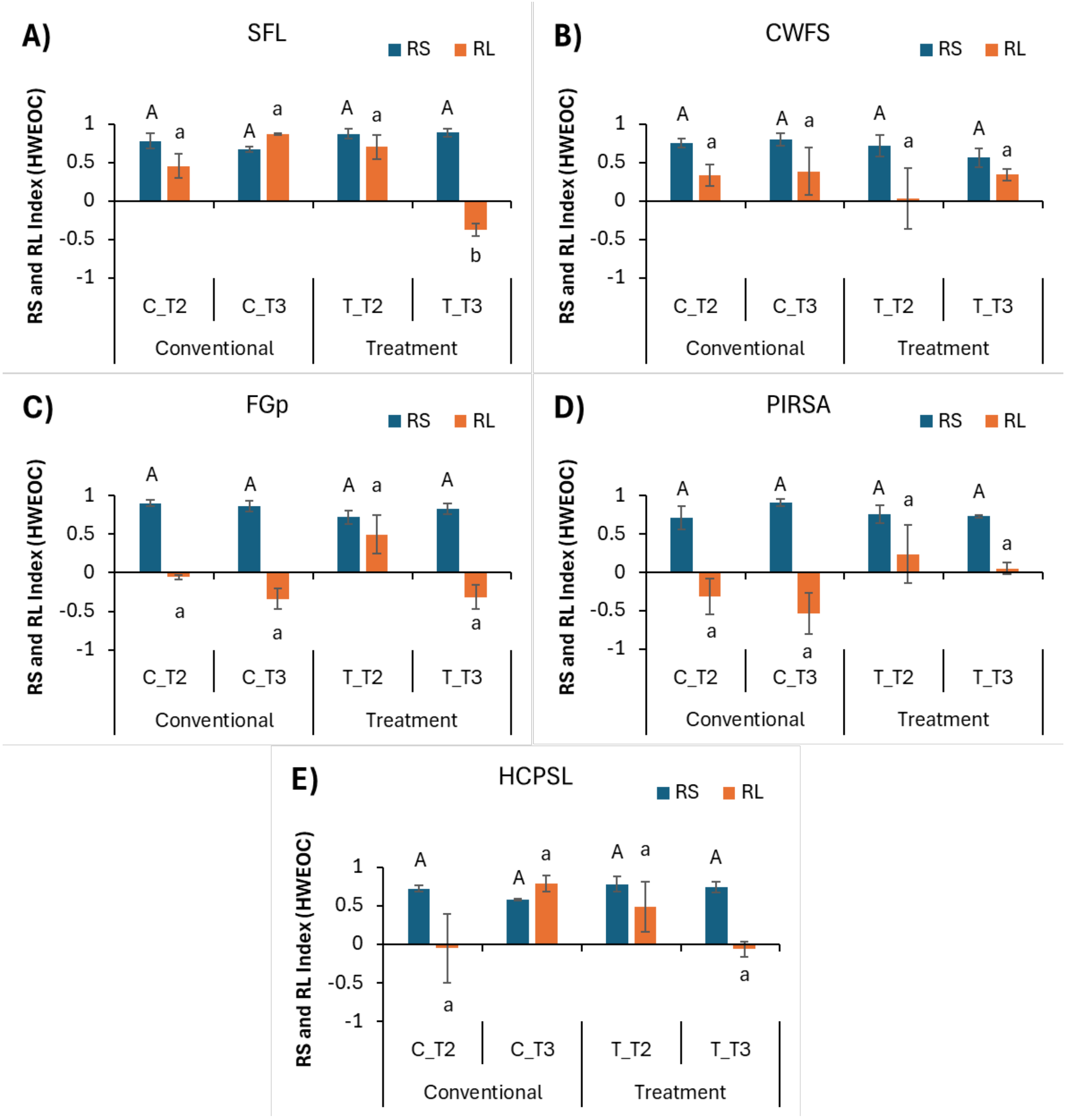

Figure 5.15. Resistance (RS) and resilience (RL) indices of HWEOC in soil across varied management histories under water stress treatments: (A) SFL (B) CWFS (C) FGp (D) PIRSA (E) HCPSL. C = conventionally managed soil, T = Cover crop management in SFL and CWFS, Bednar treatment in FGp, Lime application in PIRSA, and minimum tillage with mixed fallow cover crop management in HCPSL. T2 = moderate stress (30% WFPS), T3 = severe stress (20% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

The observed patterns in HWEOC content across different management practices and stress treatments in the pot experiment can be attributed to several factors. The stability of organic carbon in some soils might render them less sensitive to short-term water stress, explaining the lack of difference between treatments in certain cases. Cover crops could enhance microbial activity and carbon cycling under moderate stress but deplete labile carbon pools more rapidly under severe stress. Differences in microbial activity and decomposition processes between management systems, such as Bednar and conventional practices, could also influence HWEOC levels. Lime amendments and reduced tillage practices, like minimum tillage with mixed fallow cover crops, may help maintain labile carbon pools by stabilising organic matter and reducing soil disturbance. The uniform sieving of soil samples and controlled growth conditions in the pot experiment also play a role in these dynamics. Overall, the complex interplay between soil properties, management practices, and stress conditions underscores the need to consider these factors when evaluating soil carbon dynamics.

5.4.3.2 Hot Water Extractable Total Nitrogen Content

At the end of the 14-day period, hot water extractable total nitrogen (HWETN) content in soil exhibited distinct patterns across various management practices. In SFL, there was no significant difference in HWETN content between stress treatments and the control under conventional management, while severe water stress under cover crop management resulted in lower HWETN content than the no stress treatment. Additionally, no stress and moderate stress treatments under cover crop management showed higher HWETN content compared to the corresponding treatments under conventional management (Figure 5.16A). In CWFS, both moderate and severe stress treatments had lower HWETN content than the no stress treatment under conventional management, whereas only severe water stress under cover crop management led to lower HWETN content compared to the no stress treatment (Figure 5.16B). In FGp, no difference in HWETN content was observed between stress treatments and the control under either Bednar or conventional management, yet all treatments under Bednar management exhibited lower HWETN content compared to those under conventional management (Figure 5.16C). In PIRSA, HWETN content did not differ between stress treatments and the control under conventional management; however, severe water stress under lime management showed higher HWETN content than no stress and moderate stress treatments (Figure 5.16D). In HCPSL, there was no difference in HWETN content between stress treatments and the control under either excessive tillage or minimum tillage with mixed fallow cover crop management (Figure 5.16E).

After wheat plants were exposed to 14 days of water stress followed by 14 days of stress release, HWETN content showed further variations. In SFL, no difference in HWETN content was observed between stress treatments and the control under either management practice, although all treatments under cover crop management had higher HWETN content than those under conventional management (Figure 5.16A). In CWFS, no significant difference in HWETN content was found between stress treatments and the control under cover crop management, but moderate water stress under conventional management resulted in higher HWETN content than the no stress treatment (Figure 5.16B). In FGp, moderate water stress under conventional management led to higher HWETN content than the no stress treatment, while both moderate and severe water stress under Bednar management showed higher HWETN content compared to the no stress treatment (Figure 5.16C). In PIRSA, HWETN content did not differ between stress treatments and the control under either lime or conventional management (Figure 5.16D). Lastly, in HCPSL, no difference in HWETN content was observed between stress treatments and the control under excessive tillage management, but severe water stress under minimum tillage with mixed fallow cover crop management exhibited higher HWETN content compared to the moderate stress treatment (Figure 5.16E).

Severe water stress treatment under both conventional and cover crop management histories in SFL showed a lower resistance index for HWETN compared to the moderate stress treatment (Figure 5.17A). In contrast, at all other sites (CWFS, FGp, PIRSA, and HCPSL), there was no notable difference in the resistance index for HWETN between the stress treatments under the two management approaches (Figure 5.17 B, C, D, and E). Similarly, the resilience index for HWETN did not exhibit significant differences in water stress treatments between management practices at any site (Figure 5.17 A, B, D, and E), except for FGp, where the moderate stress treatment under Bednar management exhibited the highest resilience index (Figure 5.17C).

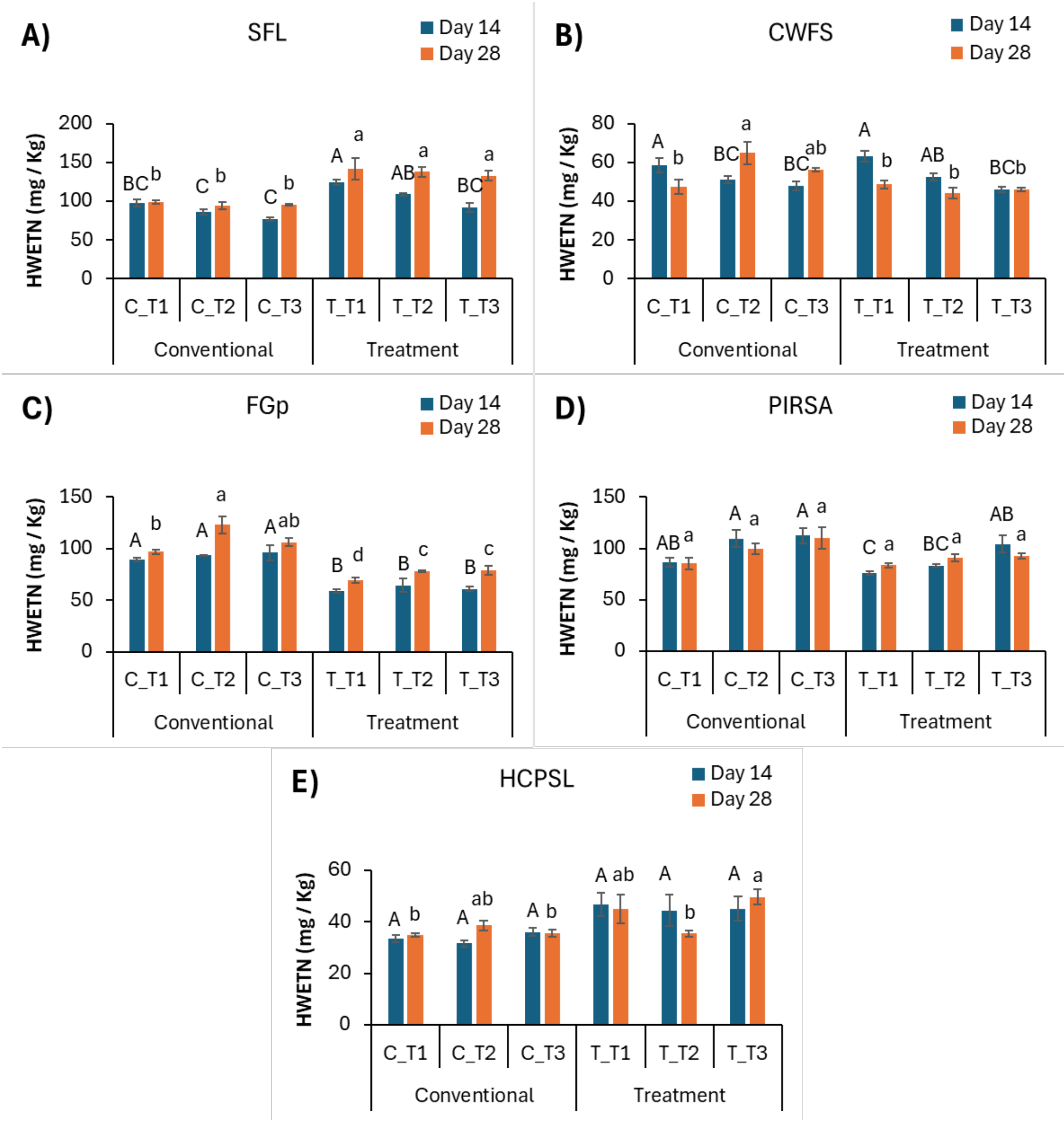

Figure 5.16. HWETN in soil across varied management histories under water stress treatments: (A) SFL (B) CWFS (C) FGp (D) PIRSA (E) HCPSL. C = conventionally managed soil, T = Cover crop management in SFL and CWFS, Bednar treatment in FGp, Lime application in PIRSA, and minimum tillage with mixed fallow cover crop management in HCPSL. T1 = no stress (50% WFPS), T2 = moderate stress (30% WFPS), T3 = severe stress (20% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

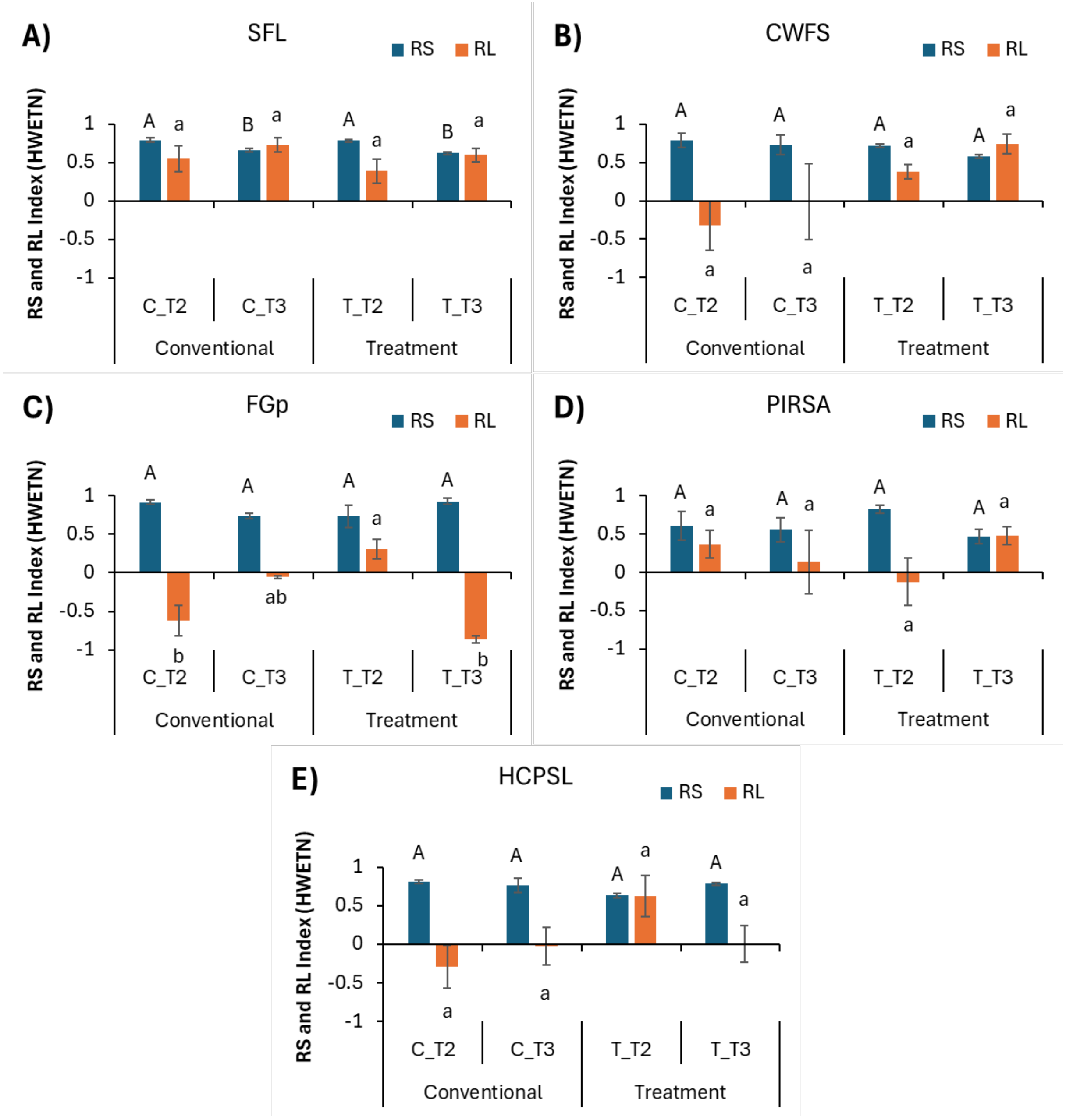

Figure 5.17. Resistance (RS) and resilience (RL) indices of HWETN in soil across varied management histories under water stress treatments: (A) SFL (B) CWFS (C) FGp (D) PIRSA (E) HCPSL. C = conventionally managed soil, T = Cover crop management in SFL and CWFS, Bednar treatment in FGp, Lime application in PIRSA, and minimum tillage with mixed fallow cover crop management in HCPSL. T2 = moderate stress (30% WFPS), T3 = severe stress (20% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

The distinct patterns in HWETN content observed in the pot experiment are influenced by the different soil management practices and their effects on soil nitrogen dynamics and plant interactions. Conventional management may not significantly affect nitrogen availability under water stress, whereas cover crop management seems to enhance nitrogen retention under optimal conditions but not under severe stress. This suggests that cover crops contribute to higher soil nitrogen content due to their nitrogen-fixing abilities and organic matter inputs. In some cases, conventional practices may deplete soil nitrogen more rapidly under stress, while cover crops can mitigate moderate stress impacts on nitrogen availability. Specific management practices like Bednar treatment may result in lower nitrogen mineralisation, highlighting the differential effects of these practices on soil nitrogen dynamics. Lime application appears to buffer the negative effects of severe water stress by enhancing microbial activity and nitrogen mineralisation. Both excessive tillage and minimum tillage with mixed fallow cover crop management maintain stable nitrogen levels under varying stress conditions. After the stress release period, cover crops continue to promote nitrogen retention and recovery, while moderate stress release conditions under conventional management can enhance nitrogen availability. In Bednar management, there may be delayed nitrogen release or enhanced mineralisation during the stress release period. Lime stabilises nitrogen content effectively, and minimum tillage with mixed fallow cover crops can increase nitrogen availability under severe stress. Severe water stress negatively impacts nitrogen retention more than moderate stress, and the resilience index shows that certain management practices, like Bednar treatment, can have superior nitrogen recovery capacities.

5.4.3.3 Soil Mineral Nitrogen

At the end of the 14-day period, the total mineral nitrogen content in soil exhibited distinct patterns across various management practices. In SFL, there was no difference in total mineral nitrogen content between stress treatments and the control under conventional management history, whereas severe water stress under cover crop management resulted in lower total mineral nitrogen content than the no stress treatment. Additionally, no stress and moderate stress treatments under cover crop management showed higher total mineral nitrogen content than all stress treatments under conventional management, and severe stress treatment under cover crop management showed higher total mineral nitrogen content than both stress treatments under conventional management (Figure 5.18A). In CWFS, there was no difference in total mineral nitrogen content between stress treatments and the control under conventional management history. However, under cover crop management history, both moderate and severe water stress treatments showed lower total mineral nitrogen content than the no stress treatment, and the no stress treatment under cover crop management showed the highest total mineral nitrogen content compared to all other treatments in conventional management history (Figure 5.18B). In FGp, no difference in total mineral nitrogen content was observed between stress treatments and the control under either Bednar treatment management history or conventional management history. Comparing the two management approaches, no stress and moderate stress treatments under Bednar treatment history showed lower total mineral nitrogen content compared to the no stress treatment under conventional management history (Figure 5.18C). In PIRSA, there was no difference in total mineral nitrogen content between stress treatments and the control under either conventional management history or lime management history. However, under lime management history, the severe water stress treatment showed higher total mineral nitrogen content than the no stress treatment under conventional management history (Figure 5.18D). In HCPSL, there was no difference in total mineral nitrogen content between stress treatments and the control under either excessive tillage history or minimum tillage with mixed fallow cover crop management history. However, moderate and severe water stress treatments under minimum tillage with mixed fallow cover crop management history showed higher total mineral nitrogen content compared to all treatments under excessive tillage history (Figure 5.18E).

After wheat plants were exposed to 14 days of water stress followed by 14 days of stress release, total mineral nitrogen content showed further variations. In SFL, there was no difference in total mineral nitrogen content between stress treatments and the control under either cover crop management history or conventional management history. However, when comparing the two management approaches, all treatments under cover crop management history showed higher total mineral nitrogen content compared to the moderate and severe water treatments under conventional management history (Figure 5.18A). In CWFS, there was no difference in total mineral nitrogen content between stress treatments and the control under either cover crop management history or conventional management history. Under conventional management history, moderate water stress treatment showed higher total mineral nitrogen content than all treatments under cover crop management history (Figure 5.18B). In FGp, under conventional management history, moderate and severe water stress treatments showed higher total mineral nitrogen content than the no stress treatment. Under Bednar treatment history, there was no difference in total mineral nitrogen content between stress treatments and the control, but a lower total mineral nitrogen content was observed in all treatments under Bednar treatment history compared to moderate and severe stress treatments under conventional management history (Figure 5.18C). In PIRSA, severe stress treatment showed higher total mineral nitrogen content than the no stress treatment under conventional management history, while under lime treatment history, both moderate and severe stress treatments showed higher total mineral nitrogen content than the no stress treatment (Figure 5.18D). Lastly, in HCPSL, there was no difference in total mineral nitrogen content between stress treatments and the control under excessive tillage management history, but under minimum tillage with mixed fallow cover crop management history, the severe water stress treatment showed higher total mineral nitrogen content than the no stress and moderate stress treatments (Figure 5.18E).

In SFL, the highest resistance index for total mineral nitrogen was observed in the moderate stress treatment under cover crop management history (Figure 5.19A). No notable difference in the resistance index of total mineral nitrogen was found between stress treatments and management approaches in CWFS (Figure 5.19B), FGp (Figure 5.19C), and PIRSA (Figure 5.19D). In HCPSL, the resistance index of total mineral nitrogen under severe stress treatment did not significantly differ between excessive tillage management and minimum tillage with mixed fallow cover crop management. However, in the moderate stress treatment, the resistance index was higher under excessive tillage management compared to minimum tillage with mixed fallow cover crop management (Figure 5.19E). The resilience index of total mineral nitrogen did not show significant differences in water stress treatments between management practices at any site (Figure 5.19A, B, C and D), except for HCPSL, where the moderate stress treatment under minimum tillage with mixed fallow cover crop management exhibited a higher resilience index than both moderate and severe stress treatments under excessive tillage management (Figure 5.19E).

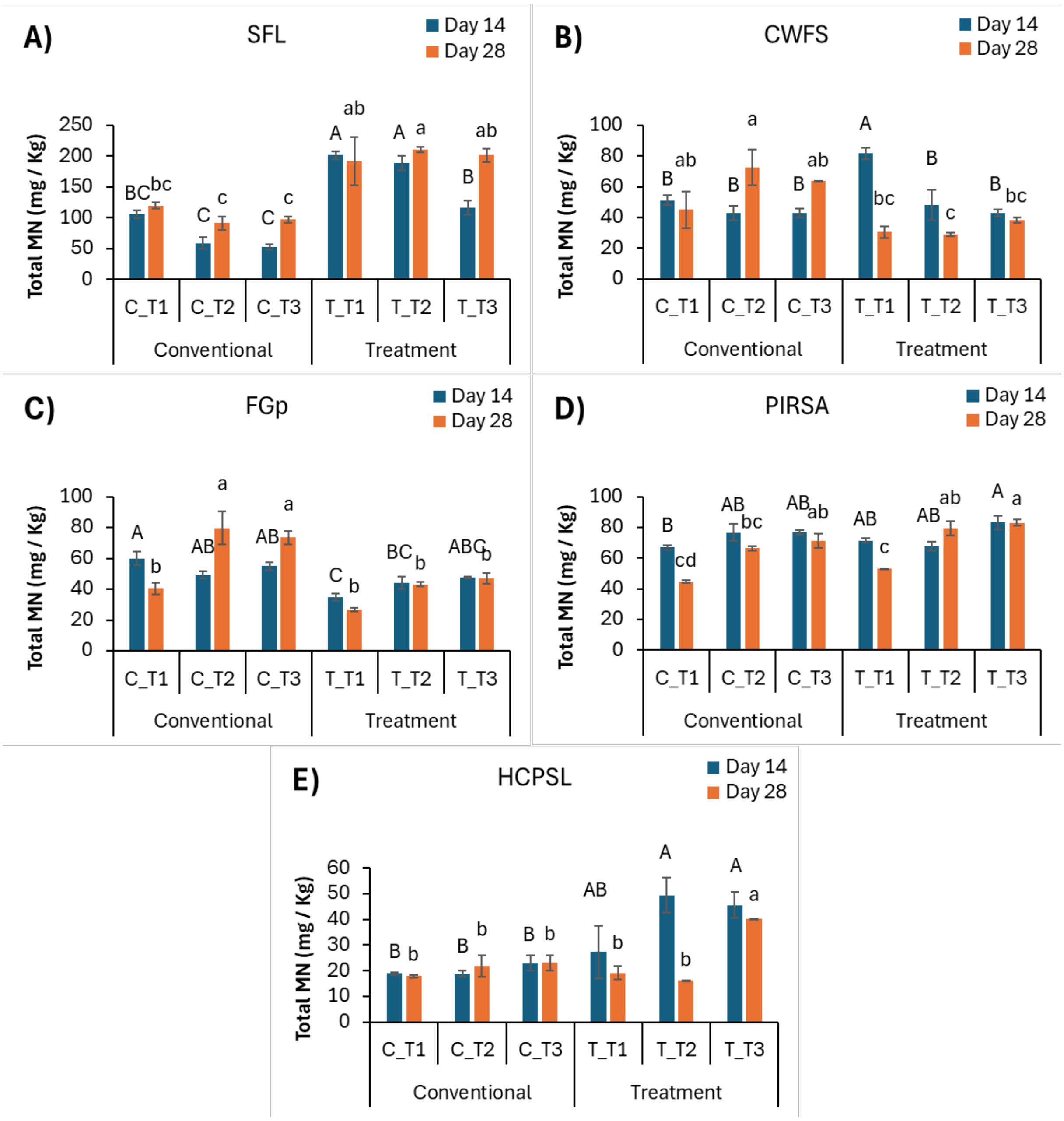

Figure 5.18. Total mineral nitrogen content (Total MN) in soil across varied management histories under water stress treatments: (A) SFL (B) CWFS (C) FGp (D) PIRSA (E) HCPSL. C = conventionally managed soil, T = Cover crop management in SFL and CWFS, Bednar treatment in FGp, Lime application in PIRSA, and minimum tillage with mixed fallow cover crop management in HCPSL. T1 = no stress (50% WFPS), T2 = moderate stress (30% WFPS), T3 = severe stress (20% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

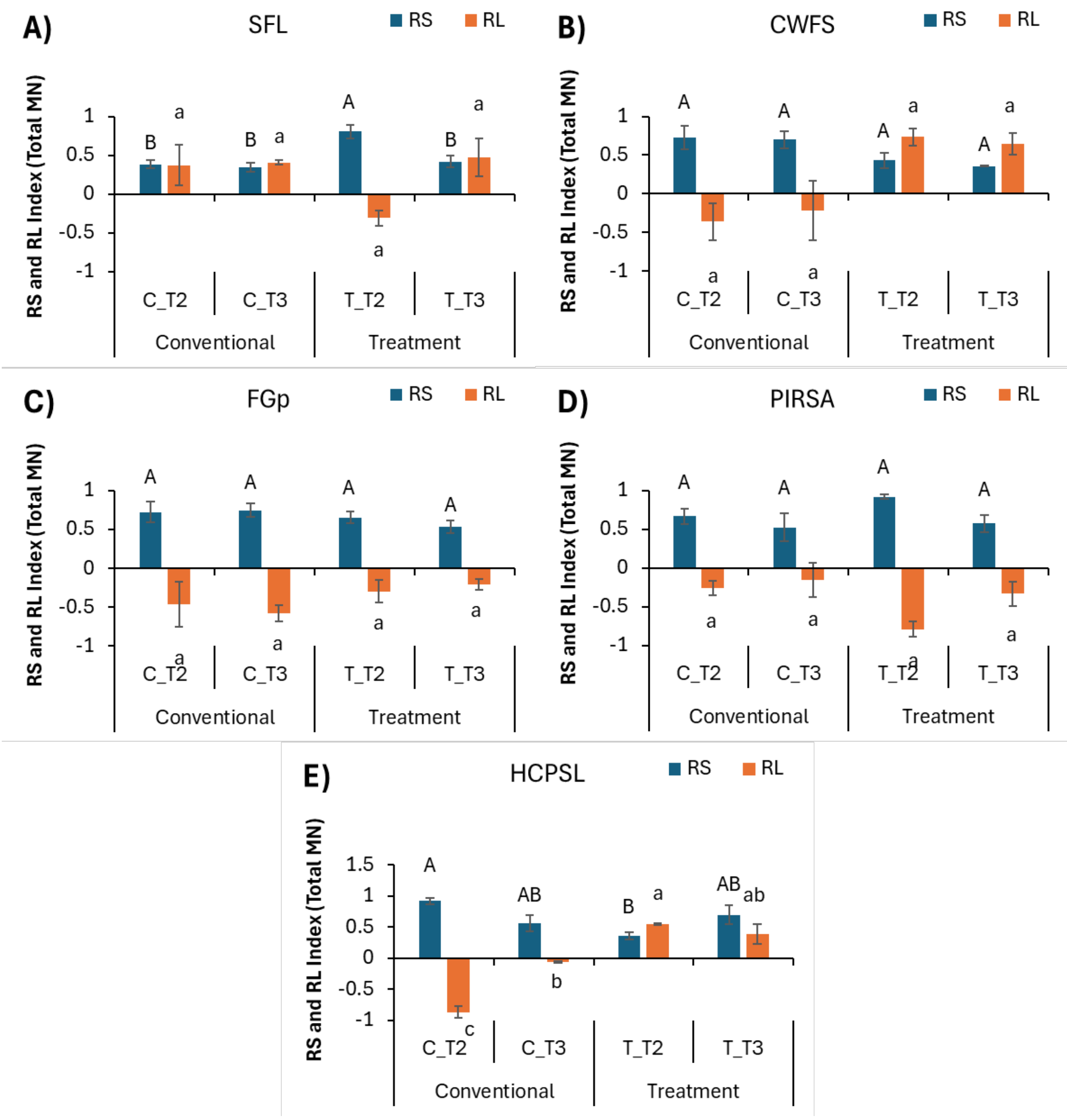

Figure 5.19. Resistance (RS) and resilience (RL) indices of Total mineral nitrogen content (Total MN) in soil across varied management histories under water stress treatments: (A) SFL (B) CWFS (C) FGp (D) PIRSA (E) HCPSL. C = conventionally managed soil, T = Cover crop management in SFL and CWFS, Bednar treatment in FGp, Lime application in PIRSA, and minimum tillage with mixed fallow cover crop management in HCPSL. T2 = moderate stress (30% WFPS), T3 = severe stress (20% WFPS). Error bars represent standard error. Means of the treatments the same capital letter is not different at the 5% level of significance for the resistance. Means of the treatments the same simple letter is not different at the 5% level of significance for the resilience.

The distinct patterns of total mineral nitrogen content observed across various management practices in the pot experiment can be attributed to the differing impacts of soil management history and water stress on soil nutrient dynamics. Soil samples from paired locations with conventional and ecosystem-based management histories likely had inherent differences in soil structure, organic matter content, and microbial activity, which influenced their response to water stress and recovery. Under cover crop management, the lower total mineral nitrogen content under severe water stress can be explained by the disruption of microbial processes responsible for nitrogen mineralisation, while the higher total mineral nitrogen content under no and moderate stress treatments suggests enhanced nitrogen retention and availability under non-stress conditions. In contrast, conventional management showed stable total mineral nitrogen content across stress treatments, indicating a less dynamic nutrient pool. The overall lower total mineral nitrogen under Bednar management compared to conventional practices implies less effective nitrogen conservation, while lime management in PIRSA mitigated the impact of severe water stress, likely due to improved soil pH and microbial activity. Minimum tillage with mixed fallow cover crop management in HCPSL maintained higher total mineral nitrogen content under stress, reflecting better soil structure and organic matter content. The resistance and resilience indices highlight that cover crop management in SFL enhances stability and nutrient retention, while conventional tillage in HCPSL offers temporary stability. The resilience indices suggest that except for HCPSL, where minimum tillage with mixed fallow cover crop management showed superior recovery, other sites did not exhibit significant differences between management practices, indicating complex interactions between soil management, stress, and recovery processes.

5.4.4 The Effect of Moisture Stress on Plant Parameters

5.4.4.1 Shoot Biomass

At the end of the 14-day drought exposure period, following 14 days under drought release conditions in the pot experiment, shoot dry matter content varied across soils with different management practices. In the SFL soil, there was no difference in shoot dry matter content between stress treatments and the control, regardless of whether the soil had a conventional or cover crop management history. However, under moderate water stress treatment in cover crop management history, shoot dry matter content was higher compared to severe water stress treatments under both conventional and cover crop management histories (Figure 5.20A). In the CWFS soil, severe water stress treatment under conventional management resulted in lower shoot dry matter content compared to the no stress treatment, while soils from the cover crop management history showed higher shoot dry matter content under no stress treatment than both moderate and severe water stress treatments in soils with conventional and cover crop management histories (Figure 5.20B). For FGp soil, moderate and severe water stress treatments under both conventional and Bednar management resulted in lower shoot dry matter content compared to the no stress treatment (Figure 5.20C). In PIRSA soil, moderate and severe water stress treatments under conventional management showed lower shoot dry matter content compared to the no stress treatment. In contrast, under lime treatment history, only severe stress treatment resulted in lower shoot dry matter content compared to the no stress treatment, while moderate stress treatment did not show a significant difference in shoot dry matter content compared to the no stress treatment (Figure 5.20D). For HCPSL soil, severe water stress treatment under both excessive tillage management history and minimum tillage with mixed fallow cover crop management history showed lower shoot dry matter content compared to the no stress treatment. Additionally, both no stress and moderate stress treatments under minimum tillage with mixed fallow cover crop management history exhibited higher shoot dry matter content compared to all treatments under soil from excessive tillage management history. These findings suggest that soil management history influences plant response to drought stress, with cover crop and minimum tillage practices generally supporting higher shoot dry matter content under no or moderate stress conditions (Figure 5.20E).

The observed variation in shoot dry matter content across different soil management histories in the pot experiment can be attributed to several scientific factors. Soils from ecosystem-based management practices, such as cover cropping and minimum tillage, often have better soil structure and higher organic matter content compared to conventionally managed soils, enhancing water retention and nutrient availability. For example, the higher shoot dry matter content in SFL soils under moderate water stress in cover crop management suggests that these practices improve soil resilience to drought by enhancing soil organic matter and microbial activity. Similarly, in the CWFS soils, cover crop management resulted in higher shoot dry matter content under no stress conditions, indicating improved nutrient cycling and soil physical properties. In FGp and PIRSA soils, the reduced shoot dry matter under water stress treatments highlights the impact of water availability, while the lime treatment in PIRSA soils suggests that improved soil pH and nutrient availability can mitigate drought effects. The HCPSL results, where minimum tillage with mixed fallow cover cropping showed higher shoot dry matter content, emphasise the benefits of these practices in improving soil health, water infiltration, and root growth, supporting better plant biomass even under moderate drought stress. These findings highlight the role of sustainable soil management practices in enhancing plant resilience to drought and improving crop productivity.

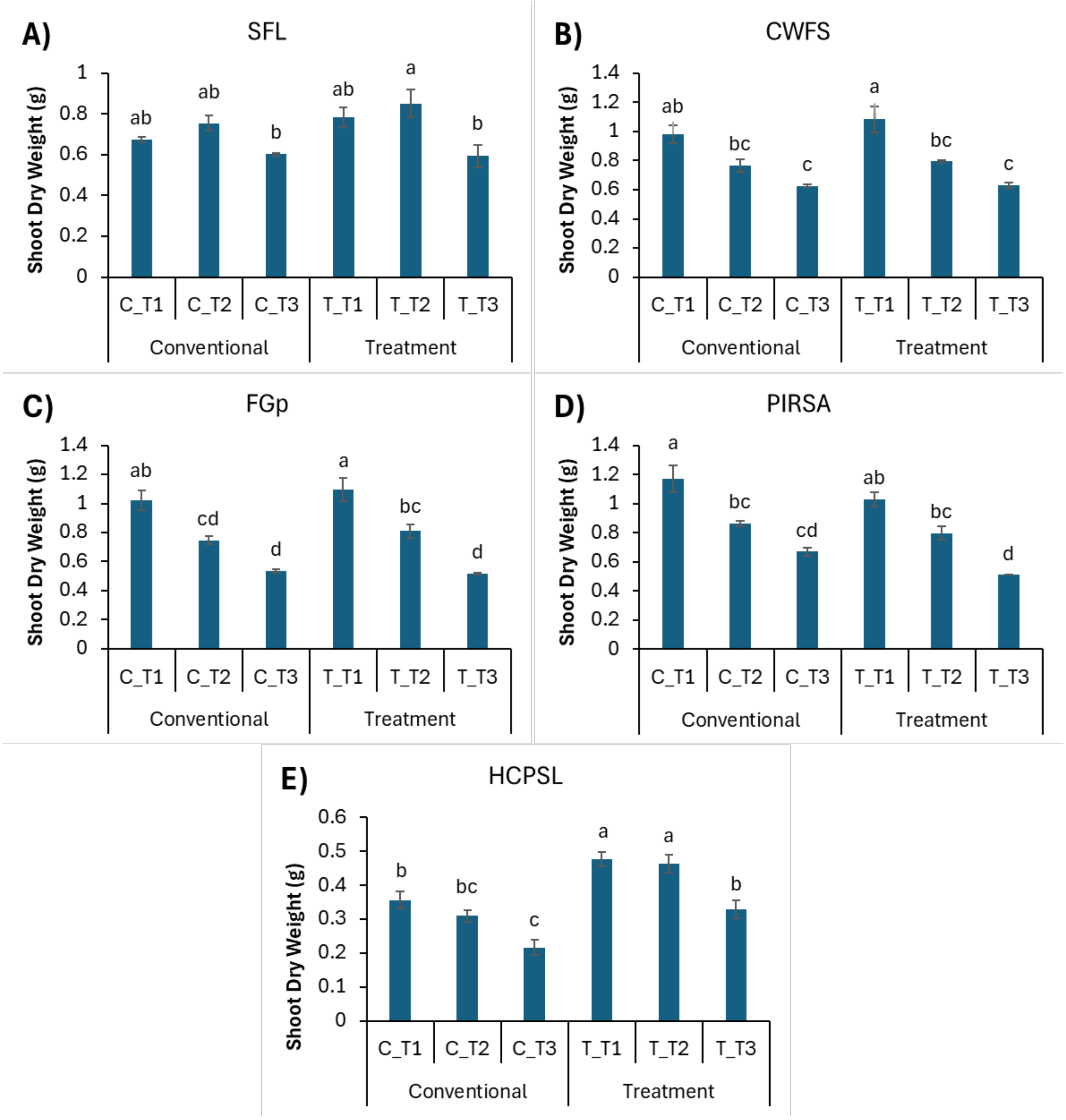

Figure 5.20. Dry matter content in wheat shoot grown in soil across varied management histories under water stress treatments: (A) SFL (B) CWFS (C) FGp (D) PIRSA (E) HCPSL. C = conventionally managed soil, T = Cover crop management in SFL and CWFS, Bednar treatment in FGp, Lime application in PIRSA, and minimum tillage with mixed fallow cover crop management in HCPSL. T1 = no stress (50% WFPS), T2 = moderate stress (30% WFPS), T3 = severe stress (20% WFPS). Error bars represent standard error. Means of the treatments the same letter is not different at the 5% level of significance.

5.4.4.2 Root Biomass

At the end of the 14-day drought exposure period, following 14 days under drought release conditions in the pot experiment, root dry matter content varied across soils with different management practices. In the SFL soil, there was no difference in root dry matter content between stress treatments and the control, regardless of whether the soil had a conventional or cover crop management history (Figure 5.21A). In the CWFS soil, the no stress treatment under cover crop management showed higher root dry matter content compared to the moderate water stress treatment under cover crop management history and all treatments under conventional management history (Figure 5.21B). For FGp soil, both no stress and moderate water stress treatments under Bednar management exhibited higher root dry matter content compared to the severe water stress treatment under Bednar management and all treatments under conventional management history (Figure 5.21C). In PIRSA soil, there was no difference in root dry matter content between stress treatments and the no stress treatment in lime management history. However, under conventional management history, the severe stress treatment showed lower root dry matter content compared to the no stress treatment (Figure 5.21D). In HCPSL soil, there was no difference in root dry matter content between stress treatments and the no stress treatment under minimum tillage with mixed fallow cover crop management history. Conversely, under excessive tillage management history, the severe water stress treatment resulted in lower root dry matter content compared to the no stress treatment (Figure 5.21E).

The observed differences in root dry matter content across soils with various management histories in the pot experiment can be attributed to the impact of soil management practices on soil structure, organic matter content, and nutrient availability. Soils from ecosystem-based management practices, such as cover cropping and minimum tillage, generally have better soil health, leading to enhanced root growth and resilience under stress conditions. For instance, in the SFL soil, the lack of difference in root dry matter content suggests balanced soil fertility and structure in both management histories. Higher root dry matter content in CWFS soil under no stress with cover crop management indicates improved soil conditions due to increased organic matter and microbial activity. Similarly, in FGp soil, Bednar management practices supported better root development compared to conventional management, likely due to improved soil aeration and reduced compaction. In PIRSA soil, lime management buffered drought effects, maintaining consistent root growth, while conventional management showed reduced root growth under severe stress. HCPSL soil results highlighted that minimum tillage with cover cropping maintained stable root growth, whereas excessive tillage led to reduced root growth under severe stress. These findings emphasise the role of sustainable soil management in enhancing root growth and plant resilience to drought.

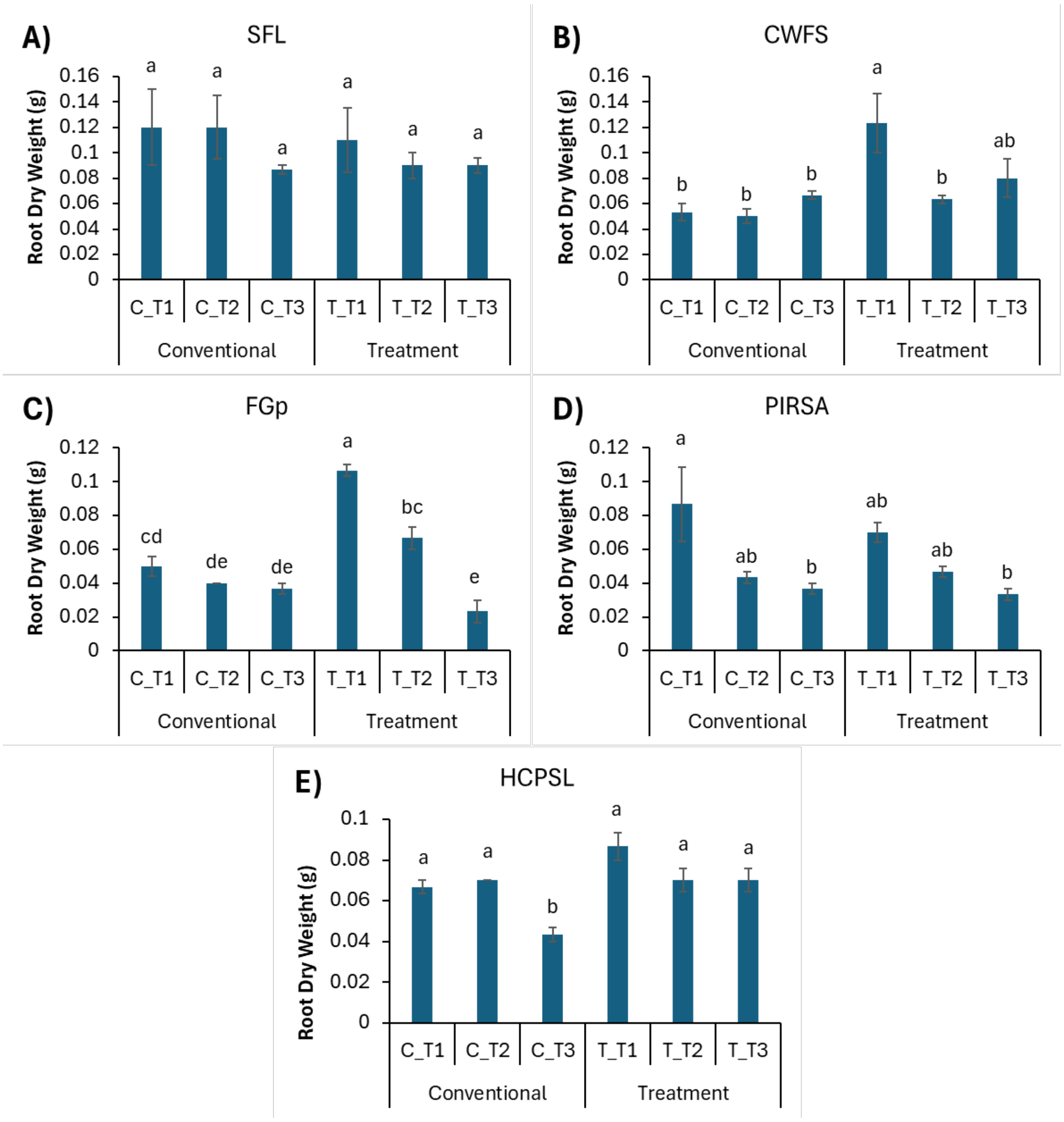

Figure 5.21. Dry matter content in wheat root grown in soil across varied management histories under water stress treatments: (A) SFL (B) CWFS (C) FGp (D) PIRSA (E) HCPSL. C = conventionally managed soil, T = Cover crop management in SFL and CWFS, Bednar treatment in FGp, Lime application in PIRSA, and minimum tillage with mixed fallow cover crop management in HCPSL. T1 = no stress (50% WFPS), T2 = moderate stress (30% WFPS), T3 = severe stress (20% WFPS). Error bars represent standard error. Means of the treatments the same letter is not different at the 5% level of significance

5.4.4.3 Shoot and Root Carbon and Nitrogen Content

At the end of the 14-day drought exposure followed by 14 days of recovery, the shoot carbon and nitrogen content in wheat plants varied depending on soil management history across the different sites (Table 5.7). In the SFL site, no significant differences were observed in shoot carbon and nitrogen percentages between stress treatments and control for soils from both conventional and cover crop management histories. However, soil carbon content was generally higher in all treatments under conventional management soils compared to cover crop soils. Interestingly, shoot nitrogen content under no stress and severe stress conditions was higher in cover crop management soils than in conventional soils, with the moderate water stress treatment in cover crop soils showing higher shoot nitrogen content than both moderate and severe stress treatments in conventional soils. For the CWFS site, no differences in shoot carbon and nitrogen percentages were found in conventional management soils, while in cover crop soils, the no stress treatment had higher shoot carbon content compared to moderate and severe stress treatments. In contrast, shoot nitrogen was lower in the no-stress condition compared to the moderate and severe water stress treatments. At the FGp site, shoot carbon content remained consistent between the no-stress and moderate stress treatments in Bednar management soils, but severe water stress significantly reduced shoot carbon. In conventional soils, both moderate and severe stress reduced shoot carbon compared to no stress. Shoot nitrogen percentages did not differ between stress treatments and control in conventional soils, though severe water stress increased shoot nitrogen compared to no stress in Bednar management soils. At the PIRSA site, no differences were observed in shoot carbon and nitrogen percentages across all stress treatments in both conventional and lime management soils. Meanwhile, at HCPSL, shoot carbon content remained stable across treatments in both minimum tillage with mixed fallow cover crop and excessive tillage soils. However, severe water stress in the minimum tillage soil resulted in higher shoot nitrogen content compared to moderate stress and no stress treatments.

In terms of root carbon and nitrogen content, results were more consistent across sites (Table 5.7). At the SFL and CWFS sites, no significant differences in root carbon and nitrogen percentages were observed between stress treatments and control, regardless of whether soils were from conventional or cover crop management histories. A similar pattern was seen at the FGp site, where no differences were found across stress treatments in either conventional or Bednar management soils. However, under severe water stress, conventional management soils exhibited higher root nitrogen content than moderate and no stress treatments in Bednar soils.

At the PIRSA site, while root carbon content remained consistent across stress treatments in conventional soils, soils from lime management history showed a higher root carbon content under severe water stress compared to no stress. Root nitrogen percentages did not differ across stress treatments in either conventional or lime management soils. Finally, at HCPSL, no significant differences in root carbon and nitrogen content were found between stress treatments and control in soils from either minimum tillage with mixed fallow cover crop management or excessive tillage management.

Table 5.7. Carbon and nitrogen percentages in wheat shoot and root grown in soil across varied management histories under water stress treatments

Site	Treatment	Shoot		Root	
		Carbon (%)	**Nitrogen (%)**	**Carbon (%)**	**Nitrogen (%)**
SFL	C_T1	36.72 ± 0.03[a]	3.90 ± 0.04[bc]	33.91 ± 5.99[a]	2.10 ± 0.31[a]
	C_T2	37.21 ± 0.20[a]	3.69 ± 0.03[c]	37.40 ± 2.59[a]	2.21 ± 0.17[a]
	C_T3	36.54 ± 0.13[a]	3.76 ± 0.05[c]	39.81 ± 1.28[a]	2.31 ± 0.12[a]
	T_T1	33.90 ± 0.24[b]	4.32 ± 0.03[a]	37.61 ± 3.31[a]	2.37 ± 0.22[a]
	T_T2	34.83 ± 0.35[b]	4.06 ± 0.08[ab]	38.84 ± 0.70[a]	2.62 ± 0.12[a]
	T_T3	33.91 ± 0.23[b]	4.16 ± 0.05[a]	31.63 ± 0.64[a]	2.07 ± 0.07[a]
CWFS	C_T1	33.05 ± 0.10[c]	4.19 ± 0.02[ab]	39.32 ± 0.76[a]	2.95 ± 0.15[a]
	C_T2	32.98 ± 0.43[c]	4.28 ± 0.03[a]	40.40 ± 0.43[a]	2.90 ± 0.06[a]
	C_T3	32.68 ± 0.22[c]	4.40 ± 0.06[a]	40.48 ± 0.20[a]	3.27 ± 0.05[a]
	T_T1	36.30 ± 0.39[a]	3.12 ± 0.23[c]	35.04 ± 5.65[a]	1.98 ± 0.41[a]
	T_T2	34.60 ± 0.16[b]	3.75 ± 0.05[b]	40.99 ± 0.12[a]	2.42 ± 0.06[a]
	T_T3	33.84 ± 0.32[bc]	3.93 ± 0.09[ab]	35.59 ± 5.99[a]	2.29 ± 0.49[a]
FGp	C_T1	35.42 ± 0.59[b]	4.02 ± 0.14[ab]	42.60 ± 0.41[a]	3.05 ± 0.17[ab]
	C_T2	33.57 ± 0.34[c]	4.24 ± 0.05[ab]	42.78 ± 0.21[a]	3.56 ± 0.05[ab]
	C_T3	33.19 ± 0.07[cd]	4.35 ± 0.03[a]	43.78 ± 0.70[a]	3.76 ± 0.29[a]
	T_T1	37.48 ± 0.53[a]	3.78 ± 0.19[b]	40.59 ± 0.84[a]	2.76 ± 0.12[b]
	T_T2	35.73 ± 0.52[ab]	4.15 ± 0.09[ab]	40.68 ± 1.26[a]	2.87 ± 0.05[b]
	T_T3	33.74 ± 0.26[bc]	4.43 ± 0.07[a]	43.61 ± 0.94[a]	3.56 ± 0.19[ab]
PIRSA	C_T1	33.11 ± 0.47[a]	4.27 ± 0.05[a]	40.72 ± 0.20[b]	2.63 ± 0.20[a]
	C_T2	33.48 ± 0.14[a]	4.04 ± 0.07[a]	41.78 ± 0.65[ab]	2.86 ± 0.11[a]
	C_T3	32.75 ± 0.17[a]	4.09 ± 0.03[a]	41.64 ± 0.37[ab]	2.84 ± 0.17[a]
	T_T1	33.22 ± 0.59[a]	4.06 ± 0.08[a]	41.22 ± 0.09[b]	2.55 ± 0.06[a]
	T_T2	33.71 ± 0.08[a]	4.04 ± 0.05[a]	42.42 ± 0.10[ab]	2.88 ± 0.15[a]
	T_T3	32.87 ± 0.07[a]	4.04 ± 0.06[a]	43.03 ± 0.41[a]	3.11 ± 0.08[a]
HCPSL	C_T1	38.39 ± 0.22[a]	2.45 ± 0.12[b]	37.75 ± 2.87[a]	1.69 ± 0.18[a]
	C_T2	38.05 ± 0.07[a]	2.72 ± 0.03[b]	37.33 ± 2.73[a]	1.63 ± 0.06[a]
	C_T3	37.77 ± 0.24[a]	3.21 ± 0.09[ab]	39.27 ± 1.43[a]	2.22 ± 0.23[a]
	T_T1	37.71 ± 0.97[a]	2.79 ± 0.15[b]	40.68 ± 0.78[a]	1.58 ± 0.04[a]
	T_T2	37.42 ± 0.48[a]	2.87 ± 0.14[b]	40.9 ± 0.46[a]	2.13 ± 0.14[a]
	T_T3	36.66 ± 0.16[a]	3.62 ± 0.14[a]	36.54 ± 2.81[a]	2.04 ± 0.09[a]

SFL - Soils for Life Trust - Canberra, ACT, CWFS - Central West Farming Systems Inc. - Condobolin, NSW, FGp - Facey Group Inc. - Wickepin WA, PIRSA - Department of Primary Industries and Regions in South Australia, HCPSL - Herbert Cane Productivity Services Ltd. - Ingham, QLD. C = conventionally managed soil, T = Cover crop management in SFL and CWFS, Bednar treatment in FGp, Lime application in PIRSA, and minimum tillage with mixed fallow cover crop management in HCPSL. T1 = no stress (50% WFPS), T2 = moderate stress (30% WFPS), T3 = severe stress (20% WFPS). Means of the treatments the same letter is not different at the 5% level of significance for the resistance in each comparison.

The differences in shoot and root carbon and nitrogen content across soil management histories and stress treatments may be attributed to variations in soil structure, nutrient availability, and microbial activity influenced by the management practices. Conventional management soils, often associated with lower organic matter and biological diversity, may have limited nutrient cycling, which could explain the generally lower shoot nitrogen content compared to cover crop soils, which promote organic matter accumulation and microbial diversity. The higher carbon content in conventional management soils under all treatments might be due to reduced microbial decomposition of organic matter under stressed conditions, leading to its accumulation. Conversely, cover crop management, which enhances soil organic matter and water retention, could facilitate better nitrogen uptake, particularly under stress conditions. The lack of significant differences in root carbon and nitrogen across most treatments suggests that roots may be less sensitive to short-term drought and recovery, or that root adaptations, such as increased nitrogen assimilation under severe stress in some soils, compensate for the variations in soil properties. These findings highlight the complex interactions between soil management practices, plant nutrient dynamics, and water stress responses.

5.5 Conclusion

This study highlights how different soil management practices influence soil biological properties and nutrient dynamics, particularly under varying stress conditions. The observed changes in MBC and MBN reflect the impact of historical soil management on microbial communities. Soils with cover cropping generally exhibited enhanced microbial resilience and nutrient availability, while practices such as excessive tillage led to decreased microbial biomass and nutrient content. Lime application showed benefits in stabilising soil pH and microbial activity, though its impact varied with stress conditions. The pot experiment demonstrated how soil management practices affect microbial respiration, metabolic efficiency, and enzyme activities under water stress. Cover cropping and minimum tillage improved microbial resilience and enzyme stability, enhancing soil health and nutrient cycling. Conversely, conventional management and excessive tillage were associated with reduced microbial activity and enzyme functions under stress, highlighting the advantages of sustainable practices in maintaining soil functionality. Additionally, variations in HWEOC and HWETN content, along with total mineral nitrogen and shoot/root dry matter, further illustrated how management practices influence soil nutrient dynamics and plant growth. Cover cropping and minimum tillage generally led to better nitrogen retention and higher plant biomass, and improve nitrogen retention and plant nutrient uptake under stress whereas conventional and excessively tilled soils showed less resilience and lower nutrient availability. Overall, these findings underscore the importance of adopting sustainable soil management practices to enhance soil health, microbial resilience, and crop productivity. Practices like cover cropping and minimum tillage offer significant benefits in maintaining soil functionality and nutrient availability under stress, supporting more stable and productive agricultural systems.

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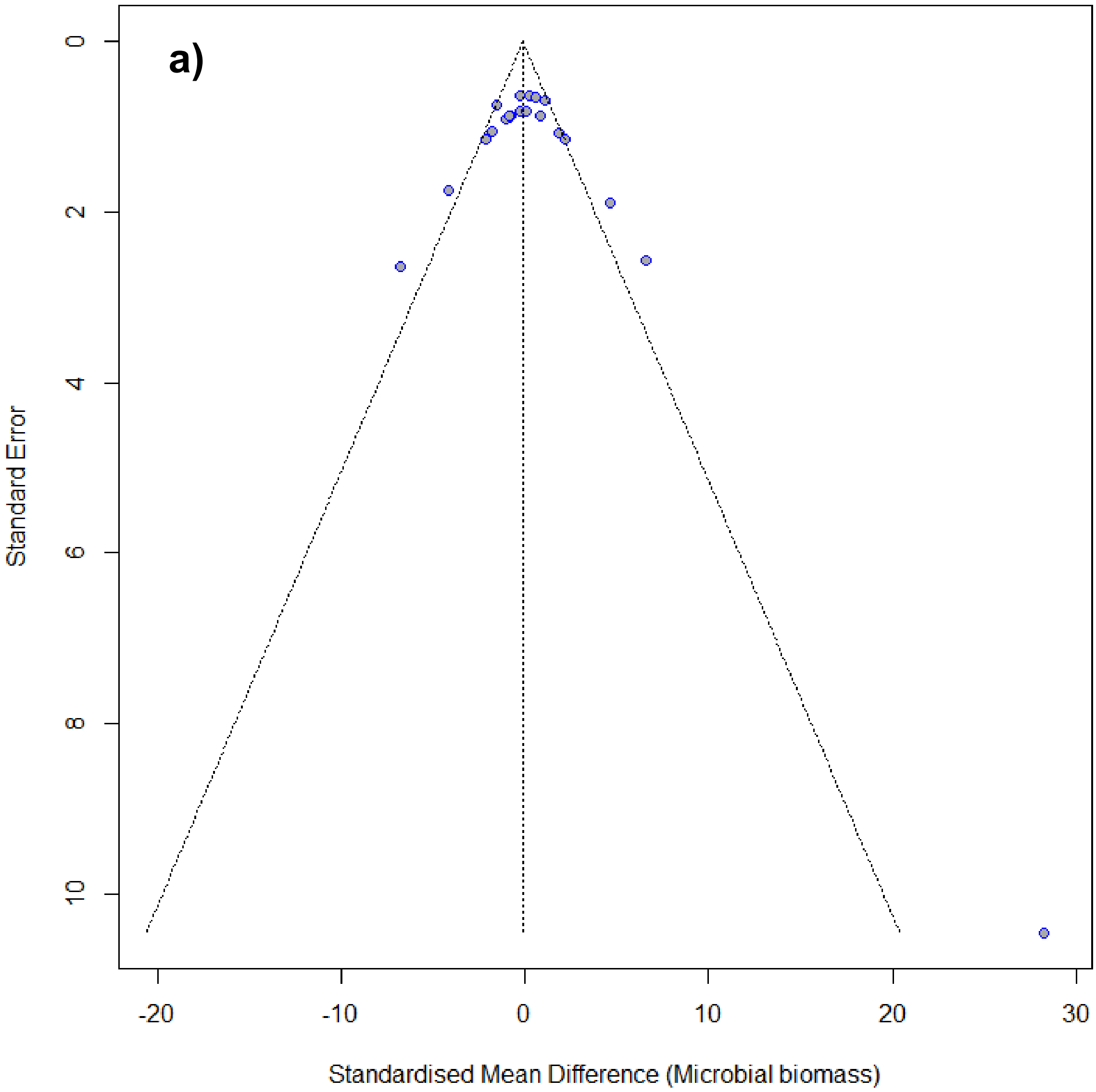
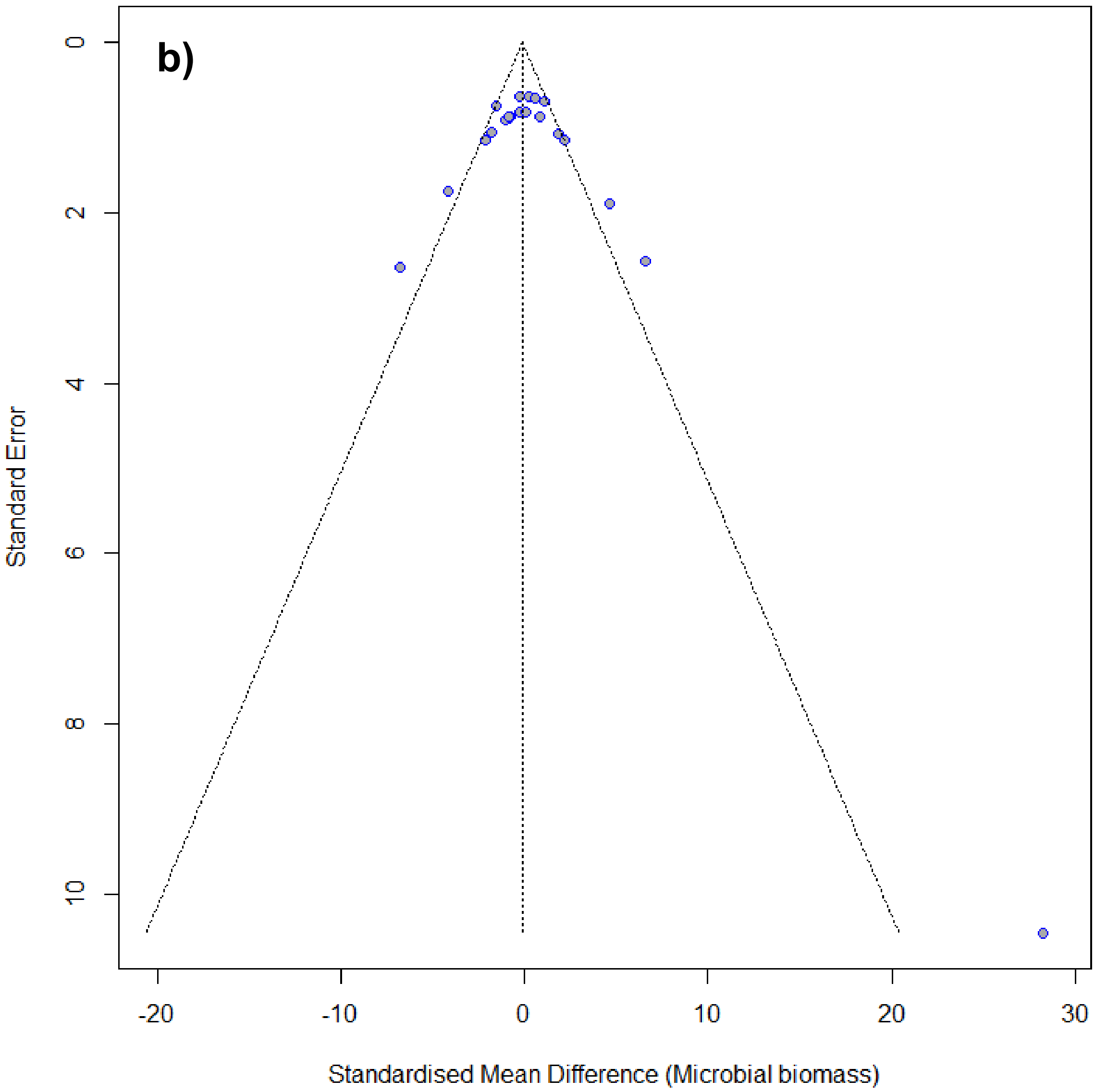
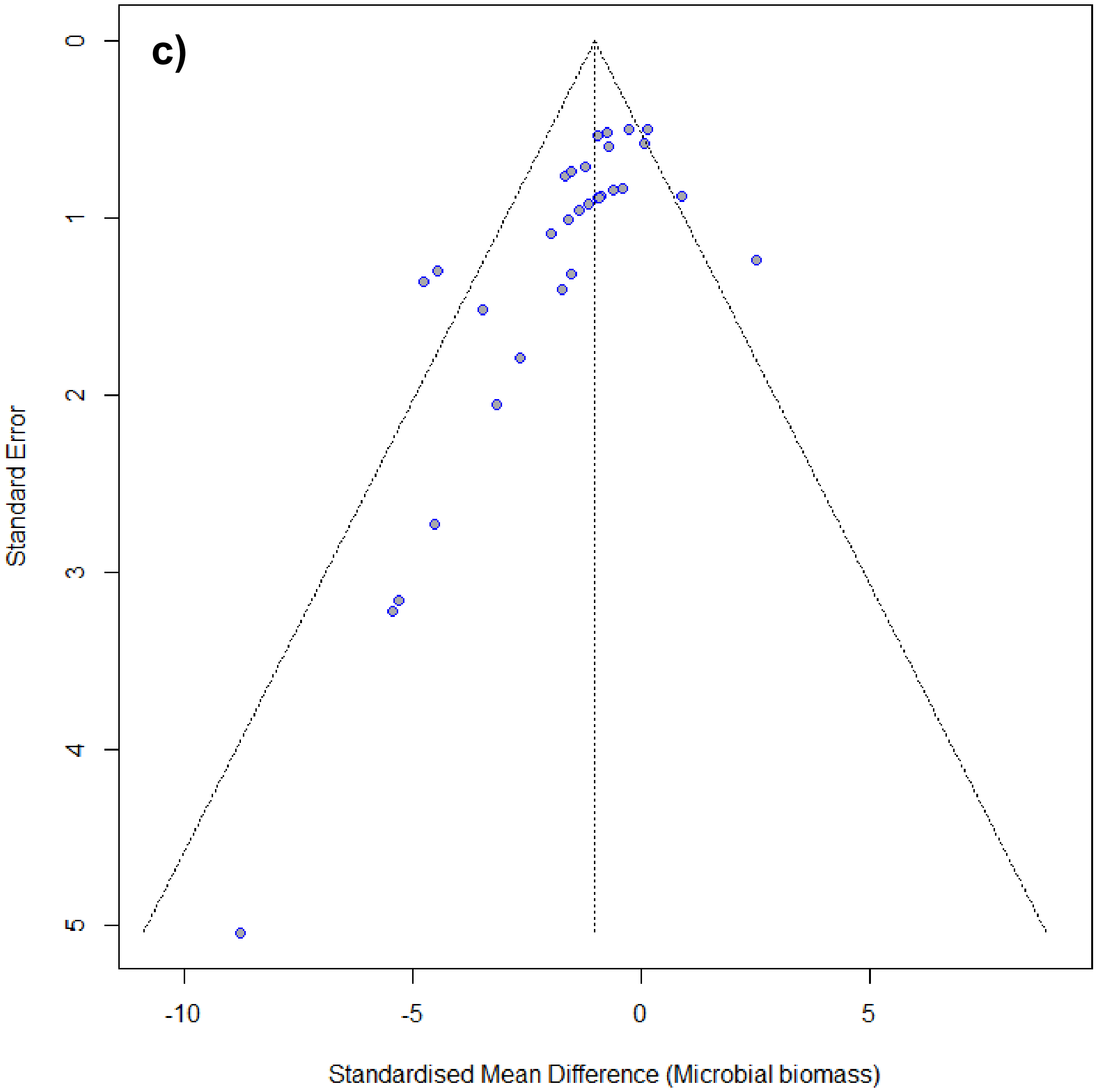
APPENDIX A: SUPPLEMENTARY MATERIALS




b)
Standard Error
0
2
4
6
8
10
-20
-10
0
10
20
30
Standardised Mean Difference (Microbial biomass)



c)
0
1
2
3
4
5
Standard Error
-10
-5
0
5
Standardised Mean Difference (Microbial biomass)

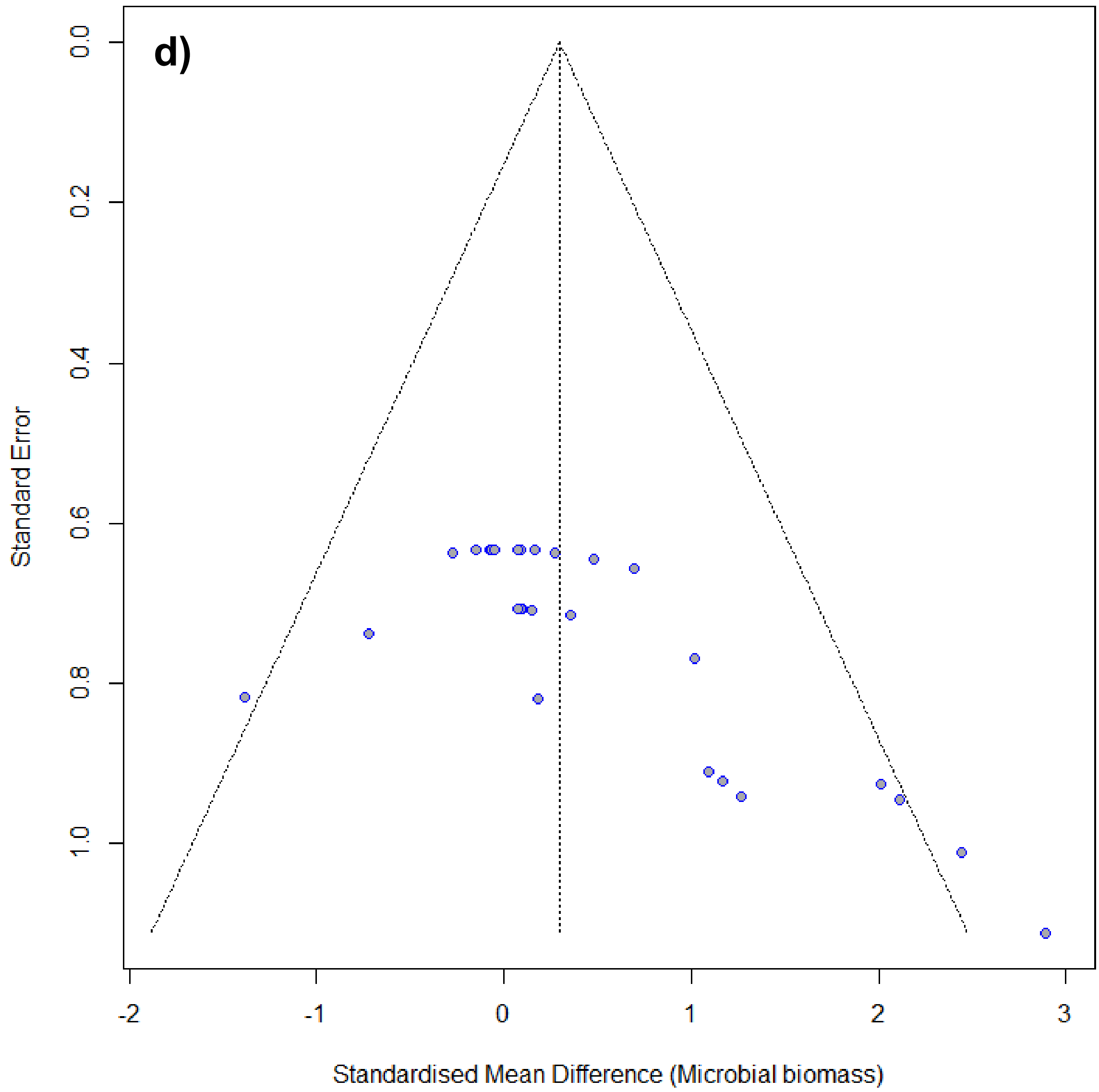


Figure S1. Funnel plot of publication bias (a) microbial biomass (resistance phase), (b) microbial biomass (resilience phase), (c) microbial respiration (resistance phase), and (d) microbial respiration (resilience phase).

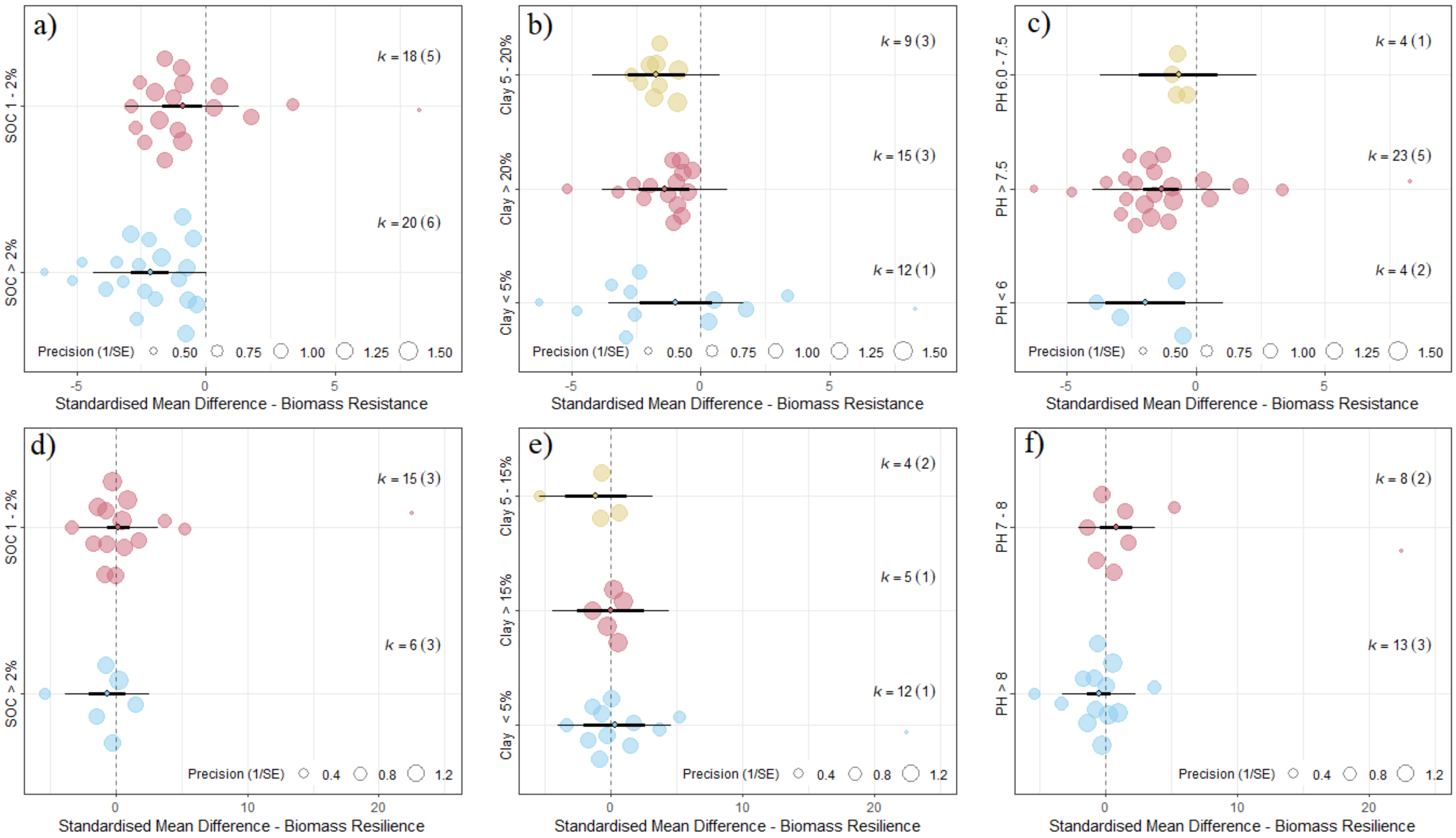

a)
SOC 1 - 2%
SOC > 2%
k = 18 (5)
k = 20 (6)
Precision (1/SE) 0.50 0.75 1.00 1.25 1.50
-5 0 5
Standardised Mean Difference - Biomass Resistance
b)
Clay 5 - 20%
Clay > 20%
Clay < 5%
k = 9 (3)
k = 15 (3)
k = 12 (1)
Precision (1/SE) 0.50 0.75 1.00 1.25 1.50
-5 0 5
Standardised Mean Difference - Biomass Resistance
c)
PH 6.0 - 7.5
PH > 7.5
PH < 6
k = 4 (1)
k = 23 (5)
k = 4 (2)
Precision (1/SE) 0.50 0.75 1.00 1.25 1.50
-5 0 5
Standardised Mean Difference - Biomass Resistance
d)
SOC 1 - 2%
SOC > 2%
k = 15 (3)
k = 6 (3)
Precision (1/SE) 0.4 0.8 1.2
0 10 20
Standardised Mean Difference - Biomass Resilience
e)
Clay 5 - 15%
Clay > 15%
Clay < 5%
k = 4 (2)
k = 5 (1)
k = 12 (1)
Precision (1/SE) 0.4 0.8 1.2
0 10 20
Standardised Mean Difference - Biomass Resilience
f)
PH 7 - 8
PH > 8
k = 8 (2)
k = 13 (3)
Precision (1/SE) 0.4 0.8 1.2
0 10 20
Standardised Mean Difference - Biomass Resilience

Figure S2. Orchard plots for the meta-regression model showing the response of microbial biomass to drought between the control and treatment during resistance (a, b, and c) and resilience (d, e, and f) phases under different SOC levels (a and d), under difference clay contents (b and e) and under different pH levels (c and f). Mean effect sizes with 95% confidence intervals (bold lines) are shown in the middle of the dot charts, where individual dots are individual (i.e., different data points in the studies) effect sizes. The size of dots shows the precision (inverse of standard error) of the effect size estimate. Negative effect sizes larger values in control, whilst positive values mark larger values in treatment in the respective parameter.

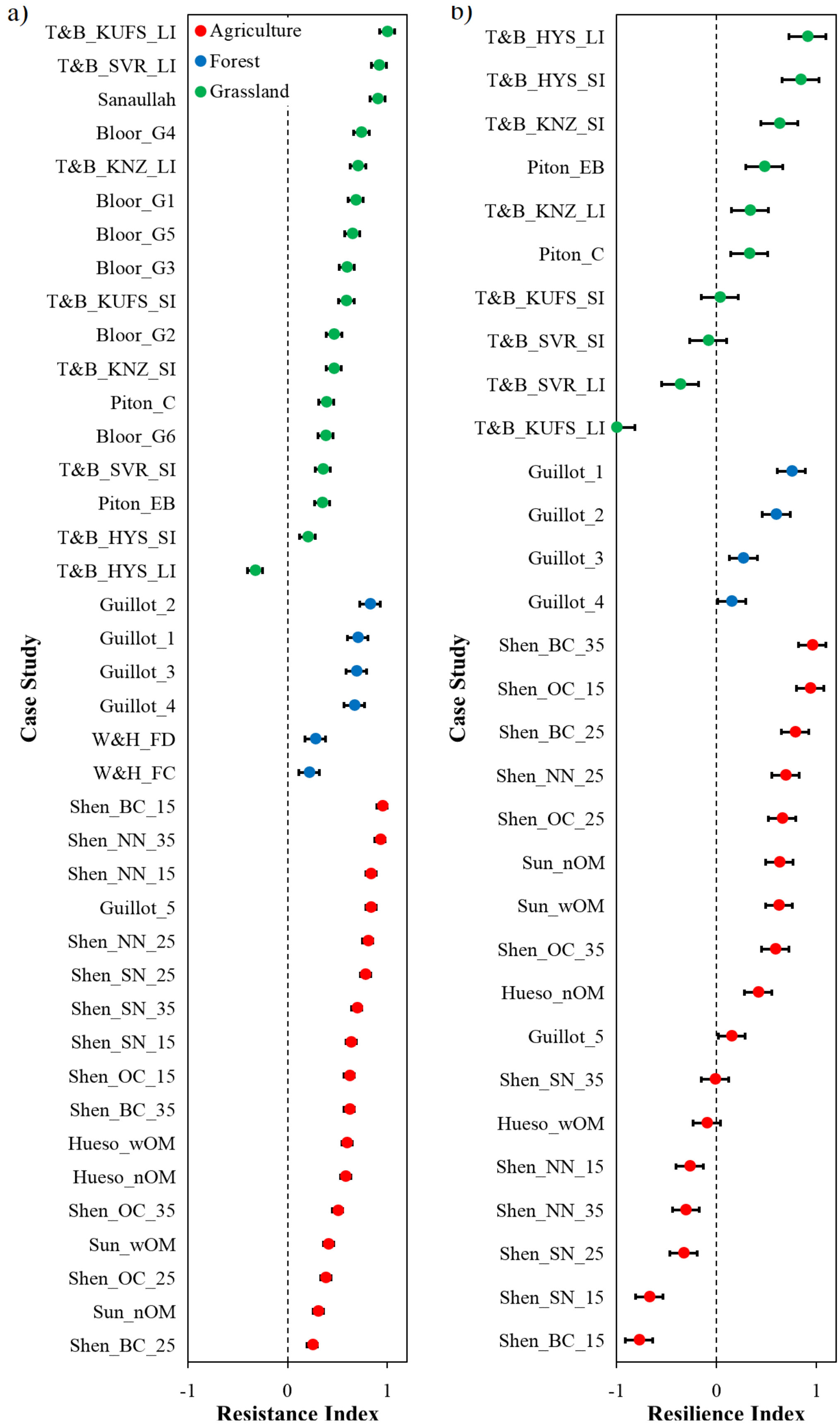


Figure S3. Resistance (a) and resilience (b) indices of soil microbial biomass to drought stress

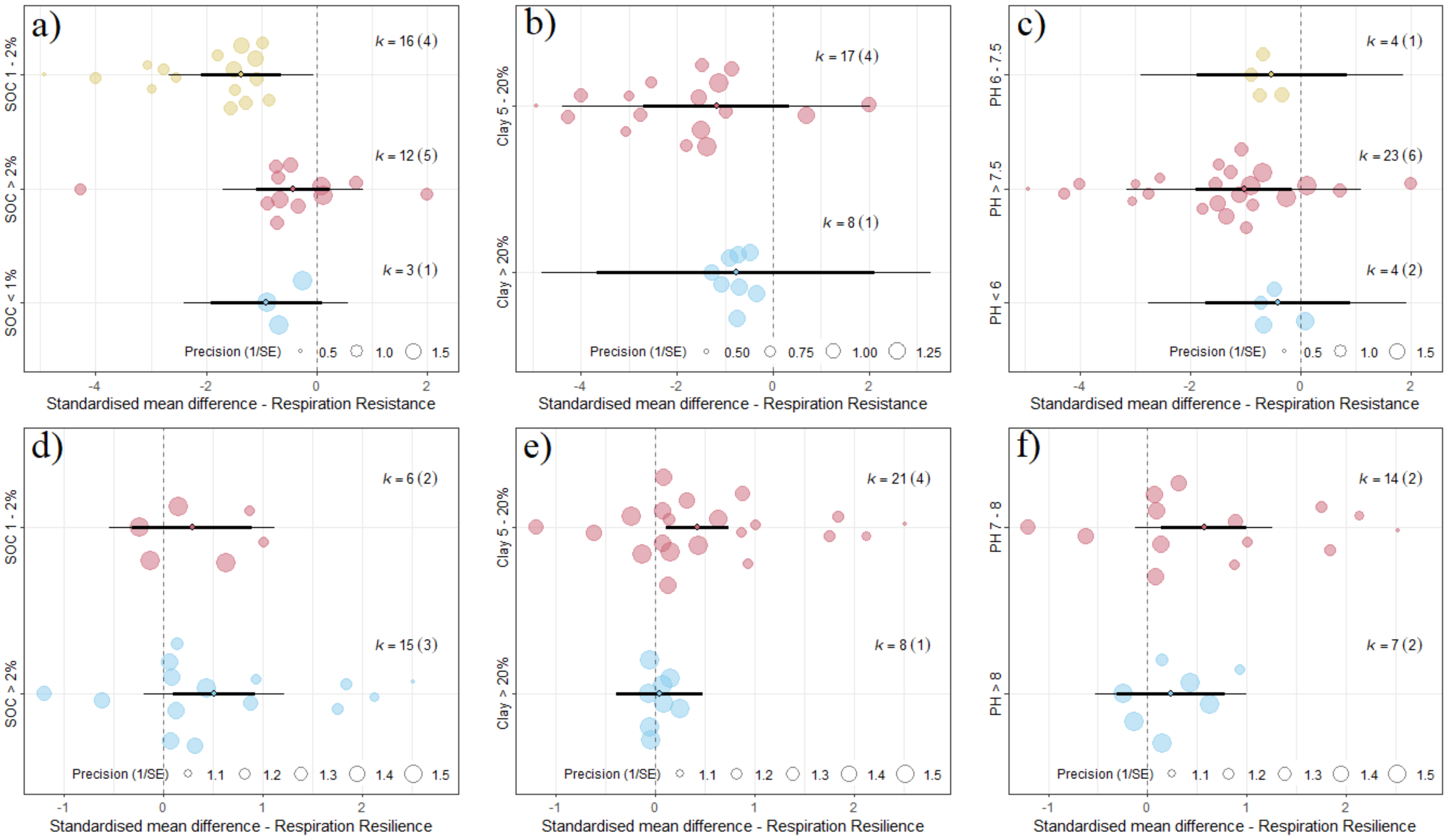

a)
k = 16 (4)
k = 12 (5)
k = 3 (1)
SOC 1 - 2%
SOC > 2%
SOC < 1%
Precision (1/SE) 0.5 1.0 1.5
-4 -2 0 2
Standardised mean difference - Respiration Resistance
b)
k = 17 (4)
k = 8 (1)
Clay 5 - 20%
Clay > 20%
Precision (1/SE) 0.50 0.75 1.00 1.25
-4 -2 0 2
Standardised mean difference - Respiration Resistance
c)
k = 4 (1)
k = 23 (6)
k = 4 (2)
PH 6 - 7.5
PH > 7.5
PH < 6
Precision (1/SE) 0.5 1.0 1.5
-4 -2 0 2
Standardised mean difference - Respiration Resistance
d)
k = 6 (2)
k = 15 (3)
SOC 1 - 2%
SOC > 2%
Precision (1/SE) 1.1 1.2 1.3 1.4 1.5
-1 0 1 2
Standardised mean difference - Respiration Resilience
e)
k = 21 (4)
k = 8 (1)
Clay 5 - 20%
Clay > 20%
Precision (1/SE) 1.1 1.2 1.3 1.4 1.5
-1 0 1 2
Standardised mean difference - Respiration Resilience
f)
k = 14 (2)
k = 7 (2)
PH 7 - 8
PH > 8
Precision (1/SE) 1.1 1.2 1.3 1.4 1.5
-1 0 1 2
Standardised mean difference - Respiration Resilience

Figure S4. Orchard plots for the meta-regression model showing the response of microbial respiration to drought between the control and treatment during resistance (a, b, and c) and resilience (d, e, and f) phases under different SOC levels (a and d), under difference clay contents (b and e) and under different pH levels (c and f). Mean effect sizes with 95% confidence intervals (bold lines) are shown in the middle of the dot charts, where individual dots are individual (i.e., different data points in the studies) effect sizes. The size of dots shows the precision (inverse of standard error) of the effect size estimate. Negative effect sizes larger values in control, whilst positive values mark larger values in treatment in the respective parameter.

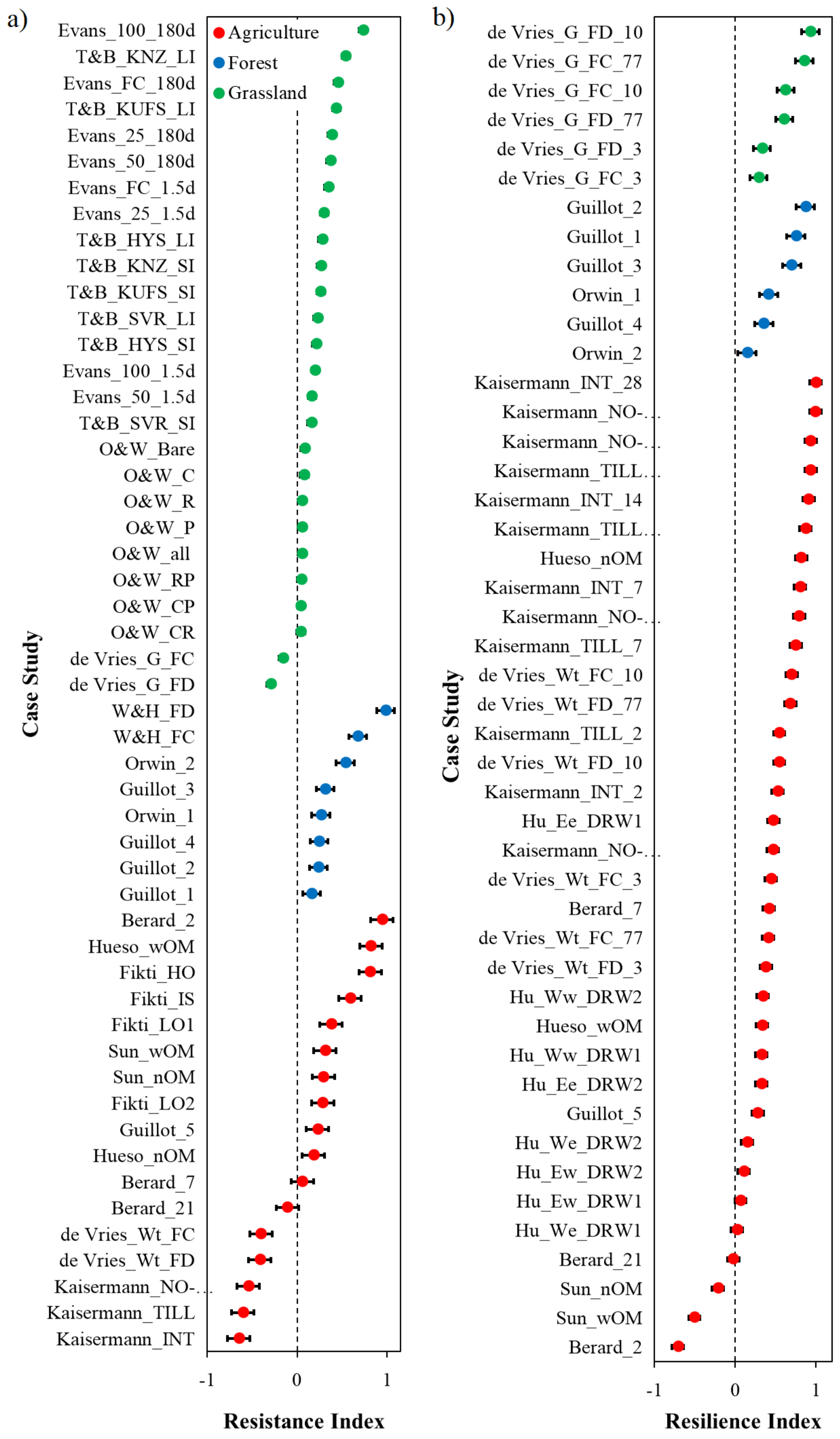


Figure S5. Resistance (a) and resilience (b) indices of soil microbial respiration to drought stress.

Table S1. Selected studies for the data extraction

Author	Microbial Biomass		Microbial Respiration	
	Resistance	Resilience	Resistance	Resilience
Bérard et al. (2011)			✓	✓
Bloor et al. (2018)	✓			
De Vries et al. (2012b)			✓	✓
Evans et al. (2014)			✓	
Fikri et al. (2021)			✓	
Guillot et al. (2019b)	✓	✓	✓	✓
Hu et al. (2021a)				✓
Hueso et al. (2011)	✓	✓	✓	✓
Kaisermann et al. (2013a)			✓	
Orwin and Wardle (2005)			✓	
Orwin et al. (2006)			✓	✓
Piton et al. (2021)	✓	✓		
Sanaullah et al. (2011)	✓			
Shen et al. (2022)	✓	✓		
Sun et al. (2017a)	✓	✓	✓	✓
Tiemann and Billings (2011)	✓	✓	✓	
Waring and Hawkes (2015)	✓		✓	

Table S2. Soil organic carbon (SOC), Clay content, pH and description of the study code in Selected studies for the analysis.

Author	Land Use	Treatment	Treatment code	SOC (%)	Clay content (%)	pH
Berard et al 2011	agriculture	2 days drought	Berard_2	1.16	32.60	8.37
Berard et al 2011	agriculture	7 days drought	Berard_7	1.16	32.60	8.37
Berard et al 2011	agriculture	21 days dought	Berard_21	1.16	32.60	8.37
Bloor et al 2018	grassland	Vegetation 1	Bloor_G1	4.30	20.80	NA
Bloor et al 2018	grassland	Vegetation 2	Bloor_G2	4.30	20.80	NA
Bloor et al 2018	grassland	Vegetation 3	Bloor_G3	4.30	20.80	NA
Bloor et al 2018	grassland	Vegetation 4	Bloor_G4	4.30	20.80	NA
Bloor et al 2018	grassland	Vegetation 5	Bloor_G5	4.30	20.80	NA
Bloor et al 2018	grassland	Vegetation 6	Bloor_G6	4.30	20.80	NA
de Vries et al 2012	agriculture	Wheat field drought	de Vries_Wt_FD	4.10	20.00	7.80
de Vries et al 2012	agriculture	Wheat field control	de Vries_Wt_FC	4.10	20.00	7.80
de Vries et al 2012	grassland	Grassland field drought	de Vries_G_FD	4.10	20.00	7.80
de Vries et al 2012	grassland	Grassland field control	de Vries_G_FC	4.10	20.00	7.80
de Vries et al 2012	agriculture	Wheat field drought_3 days	de Vries_Wt_FD_3	4.10	20.00	7.80
de Vries et al 2012	agriculture	Wheat field control_3 days	de Vries_Wt_FC_3	4.10	20.00	7.80
de Vries et al 2012	grassland	Grassland field control_3 days	de Vries_G_FC_3	4.10	20.00	7.80
de Vries et al 2012	grassland	Grassland field drought_3 days	de Vries_G_FD_3	4.10	20.00	7.80
de Vries et al 2012	agriculture	Wheat field drought_10 days	de Vries_Wt_FD_10	4.10	20.00	7.80
de Vries et al 2012	agriculture	Wheat field control_10 days	de Vries_Wt_FC_10	4.10	20.00	7.80
de Vries et al 2012	grassland	Grassland field drought_10 days	de Vries_G_FD_10	4.10	20.00	7.80
de Vries et al 2012	grassland	Grassland field control_10 days	de Vries_G_FC_10	4.10	20.00	7.80
de Vries et al 2012	agriculture	Wheat field drought_77 days	de Vries_Wt_FD_77	4.10	20.00	7.80

de Vries et al 2012	agriculture	Wheat field control_77 days	de Vries_Wt_FC_77	4.10	20.00	7.80
de Vries et al 2012	grassland	Grassland field drought_77 days	de Vries_G_FD_77	4.10	20.00	7.80
de Vries et al 2012	grassland	Grassland field control_77 days	de Vries_G_FC_77	4.10	20.00	7.80
Evans et al 2014	grassland	Field control	Evans_FC_1.5d	1.40	15.00	7.70
Evans et al 2014	grassland	25% rain reduction	Evans_25_1.5d	1.40	15.00	7.70
Evans et al 2014	grassland	50% rain reduction	Evans_50_1.5d	1.40	15.00	7.70
Evans et al 2014	grassland	100% rain reduction	Evans_100_1.5d	1.40	15.00	7.70
Evans et al 2014	grassland	Field control	Evans_FC_180d	1.40	15.00	7.70
Evans et al 2014	grassland	25% rain reduction	Evans_25_180d	1.40	15.00	7.70
Evans et al 2014	grassland	50% rain reduction	Evans_50_180d	1.40	15.00	7.70
Evans et al 2014	grassland	100% rain reduction	Evans_100_180d	1.40	15.00	7.70
Fikri et al 2021	agriculture	initial soil (IS)	Fikti_IS	0.37	3.10	9.10
Fikri et al 2021	agriculture	low organic matter soil 1 (LO1)	Fikti_LO1	0.84	14.70	7.60
Fikri et al 2021	agriculture	low organic matter soil 2 (LO2)	Fikti_LO2	0.77	15.10	7.80
Fikri et al 2021	agriculture	high organic matter soil (HO)	Fikti_HO	2.50	14.80	8.00
Guillot et al 2019	forest	Tree row	Guillot_1	2.05	17.80	8.40
Guillot et al 2019	forest	1-2 m from the middle of tree row	Guillot_2	1.55	17.30	8.50
Guillot et al 2019	forest	2-4 m from the middle of tree row	Guillot_3	1.51	18.60	8.60
Guillot et al 2019	forest	4-6.5 m from the middle of tree row	Guillot_4	1.39	18.40	8.60
Guillot et al 2019	agriculture	Conventional cropping	Guillot_5	1.52	17.70	8.60
Hu et al 2021	agriculture	We_DRW1	Hu_We_DRW1	NA	24.80	NA
Hu et al 2021	agriculture	Ww_DRW1	Hu_Ww_DRW1	NA	24.80	NA
Hu et al 2021	agriculture	Ee_DRW1	Hu_Ee_DRW1	NA	45.60	NA
Hu et al 2021	agriculture	Ew_DRW1	Hu_Ew_DRW1	NA	45.60	NA
Hu et al 2021	agriculture	We_DRW2	Hu_We_DRW2	NA	24.80	NA

Hu et al 2021	agriculture	Ww_DRW2	Hu_Ww_DRW2	NA	24.80	NA
Hu et al 2021	agriculture	Ee_DRW2	Hu_Ee_DRW2	NA	45.60	NA
Hu et al 2021	agriculture	Ew_DRW2	Hu_Ew_DRW2	NA	45.60	NA
Hueso et al 2011	agriculture	No organic matter	Hueso_nOM	2.15	15.00	8.56
Hueso et al 2011	agriculture	With organic matter	Hueso_wOM	2.15	15.00	8.56
Kaisermann et al 2013	agriculture	intensively managed (INT)	Kaisermann_INT	1.06	17.20	7.30
Kaisermann et al 2013	agriculture	without chemical inputs (TILL)	Kaisermann_TILL	0.91	18.20	7.80
Kaisermann et al 2013	agriculture	and without tillage (NO-TILL)	Kaisermann_NO-TILL	1.66	16.80	6.90
Kaisermann et al 2013	agriculture	intensively managed (INT)_2 days	Kaisermann_INT_2	1.06	17.20	7.30
Kaisermann et al 2013	agriculture	without chemical inputs (TILL)_2 days	Kaisermann_TILL_2	0.91	18.20	7.80
Kaisermann et al 2013	agriculture	and without tillage (NO-TILL) _2 days	Kaisermann_NO-TILL_2	1.66	16.80	6.90
Kaisermann et al 2013	agriculture	intensively managed (INT)_7 days	Kaisermann_INT_7	1.06	17.20	7.30
Kaisermann et al 2013	agriculture	without chemical inputs (TILL)_7 days	Kaisermann_TILL_7	0.91	18.20	7.80
Kaisermann et al 2013	agriculture	and without tillage (NO-TILL) _7 days	Kaisermann_NO-TILL_7	1.66	16.80	6.90
Kaisermann et al 2013	agriculture	intensively managed (INT)_14 days	Kaisermann_INT_14	1.06	17.20	7.30
Kaisermann et al 2013	agriculture	without chemical inputs (TILL)_14 days	Kaisermann_TILL_14	0.91	18.20	7.80
Kaisermann et al 2013	agriculture	and without tillage (NO-TILL) _14 days	Kaisermann_NO-TILL_14	1.66	16.80	6.90
Kaisermann et al 2013	agriculture	intensively managed (INT)_28 days	Kaisermann_INT_28	1.06	17.20	7.30
Kaisermann et al 2013	agriculture	without chemical inputs (TILL)_28 days	Kaisermann_TILL_28	0.91	18.20	7.80
Kaisermann et al 2013	agriculture	and without tillage (NO-TILL) _28 days	Kaisermann_NO-TILL_28	1.66	16.80	6.90
Orwin & Wardle 2005	grassland	Bare soil	O&W_Bare soil	4.00	NA	5.10
Orwin & Wardle 2005	grassland	plantain	O&W_plantain	4.00	NA	5.10
Orwin & Wardle 2005	grassland	ryegrass	O&W_ryegrass	4.00	NA	5.10

Orwin & Wardle 2005	grassland	Clover	O&W_Clover	4.00	NA	5.10
Orwin & Wardle 2005	grassland	clover+ryegrass	O&W_clover+ryegrass	4.00	NA	5.10
Orwin & Wardle 2005	grassland	clover+plantain	O&W_clover+plantain	4.00	NA	5.10
Orwin & Wardle 2005	grassland	ryegrass+plantain	O&W_ryegrass+plantain	4.00	NA	5.10
Orwin & Wardle 2005	grassland	all 3 species	O&W_all	4.00	NA	5.10
Orwin et al 2006	forest	<5000 years chronosequence	Orwin_1	NA	NA	NA
Orwin et al 2006	forest	>5000 years chronosequesnce	Orwin_2	NA	NA	NA
Piton et al 2020	grassland	ecosystem based	Piton_EB	3.02	12.90	5.08
Piton et al 2020	grassland	conventional	Piton_C	3.53	12.52	5.19
Sanaullah et al 2011	grassland		Sanaullah	1.40	NA	NA
Shen et al 2022	agriculture	No N fertilizer	Shen_NN_15	1.81	2.38	8.27
Shen et al 2022	agriculture	No N fertilizer	Shen_NN_25	1.81	2.38	8.27
Shen et al 2022	agriculture	No N fertilizer	Shen_NN_35	1.81	2.38	8.27
Shen et al 2022	agriculture	Synthesized N Fertilizer	Shen_SN_15	1.75	1.98	7.98
Shen et al 2022	agriculture	Synthesized N Fertilizer	Shen_SN_25	1.75	1.98	7.98
Shen et al 2022	agriculture	Synthesized N Fertilizer	Shen_SN_35	1.75	1.98	7.98
Shen et al 2022	agriculture	Organic fertilizer+Chemical N fertilizer	Shen_OC_15	2.34	1.80	7.59
Shen et al 2022	agriculture	Organic fertilizer+Chemical N fertilizer	Shen_OC_25	2.34	1.80	7.59
Shen et al 2022	agriculture	Organic fertilizer+Chemical N fertilizer	Shen_OC_35	2.34	1.80	7.59
Shen et al 2022	agriculture	Biofertilizer+Chemical N fertilizer	Shen_BC_15	1.94	2.04	8.15
Shen et al 2022	agriculture	Biofertilizer+Chemical N fertilizer	Shen_BC_25	1.94	2.04	8.15

Shen et al 2022	agriculture	Biofertilizer+Chemical N fertilizer	Shen_BC_35	1.94	2.04	8.15
Sun et al 2017	agriculture	no amendment	Sun_nOM	1.84	9.70	7.90
Sun et al 2017	agriculture	with amendment	Sun_wOM	1.84	9.70	7.90
Tiemann & Billings 2011	grassland	HYS_LI	T&B_HYS_LI	4.59	31.33	6.59
Tiemann & Billings 2011	grassland	HYS_SI	T&B_HYS_SI	4.59	31.33	6.59
Tiemann & Billings 2011	grassland	SVR_SI	T&B_SVR_SI	1.82	24.98	7.83
Tiemann & Billings 2011	grassland	KNZ_SI	T&B_KNZ_SI	3.40	27.63	6.11
Tiemann & Billings 2011	grassland	KUFS_SI	T&B_KUFS_SI	2.72	26.33	5.99
Tiemann & Billings 2011	grassland	KNZ_LI	T&B_KNZ_LI	3.40	27.63	6.11
Tiemann & Billings 2011	grassland	SVR_LI	T&B_SVR_LI	1.82	24.98	7.83
Tiemann & Billings 2011	grassland	KUFS_LI	T&B_KUFS_LI	2.72	26.33	5.99
Waring & Hawkes 2015	forest	Field control	W&H_FC	10.10	NA	4.50
Waring & Hawkes 2015	forest	Field drought	W&H_FD	10.10	NA	4.50

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