SOIL CRC

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Evaluating soil functional resilience to compaction and drought stresses for developing higher performance soils

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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
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- compliance with ethical guidelines
- conclusions against results
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Keywords

Resistance; resilience; compaction; drought; environmental stressors; plant residue amendment.

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EXECUTIVE SUMMARY

The environmental disturbance of agricultural soils has received significant attention in recent years, due to its impact on soil microbial activities as well as associated crop yield and soil health decline (a healthy soil has the continued capacity to supply plants with adequate and balanced nutrients, water and air as a complex biological system in a disease-free environment). However, the responses of soil microbial community to compaction and drought stresses are largely unknown. Therefore, a robust, cheap, and easy-to-understand index needs to be developed in monitoring soil microbial community responses to compaction and drought as current mainstream soil health indicators are expensive and complicated for end users. This project is designed to address a specific industry problem related to lack of practical methods for assessment of soil resistance and resilience to environmental stresses (compaction and drought) in agricultural lands. The project aims to provide an improved process-based understanding of relationships among compaction and drought stresses and soil resilience and functionality in different farming systems. In this project, soil resistance is defined as the ability of a soil to maintain its functional stability after a disturbance, while soil resilience is defined as the speed with which a soil system can return to its pre-disturbance conditions. Here we provide brief summaries of the key findings from each chapter.

Chapter 1: Soil microbial responses as an indicator of compaction resilience is governed by soil moisture status in sugarcane cropping systems

Two contrasting sandy and clayey sugarcane soils were treated with a combination of compaction and drought stresses, and soil microbial functions were investigated following the application of stress (resistance phase), and removal of the applied stress (resilience phase). A gradient of bulk densities (0.9 g cm^{-3} − 1.5 g cm^{-3}) and water-filled pore spaces (WFPS; 21% to 100%) were applied to both soils. Under high moisture contents (WFPS of 47% to 100%), low compaction levels (1.1 g cm^{-3} and 1.2 g cm^{-3}) increased soil cumulative microbial respiration in sandy soil treatments by 18% in comparison to control treatment (0.9 g cm^{-3}), while high compaction levels (1.3 cm^{-3} − 1.5 g cm^{-3}) decreased soil cumulative microbial respiration by 25% compared to the control treatment. In contrast, in clayey soil treatments with high moisture content, the highest compaction treatment (1.5 g cm^{-3}) significantly increased soil cumulative microbial respiration by 12% in comparison to the control treatment. The clayey soil had a higher resistance index of microbial carbon use efficiency, while the sandy soil had a higher resilience index of microbial carbon use efficiency to compaction and drought stresses. This could be attributed to higher aggregation potential (associated with more finely textured particles) and higher diversity of the microbial community in the clayey soils, which provide a higher functional stability to resist environmental disturbance, while fast drainage and higher adaptation of microbial communities in the sandy soils provide a faster recovery from applied stresses. This also indicates that soil microbial responses to compaction stress are mainly governed by soil texture and moisture status.

Chapter 2: Field management practices play a vital role in building soil resistance and resilience against compaction stress

Two adjacent silty loam soils (similar physicochemical properties) with conventional and improved management history were treated with compaction stress (1.4 g cm^{-3}),

and microbial functions were investigated following the application of stress, and removal of the applied stress. The farm with improved management was under minimum tillage, mound planting, and was multiple species of legume fallow, while the conventionally managed farm had furrow planting and grassy fallow. Compaction stress was applied at the commencement of the experiment (resistance phase) and removed after one month (resilience phase) via legume residue amendment combined with ploughing practice. The observed concentration of labile carbon was 42% higher in treatments with improved management history. Within treatments with the same management history, the treatments with ploughing practice following compaction stress generated the highest cumulative and net cumulative microbial respiration, followed by compaction-only and ploughing-only treatments. The legume residue incorporation to treatments with improved management history minimised the fluctuation of net cumulative microbial respiration between compacted and noncompacted treatments by the end of incubation experiment; however, the same pattern was not observed in treatments with a conventional management history. The results indicate that different field management practices may not alter soil microbial response patterns to compaction stress as soil microbial activity following compaction stress is mainly governed by soil moisture content and its water-filled pore space. However, improved field management practices can increase soil resistance and resilience to compaction stress. The application of legume residue also improved soil chemical properties, increased the supply of labile carbon and nitrogen for soil microbial community and enhanced soil nutrient cycling processes.

Chapter 3: Management history governs soil microbial responses to drought in grain cropping systems.

Two adjacent loamy soils (similar physicochemical properties) with conventional and improved management history were treated with different levels of drought stress (severe, moderate, and nil). The microbial functions were investigated following the application of stress (resistance phase), and the removal of the applied stress (resilience phase) via rewetting and wheat residue amendment. The farm with improved management was under crop rotation (wheat, lupin, barley, wheat), while the conventionally managed farm was under wheat monoculture. Without the wheat residue amendment, the treatments with improved management history showed higher (15–53%) cumulative microbial respiration in comparison to treatments with conventional management throughout the incubation period. In addition, drought stress significantly decreased cumulative microbial respiration regardless of soil management history. The hot water extractable organic carbon pool was highly related ($r = 0.56–0.78$) to the cumulative microbial respiration and microbial activity in all treatments. The crop rotation practice in treatments with improved management history increased soil resilience, as showed with higher recovery following moderate and severe drought stresses, possibly due to their higher organic matter content and complexity of microbial community. The wheat residue amendment increased soil microbial biomass content and enzyme activities by increasing soil labile carbon and nitrogen pools, regardless of soil management history. This observation confirmed that plant residue amendment would increase the supply of organic carbon and nitrogen for soil microbial community and enhance soil nutrient cycling processes.

The knowledge generated in this project is critical for Soil CRC partners and farming groups that work towards adoption of best management practices for re-engineering stressed soils for higher crop productivity and farm profitability. In the longer term, the methods produced in this project will have broader applications across Australian grower group networks and agricultural industries.

OBJECTIVES

1) To assess the mechanisms responsible for the microbial functional response of soils to compaction and drought stresses, using a soil resistance and resilience approach.

2) To use soil biological functions (e.g. respiration, microbial biomass and activity) as reliable indices for assessment of soil resistance and resilience to compaction and drought stresses.

3) To assess the effect of soil properties (e.g. soil texture or organic matter content) and management history (e.g. minimum zonal tillage, cover cropping, crop rotation, legume fallow, or plant residue amendment) on the response of different soils to compaction and drought stresses.

RESULTS

1) In low stress conditions, soil microbial activity and respiration rates are mainly governed by the bioavailability of soil carbon and nitrogen pools. Under stressed conditions, soil texture, soil water-filled pore space, and the applied stress levels (rather than soil organic matter content) regulate the resistance and resilience of soil microbial functions to compaction and drought stresses.
2) Soils with a fine texture have higher resistance of microbial carbon use efficiency, while soils with a coarse texture have higher resilience of microbial carbon use efficiency to compaction and drought stresses. This could be attributed to higher aggregation potential (associated with finely textured particles) and higher diversity of the microbial community in soils with fine texture, which provides a higher functional stability to resist environmental disturbance, while fast drainage and higher adaptation of microbial communities (associated with frequent disturbance) in soils with coarse texture would provide a faster recovery from applied stresses.
3) The response pattern of a soil microbial community to environmental disturbance is highly related to soil texture and the applied stress levels, rather than the history of field management practices. However, improved field management practices (i.e. minimum tillage, cover cropping, crop rotation, legume fallow, or organic residue amendment) can increase soil organic matter content, labile carbon and nitrogen pools, and the diversity of soil microbial community, which lead to a significant increase in soil microbial functional resistance and resilience to compaction and drought stresses.

NEXT STEPS	TIMING
1) Assessment of the effect of ecosystem processes on improvement of soil carbon stocks (quality and quantity) and soil functional resilience to environmental stresses.	1) 1–2 years

2) Quantification of the changes in soil microbial functional resistance and resilience to key environmental stresses (e.g. drought, compaction, chemical residues of fungicides and carbon decline) as induced by the ecosystem-based management practices.	2) 3–4 years
3) Providing recommendations and 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.	3) 3–4 years
4) Assessment of the limitations in the adoption of farming practices (e.g. cover cropping, regenerative agricultural management) that may result in more resilient soils and make recommendations on when these systems would be most likely to deliver positive outcomes for farmers.	4) 3–4 years

CHAPTER 1: SOIL MICROBIAL RESPONSES AS AN INDICATOR OF COMPACTION RESILIENCE IS GOVERNED BY SOIL MOISTURE STATUS IN SUGARCANE CROPPING SYSTEMS

1.1 INTRODUCTION

Climate change and human activity are altering the environment by increasing the atmospheric CO_2, seasonal temperatures, and frequency of extreme weather events (high intensity rainfall incidences or drought; Gauthier et al., 2015). This is particularly important in sugarcane production, which relies on a precipitation pattern (intensity and frequency) to achieve optimum productivity. The majority of Australian sugarcane farms are located along a 2000 km stretch of the east coast that extends south from Far North Queensland to New South Wales (Linnenluecke et al., 2020). High-intensity rainfall during prolonged parts of the growing season can result in long periods of limited oxygen availability in the sugarcane root zone, which may significantly reduce crop yield (Bell et al., 2007; Tracy and Zhang, 2008). On the other hand, uneven distribution of rainfall may lead to periodic drought despite high annual rainfall, also resulting in lower crop yield (Yu et al., 2013).

Soil compaction, a key contributing factor to global land degradation, is primarily caused by the overuse of machinery (Batey, 2009; Shah et al., 2017), particularly on wet soils, and may result in the depletion of soil health due to loss of organic matter, high bulk density, poor root zone aeration, and the physical restriction of root growth (Kaufmann et al., 2010; Stoessel et al., 2018). Previous studies in Brazil and Australia indicate that soil compaction negatively affects sugarcane growth and cause significant yield loss (20–50%), mainly in the fourth and fifth ratoon plants (Bell et al., 2002; Cherubin et al., 2016). Simultaneous occurrence of soil compaction and water stress (drought or waterlogging) significantly affects soil physicochemical and biological properties, such as soil water-filled pore space (WFPS; Logsdon and Karlen, 2004), soil C and nutrient cycles, nutrient

bioavailability (Martínez and Zinck, 2004; Mikha et al., 2005), soil microbial biomass content, soil enzyme activities, and microbial functional structure and diversity (Li et al., 2004).

Ecological systems have differing capacities to resist the impact of a disturbance or stress and to adapt and recover from the impact (Ingrisch and Bahn, 2018). However, soil biological properties are generally more sensitive to environmental stresses compared with soil physical and chemical properties (Kuzyakov et al., 2020), and the relationships between soil biological properties and their response to stressors are remarkably complex. Several indicators have been used to assess the biological responses of soils to environmental and anthropogenic stresses (Banning and Murphy, 2008; Guillot et al., 2019; Orwin and Wardle, 2004). Soil respiration and its relation to changes in soil microbial biomass and enzyme activity is considered one of the most widely used and robust biological indicators of soil health and its resistance (RS; ability of a soil to maintain its functional stability after a disturbance) and resilience (RL; speed with which a soil system can return to its pre-disturbance condition) to stressors (Guillot et al., 2019; Hu et al., 2021; Yu et al., 2021). Previous studies indicate that compaction stress can regulate soil respiration (Conlin and Driessche, 2000) by changing the microbially-mediated soil C and nutrient cycling processes (Siczek and Frąc, 2012), and the shift in soil enzyme activities, following a disturbance, can be considered as an early indicator of changes to soil metabolic capacity (Alhameid et al., 2019).

Despite widespread interest in detrimental impacts of soil compaction and water stress in agricultural systems, the response of the belowground nutrient turnover processes to soil compaction and moisture status and how such responses are mediated by soil microbial communities have been less explored, particularly in sugarcane systems. The assessment of resistance and resilience to stresses can also help provide a mechanistic understanding of soil processes, particularly those related to the provision of plant nutrients. Therefore, the objective of this study is to assess the mechanisms responsible for sugarcane soils' microbial functional response to compaction and water stresses, using a soil resistance and resilience approach. The underlying hypotheses are that: a) soil biological functions,

such as respiration, microbial biomass, and activity, are reliable indices for assessment of soil resistance and resilience to compaction and water stresses; b) soil WFPS regulates soil microbial activity by increasing diffusion of organic C and limiting soil air availability; and c) the Nitisols soil has a greater resistance to compaction while the Planosols soil tends to have higher resilience to compaction.

1.2 MATERIALS AND METHODS

1.2.1 Sample preparation and experimental design

Soil samples were collected from 0–10 cm depth of two sugarcane farms at Rocky Point (Planosols; 27°43'51"S, 153°17'20"E) and Ingham (Nitisols; 18°39'09"S, 146°07'59"E), Queensland, Australia (WRB, 2014). Each soil sample had an initial water content of around 15% (w/w). At each site, the soil samples were randomly collected from five locations within a sugarcane growing field and composited right after sugarcane was harvested. All soil samples collected from experimental sites were well mixed, sieved (< 2 mm), and large roots removed before experiment. The Planosols contained 22% coarse sand, 48% fine sand, 15% silt and 15% clay and had an initial pH (1:5 water) of 6.2. Total C (TC) and total N (TN) contents were 10.9 g kg^{-1} and 0.8 g kg^{-1}, respectively. The Nitisols contained 5% coarse sand, 24% fine sand, 26% silt and 45% clay and had an initial pH (1:5 water) of 5.7. The TC and TN contents were 12.2 g kg^{-1} and 0.9 g kg^{-1}, respectively.

Fresh soils (120 g oven-dry equivalent) were moistened to 15% gravimetric water content (GWC; low water content group that represents drought stress) or 35% GWC (high water content group that represents excess water and waterlogging stress) with distilled water. The moistened samples were then transferred to 150 mL flat end polypropylene jars, compacted to bulk densities of 0.9, 1.1, 1.2, 1.3, 1.4 and 1.5 g cm^{-3} using a modified compactor (the compression process was performed in three soil layers to generate a uniform compaction throughout the soil profile), and incubated in the dark at 22 ± 0.5 °C for the first 35 days of the experiment (the RS phase). At the end of the RS phase, the compaction stress was removed by a replicated ploughing process that reduced the bulk density in all

samples to 0.9 g cm^{-3}. The samples were then further incubated in the dark at 22 ± 1°C until the end of the experiment at day 74 (the RL phase). The experiment had 24 treatments in total (two soil types, two water content levels, six compaction levels) with three replicates for each treatment (in total 18 replicates for six soil sampling times). The treatments were divided into four groups: a) Planosols treatments with low water content (L0.9, L1.1, L1.2, L1.3, L1.4 and L1.5; the letter 'L' denotes low water content, followed by the bulk density, from 0.9 g cm^{-3} to 1.5 g cm^{-3}, where 0.9 represents nil compaction); b) Planosols treatments with excess water or waterlogging stress (H0.9, H1.1, H1.2, H1.3, H1.4 and H1.5; the letter 'H' denotes high water content, followed by the bulk density from 0.9 g cm^{-3} to 1.5 g cm^{-3}, where 0.9 represents nil compaction); c) Nitisols treatments with low water content or drought stress (L0.9, L1.1, L1.2, L1.3, L1.4 and L1.5); and d) Nitisols treatments with excess water or waterlogging stress (H0.9, H1.1, H1.2, H1.3, H1.4 and H1.5). The application of combined compaction and water stress to the samples resulted in a range of WFPS values, from 21% (representing drought stress and soil aerobic status) to over 100%. The low and high water content groups of treatments had WFPS values from 21% to 52%, and from 47% to over 100%, respectively, with an overlap between L1.5 (low water content and high compaction) and H0.9 (high water content and nil compaction) treatments. In this study, the treatments with bulk densities of 1.3, 1.4, and 1.5 g cm^{-3} were considered as having a high level of compaction. The H0.9 treatments (nil compaction and no water stress) in both Planosols and Nitisols were considered as the control.

Soil samples were collected at 7, 14, 35, 42, 49 and 74 days after the commencement of the incubation. Three replicates of each treatment were randomly selected and destructively sampled for measurement of mineral N (NH_4^+-N and NO_3^--N), hot water extractable organic C (HWEOC), hot water extractable total N (HWETN), microbial biomass C (MBC), microbial biomass N (MBN), and β-glucosidase and chitinase enzyme activities. Soil respiration samples were collected at 27 sampling dates (gas sampling was undertaken every one-to-four days depending on the expected levels of CO_2 emissions) during the incubation experiment, using three randomly selected replicates of each treatment.

1.2.2 Analysis of soil chemical properties

Soil pH (1:5 soil to water ratio) was measured using a glass electrode (Rayment and Lyons, 2011). Soil mineral N was extracted using 2M potassium chloride (KCl) at a 1:4 ratio of soil to extractant using an end-over-end shaker for one hour, filtered by a Whatman 42 filter paper and concentrations of NH_4^+-N and NO_3^--N, which were measured by a SEAL AA3 Continuous Segmented Flow Analyzer (SEAL Analytical Limited, USA). The TC and TN contents of soil samples were measured by dry-combustion using a LECO CNS-2000 analyser (LECO Corporation, MI, USA). The HWEOC and HWETN contents of the samples were measured using the method described by Chen et al. (2000). Briefly, 4.0 g (oven-dry equivalent) of fresh soil was incubated with 20 mL of water in a capped falcon-tube at 70°C for 18 hours. After incubation, tubes were shaken on an end-over-end shaker for five minutes and filtered through a Whatman 42 filter paper (Whatman Ltd, Maidstone, UK), followed by a 0.45-µm filter membrane. Concentrations of organic C and total extractable N in the filtrate were determined using a SHIMADZU TOC-VCPH (Shimazu, Kyoto, Japan) TOCN analyser. The results were expressed on an oven-dry basis. Water fill pore space (WFPS) was calculated by following equation:

$$WFPS = (GWC \times BD) / [1 - (BD / PD)]$$

Where GWC is gravimetric water content, BD is bulk density, and PD is particle density, which is estimated as 2.65 g cm^{-3} in this study.

1.2.3 Analysis of soil biological properties

Soil microbial biomass C (MBC) and microbial biomass N (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 (Wilson, 1988) as described by (Liu et al., 2018). Concentrations of dissolved organic C and total N in fumigated and unfumigated samples were determined using a Shimadzu TOC-VCSH/CSN total organic C and N analyser (Shimadzu Scientific Instruments, Japan). Soil respiration was measured using a gas sampling method, as described by Rezaei Rashti

et al. (2016). At each gas sampling event, three replicates of each treatment were placed into 2 L airtight glass jars that were then flushed with ambient compressed air for one minute before closure. Gas samples were collected from the headspace of the glass jars eight hours after closure using a 25 mL gas-tight syringe and immediately transferred to pre-evacuated 12 mL glass vials (Labco, UK). Gas samples were analysed for CO_2 concentration using a gas chromatograph (Shimadzu GC-2010 Plus). Linearity tests on CO_2 concentration increases were performed on all treatments over a subset of sampling times during the incubation. These samples were taken initially after closure of the glass jars and then every 60 minutes for 10 hours. The emissions for days without gas sampling were estimated using the arithmetic mean of the measurements on the two closest days (Rezaei Rashti et al., 2016). The cumulative emissions were calculated by summing the daily emission measurements. The microbial metabolic quotient (qCO_2) was defined as the C respired per unit of MBC per day, and calculated from the ratio of CO_2-C emission rate (mg CO_2-C kg^{-1} soil day^{-1}) to MBC (mg C kg^{-1} soil) content of each treatment. The activities of β-glucosidase (hydrolysing cellulose to glucose) at pH 6.0 and chitinase (hydrolysing chitin and other glucosamine polymers) at pH 5.0 in soil samples were measured according to Tabatabai (1994).

1.2.4 Soil microbial resistance (RS) and resilience (RL) indices

To assess the functional RS and RL of C use efficiency (metabolic quotient) in soil microbial communities under compaction and water stresses, the following indices were used according to Orwin and Wardle (2004):

$$RS = 1 - [(2 \times |D_{35}|) / (C_{35} + |D_{35}|)]$$

D_{35} is the difference between soil metabolic quotient value in the control (H0.9 in Planosols and Nitisols) treatment (C_{35}) and the stressed treatment (combined compaction and water stresses) at the end of the RS phase (day 35). The RL index for the microbial C use efficiency of each treatment was calculated at the end of the incubation experiment (day 74), according to:

$$RL = [(2 \times |D_{35}|) / (|D_{35}| + |D_{74}|)] - 1$$

D_{74} is the difference between soil metabolic quotient value in the control (H0.9 in Planosols and Nitisols) treatment and the stressed treatment at the end of the RL phase (day 74). 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 C use efficiency to compaction and water stresses. Negative values of the RS index indicate a change greater than 100% in the response variable compared with that in the control treatment. If the absolute value of D_{74} is higher than the absolute value of D_{35}, then the RL index has a negative value. Mathematically, the RL index of the control treatment is infinitely close to +1, while it has been considered as +1 in the RL equation.

1.2.5 Statistical analysis

Differences at $P < 0.05$ between treatments were considered significant and all variables were tested for normality of distribution using the Kolmogrov–Smirnov test. The effects and interactions of water content, compaction level, and soil types on soil properties were tested using three-way ANOVA. Soil properties were assessed using principal component analysis (PCA) to distinguish the effects of water and compaction levels at the end of RS and RL phases, using the IBM SPSS Statistics 26 software package (IBM Corp., released 2020, IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp). Stepwise multiple linear regression analysis was used to identify the relationships between cumulative CO_2 emissions and soil properties.

1.3 RESULTS

Both soils were slightly acidic (pH ranged from 5.5–6.5) at the end of incubation period. However, there were no significant differences in soil pH values among treatments. The initial TC and TN contents were found to be significantly ($P < 0.05$) higher in the Nitisols soil treatments than the Planosols soil treatments, while no significant differences were observed between the initial and final concentrations of TC and TN across treatments.

1.3.1 Soil bioavailable carbon and nitrogen contents

The Planosols treatments with high water content (H1.1 to H1.5) showed an increase in HWEOC contents in the first week of the experiment (from 480 mg kg^{-1} to 541 mg kg^{-1}), while those with low water content (L0.9 to L1.5) resulted in a decrease in HWEOC contents. However, regardless of water content, the overall concentrations of HWEOC in all Planosols treatments decreased sharply in the second week of incubation and continuously decreased (with a gentle slope) until the end of the RS phase at day 35, and before the removal of compaction stress by ploughing (Fig. 1.1a). During the RL phase, which started at day 35, the concentration of HWEOC significantly ($P < 0.05$) increased in all treatments (days 35–42), and then gradually decreased from day 49 until the end of the incubation. There were no significant differences in HWEOC concentrations of Planosols treatments with low water content (L0.9 to L1.5) in the RS phase of the experiment (first 35 days), while the concentration of HWEOC was significantly ($P < 0.05$) higher in treatments with low compaction (L0.9, L1.1 and L1.2) than higher compaction (L1.3, L1.4 and L1.5) in the RL phase (days 42–74).

In the Planosols treatments with high water content, the more compacted treatments (H1.3, H1.4 and H1.5) tended to have a higher concentration of HWEOC compared with lower compaction levels (H0.9, H1.1 and H1.2) throughout the experiment period (days 7–74). At the end of incubation, the concentration of HWEOC in low compacted treatments with low water content (L0.9, L1.1 and L1.2) was significantly ($P < 0.05$) higher than the high compacted treatments (L1.3, L1.4 and L1.5), while an opposite pattern was observed in treatments with high water content. This may indicate the significantly ($P < 0.05$) higher release of labile C in the RL phase of the experiment, in Planosols under low levels of compaction and low water content (L0.9, L1.1 and L1.2) compared with other treatments.

The observed pattern of HWEOC concentration shifts in Nitisols, during RS and RL phases, was similar to Planosols. The HWEOC content in Nitisols treatments with low water content (L0.9 to L1.5) tended to decrease with increasing the compaction level, during RS phase, with the values

decreased gradually from day 7 (average of 368 mg kg^{-1}) to day 35 (average of 162 mg kg^{-1}) of the experiment (Fig. 1.1b). However, there was no significant difference among all treatments at the end of the RS phase (day 35). After removal of compaction stress at day 35, the HWEOC content in Nitisols treatments increased to levels close to its initial concentration (average of 406 mg kg^{-1}) within one week, and then gradually decreased towards the end of the experiment. Regardless of soil type, all treatments with high water content showed lower concentration of HWEOC compared with other treatments, at the end of the RL phase (Figs. 1.1a and 1.1b). This observation indicates that the dependency of the C mineralisation rate (both Planosols and Nitisols) to the soil moisture status during recovery forms an environmental stress.

Planosols generally showed a decrease in HWETN content during the first two weeks of incubation, while the values increased towards the end of the RS phase at day 35 (Fig. 1.1c). By the end of the RS phase, the H1.5 treatment (with highest compaction and water content) showed the lowest ($P < 0.05$) HWETN content, while no significant differences were observed between other treatments. In the RL phase, the HWETN contents in all of treatments significantly ($P < 0.05$) increased in the first week (day 42), and then showed a stable pattern until the end of incubation experiment (day 74). The H1.4 and H1.5 treatments (with highest compaction and water content) showed the lowest ($P < 0.05$) recovery in HWETN content during the RL phase, compared with other treatments. By the end of the RL phase, Planosols treatments with high water content (H1.1 to H1.5) and low water content (L0.9 to L1.5) showed significantly ($P < 0.05$) lower and higher HWETN contents compared with control treatment (H0.9), respectively.

The observed pattern of HWETN concentration shifts in Nitisols during RS and RL phases was similar to Planosols. The H1.5 treatment (with highest compaction and water content) showed the lowest ($P < 0.05$) HWETN content throughout the RS phase (Fig. 1.1d). However, the Nitisols treatments with low water content (L0.9 to L1.5) generally had higher HWETN contents compared to other treatments in the first 35 days of the incubation. At the end of the RL phase, no significant

differences were observed between the Nitisols treatments with low water content (L0.9 to L1.5) and the control treatment (H0.9). However, Nitisols treatments with high water content (H1.1 to H1.5) showed significantly ($P < 0.05$) lower recovery in HWETN contents in comparison with the control treatment, with lower values in high compacted treatments (H1.3, H1.4 and H1.5) than in low compacted treatments (H1.1 and H1.2).

The mineral N ($NH_4^+ + NO_3^-$) contents did not show any significant difference among all Planosols treatments in the first week of the RS phase (Table 1.1), while the observed values were significantly lower than the Nitisols treatments (except the H1.4 treatment). Although different Planosols treatments showed different patterns of N mineralisation during the RS phase, their mineral N concentrations were not significantly different at day 35 of incubation (the values were generally lower than Nitisols treatments). In the RL phase, the Planosols treatments with high compaction and high water content (H1.3, H1.4 and H1.5), showed significantly ($P < 0.05$) lower N mineralisation rates compared with other treatments, while the control treatment (H0.9) had the highest ($P < 0.05$) N mineralisation rate at the end of the experiment (day 74). The Nitisols treatments with low water content (L0.9 to L1.5) showed higher N mineralisation rates in the first week of the RS phase compared with the control (H0.9) and treatments with high water content (H1.1 to H1.5), while there was no significant difference among all Nitisols treatments at the end of the RS phase (day 35). Similar to Planosols, the Nitisols treatments with high compaction and high water content (H1.3, H1.4 and H1.5) showed the lowest ($P < 0.05$) mineral N concentrations in the first seven days of the RL phase. However, the Nitisols treatments with low water content (L0.9 to L1.5) generally had lower mineral N concentrations compared with the control and high water content (H1.1 to H1.5) treatments by the end of the experiment.

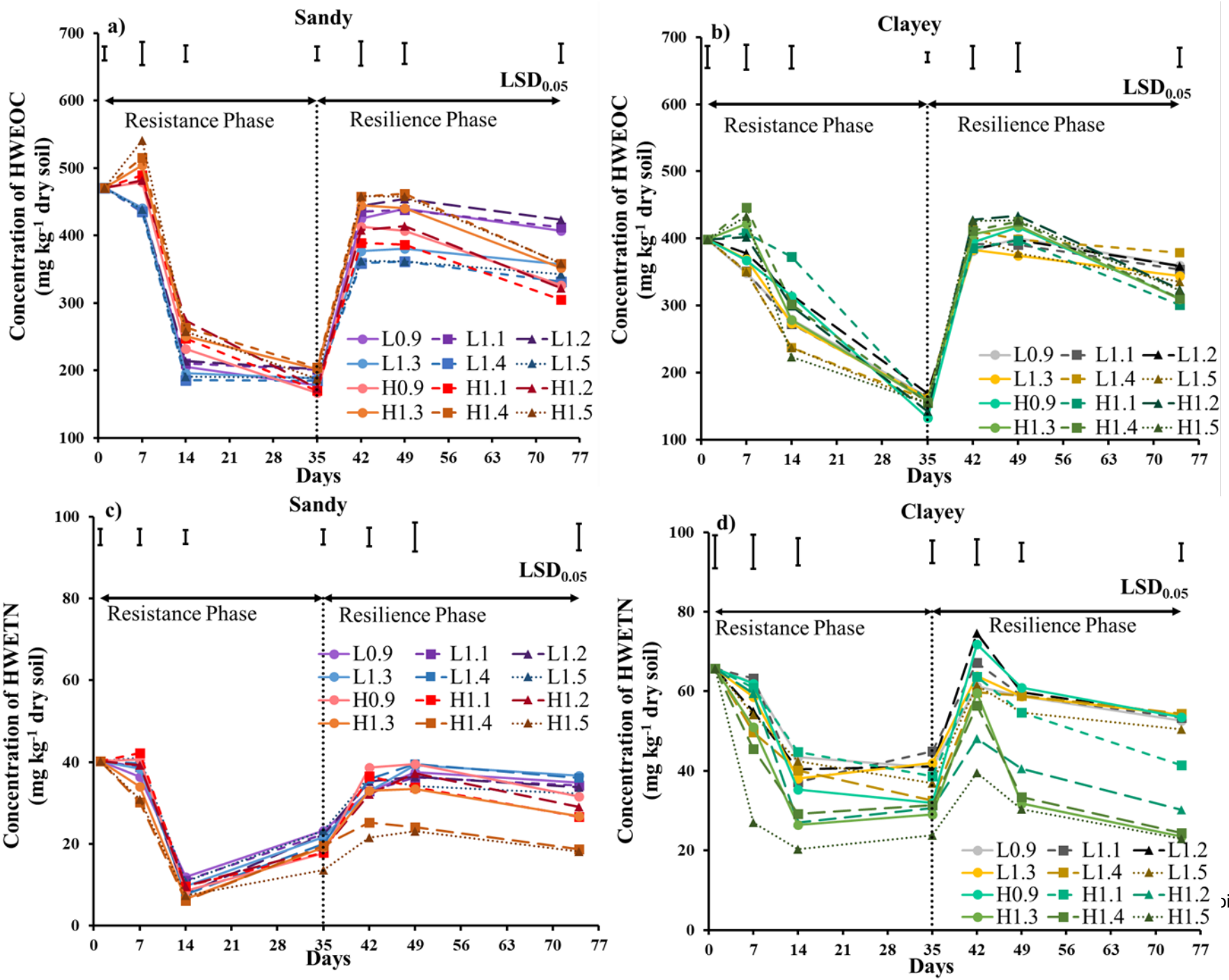

a)
Sandy
LSD0.05
Resistance Phase
Resilience Phase
Concentration of HWEOC (mg kg-1 dry soil)
Days
L0.9 L1.1 L1.2
L1.3 L1.4 L1.5
H0.9 H1.1 H1.2
H1.3 H1.4 H1.5
b)
Clayey
LSD0.05
Resistance Phase
Resilience Phase
Concentration of HWEOC (mg kg-1 dry soil)
Days
L0.9 L1.1 L1.2
L1.3 L1.4 L1.5
H0.9 H1.1 H1.2
H1.3 H1.4 H1.5
c)
Sandy
LSD0.05
Resistance Phase
Resilience Phase
Concentration of HWETN (mg kg-1 dry soil)
Days
L0.9 L1.1 L1.2
L1.3 L1.4 L1.5
H0.9 H1.1 H1.2
H1.3 H1.4 H1.5
d)
Clayey
LSD0.05
Resistance Phase
Resilience Phase
Concentration of HWETN (mg kg-1 dry soil)
Days
L0.9 L1.1 L1.2
L1.3 L1.4 L1.5
H0.9 H1.1 H1.2
H1.3 H1.4 H1.5

Fig. 1.1 Effect of combined compaction and moisture stresses on concentration of hot water extractable organic C (HWEOC) and hot water extractable total N (HWETN) in sandy (a and c) and clayey (b and d) sugarcane soils (n = 36). L, low moisture level (15% GWC); H, high moisture level (35% GWC). Numbers followed by letter L or H indicate bulk density (e.g. 0.9 means 0.9 g cm^{-3}). Low moisture treatments refer to 15% GWC with bulk density ranging from 0.9 to 1.5 g cm^3 (water fill pore space ranged from 21% to 52%) and high moisture treatments refer to 35% GWC with bulk density ranging from 0.9 to 1.5 g cm^3 (water fill pore space ranged from 47% to over 100%). For example, L0.9 in Fig. 1.1a means sandy soil with moisture content of 15% GWC and bulk density of 0.9 g cm^{-3} and H1.5 in Fig. 1.1b means clayey soil with moisture content of 35% GWC and bulk density of 1.5 g cm^{-3}. The Least Significant Difference ($LSD_{0.05}$) bars indicate significant differences between treatments. The reported data are means of three replicates.

Table 1.1 Mineral N contents of sugarcane soils under different moisture and compaction stresses.

Soil types	Treatments	Mineral N (mg kg^{-1} dry soil)					
		day7	day14	day35	day42	day49	day74
Sandy	L0.9	20.01e	29.48c	22.32e	24.84d	32.46e	52.14c
	L1.1	17.62e	26.75cd	26.06de	26.92d	36.04de	65.59c
	L1.2	16.81e	31.42c	28.71d	26.38d	41.69de	61.76c
	L1.3	19.17e	31.97c	43.67c	29.33d	35.22de	59.09c
	L1.4	19.36e	29.95c	33.75cd	40.34c	42.26de	39.81d
	L1.5	19.4e	28.27cd	28.03d	30.23d	42.33de	50.88c
	H0.9	17.98e	25.21d	34.49cd	32.19c	35.25de	85.49a
	H1.1	20.54e	24.43d	29.74d	34.61c	29.59e	77.11b
	H1.2	17.57e	23.49d	28.09d	27.98d	30.31e	54.03c
	H1.3	17.95e	28.68cd	25.66de	22.67e	25.46f	16.36f
	H1.4	16.22e	24.17d	23.83e	14.97e	15.01f	18.99f
	H1.5	19.49e	24.87d	25.19de	9.89f	12.17f	23.64e
Clayey	L0.9	46.73b	54.60a	36.34cd	59.86b	66.55c	20.72e
	L1.1	48.04b	45.03ab	41.56c	73.78a	80.43b	21.24e
	L1.2	57.13a	57.45a	50.17b	73.34a	88.24a	29.41de
	L1.3	53.49a	53.21a	66.82a	82.22a	63.34c	32.28d
	L1.4	45.68b	51.70a	44.66c	68.3ab	50.05d	25.78de
	L1.5	42.74c	51.15a	45.36c	64.25ab	56.37cd	28.69de
	H0.9	37.08d	56.63a	49.19b	76.36a	66.72c	41.19d
	H1.1	42.21c	38.64b	49.46b	59.57b	50.40d	38.74d
	H1.2	44.43c	37.30b	44.83c	36.46c	34.26de	41.48d
	H1.3	43.15c	46.48ab	40.18cd	23.85e	22.32f	42.20d
	H1.4	24.23e	31.98c	30.88d	22.79e	15.66f	37.68d
	H1.5	37.14d	35.81b	31.63d	19.65e	13.03f	23.81e

The reported data are means of three replicates. Means followed by different letters within a column indicate significant differences between the treatments at $P < 0.05$.

1.3.2 Soil enzyme activities and microbial biomass carbon and nitrogen contents

The changes of β-glucosidase enzyme activity in Planosols treatments did not show any specific pattern during the RS phase (first 35 days); however, all Planosols treatments showed lower β-glucosidase enzyme activity than the control (H0.9) at the end of the RL phase (day 74; Table 1.2). In contrast to Planosols, compaction stress did not show any significant effect on β-glucosidase enzyme activity of Nitisols treatments in the RS phase. All Nitisols treatments with low water content (L0.9 to L1.5) had significantly ($P < 0.05$) lower β-glucosidase enzyme activity than control (H0.9) and high water content (H1.1 to H1.5) treatments by the end of the RS phase. However, no specific pattern of recovery was observed in β-glucosidase enzyme activity of the Nitisols treatments during the RL phase. The chitinase enzyme activity in the Planosols treatments with low water content (L0.9 to L1.5) were generally higher than the control (H0.9) and high water content (H1.1 to H1.5) treatments at the end of the RS phase (Table 1.2). However, this difference increased during the RL phase and resulted in significantly ($P < 0.05$) higher chitinase enzyme activity in low water content than the control and high water content treatments (except H1.5) by the end of the experiment (day 74). In contrast, Nitisols treatments with high water content (H1.1 to H1.5) showed higher chitinase enzyme activity than low water content (L0.9 to L1.5) treatments during the RS and RL phases. There were no significant differences between chitinase enzyme activity in control and high water content Nitisols treatments during the experiment.

The MBC content in low compacted Planosols treatments with low water content (L0.9, L1.1 and L1.2) and the control treatment (H0.9) was significantly ($P < 0.05$) higher than other treatments in the first week of the RS phase (Fig. 1.2a). However, by the end of the RS phase (day 35) Planosols treatments with high water content (H1.1 to H1.5) showed significantly ($P < 0.05$) higher MBC content than the control (H0.9) and treatments with low water content (L0.9 to L1.5), except the L0.9 treatment. The changes of MBC contents in Planosols treatments did not show any specific pattern during the first two weeks of the RL phase (day 49), while the L0.9 and H1.3 treatments showed significantly ($P < 0.05$) higher values than other treatments at the end of the experiment (day 74). In

Nitisols treatments, the MBC contents in treatments with low compaction and low water content (L0.9, L1.1 and L1.2) were significantly higher than other treatments in the first seven days of the RS phase, while the treatments with high compaction and low water content (L1.3, L1.4 and L1.5) showed significantly lower values compared with other treatments at day 35 (Fig. 1.2b). By the end of the RL phase at day 74, the Nitisols control treatment (H0.9) had significantly ($P < 0.05$) higher MBC content than other treatments (except L1.1 and H1.1 treatments).

The changes of MBN content in the Planosols treatments showed a similar pattern in both the RS and RL phases (Fig. 1.2c), with generally higher values observed in treatments with high water content (H1.1 to H1.5) than low water content (L0.9 to L1.5) at the end of both phases (except L0.9 treatment at the RS phase). In the Nitisols treatments, the H1.3 treatment showed significantly ($P < 0.05$) higher MBN content in the first week of the RS phase, while no significant differences were observed between other treatments (Fig. 1.2d). At the end of the RS phase, the Nitisols treatments with high compaction with high (H1.3, H1.4 and H1.5) and low (L1.3, L1.4 and L1.5) water content showed significantly ($P < 0.05$) higher and lower MBN contents than other treatments, respectively. Similarly, Nitisols treatments under high compaction and high water content (H1.3, H1.4 and H1.5) showed higher MBN contents compared with other treatments in the first week of the RL phase, while the control treatment (H0.9) had lower MBN content than other treatments (except L1.3, L1.4 and L1.5 treatments) by the end of the experiment (day 74).

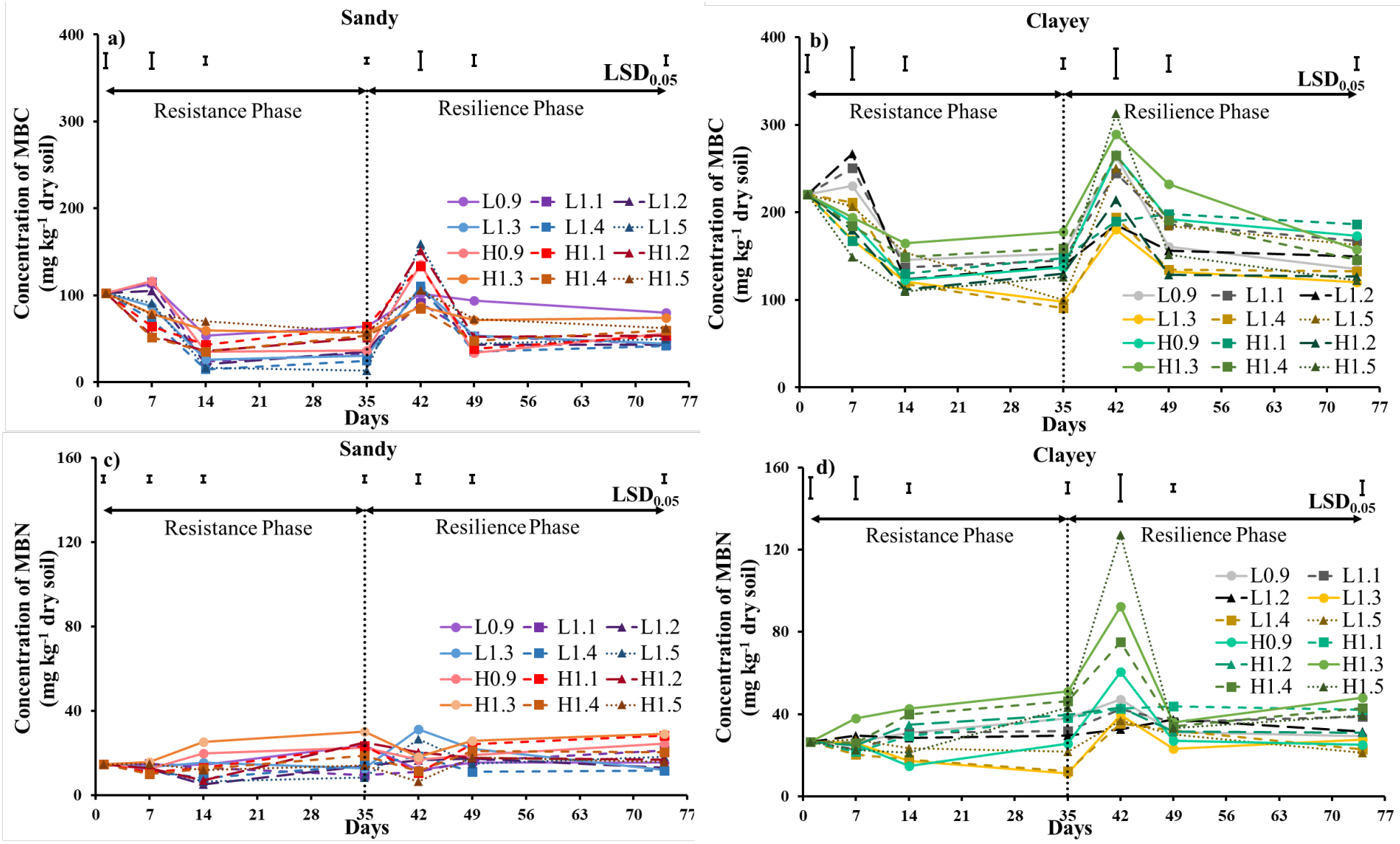

Sandy
a)
Clayey
b)
Sandy
c)
Clayey
d)
Resistance Phase
Resilience Phase
LSD0.05
Concentration of MBC (mg kg-1 dry soil)
Concentration of MBN (mg kg-1 dry soil)
Days
L0.9 L1.1 L1.2
L1.3 L1.4 L1.5
H0.9 H1.1 H1.2
H1.3 H1.4 H1.5

Fig. 1.2 Effect of combined compaction and moisture stresses on soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) contents in sandy (a and c) and clayey (b and d) sugarcane soils (n = 36). L, low moisture level (15% GWC); H, high moisture level (35% GWC). Numbers followed by the letter L or H indicate bulk density (e.g. 0.9 means 0.9 g cm^{-3}). The Least Significant Difference (LSD0.05) bars indicate significant differences between treatments. The reported data are means of three replicates.

Table 1.2 Selected enzyme activities of sugarcane soils under different moisture and compaction stresses.

Treatments	Soil types	β-Glucosidase activities (μg p-nitrophenol h^{-1} g^{-1} dry soil)						Chitinase activities (μg p-nitrophenol h^{-1} g^{-1} dry soil)					
		day7	day14	day35	day42	day49	day74	day7	day14	day35	day42	day49	day74
Sandy	L0.9	215.9c	261.4c	82.2ef	126.6f	228.0c	60.2g	269.3b	223.1a	111.8c	98.9d	157.0b	107.8c
	L1.1	180.3d	237.64d	63.4f	202.2cd	198.9d	128.8e	281.4b	210.2a	115.4c	128.1bc	138.6c	110.3c
	L1.2	194.9d	263.6c	85.4ef	205.9c	231.9c	182.0d	306.3a	204.0a	143.4a	134.1bc	157.7b	122.6c
	L1.3	188.3d	189.7e	113.8e	204.5c	167.7d	183.9d	269.1b	170.3ab	136.7ab	135.9bc	145.6c	143.1b
	L1.4	169.9d	214.4de	93.5ef	229.6b	190.2d	180.1d	264.2b	204.1a	156.3a	120.3c	143.7c	118.1c
	L1.5	150.0e	192.1e	149.1d	231.0b	159.5de	148.4de	261.5b	161.0b	143.5a	128.7bc	146.1c	131.6b
	H0.9	182.2d	157.5f	133.9d	240.7b	156.2de	209.7cd	247.1bc	197.5a	121.2b	121.6c	123.3d	96.0d
	H1.1	141.2e	168.2ef	100.8e	206.5c	245.3c	191.1d	333.4a	209.2a	120.4b	118.0c	96.9e	95.3d
	H1.2	213.7c	211.0de	157.0d	190.1cd	113.4e	151.2de	261.1b	169.4ab	86.6d	105.8d	99.2e	85.4de
	H1.3	189.6d	270.6c	152.5d	227.9b	93.2f	126.6e	293.7a	208.4a	105.8cd	107.9d	89.7f	90.3de
	H1.4	148.6e	160.5f	138.0d	258.1a	113.6e	91.1f	209.2c	142.5c	93.8d	106.7d	136.5c	93.4d
	H1.5	174.9d	164.3f	120.5e	212.0c	122.8e	135.6e	250.5bc	155.1b	104.8cd	108.3d	112.5d	105.2c
Clayey	L0.9	247.8b	308.1b	235.3c	186.7e	335.3b	241.8c	138.6e	120.1e	24.2f	52.0f	91.9f	36.7f
	L1.1	237.8b	264.2c	219.7c	196.2cd	280.1b	160.2de	153.8e	129.9d	36.5f	73.6e	82.0f	66.4e
	L1.2	241.1b	326.6b	254.4c	264.3ab	318.0b	194.6d	168.8d	125.8d	73.9e	62.8f	100.6e	105.4c
	L1.3	192.8d	185.6e	264.8c	276.2a	407.1a	262.1c	143.7e	127.2d	72.2e	103.5d	94.5e	101.6d
	L1.4	188.8d	175.0ef	229.0c	219.8bc	339.1b	275.9c	157.9e	119.0e	72.7e	76.1e	93.8e	109.5c
	L1.5	163.1de	183.4e	225.7c	220.7bc	321.3b	277.6c	159.2e	88.9f	108.2c	85.2e	85.2f	112.6c
	H0.9	218.2c	206.5de	359.8b	260.6ab	219.7cd	461.2b	218.2c	160.0b	137.3ab	145.1b	232.1a	168.6a
	H1.1	288.67a	239.1d	395.2b	210.2c	207.0cd	517.9a	146.4e	155.4b	148.8a	193.8a	228.4a	184.2a
	H1.2	342.0a	202.5de	377.6b	233.6b	296.1b	204.4cd	167.4d	157.9b	112.6c	138.6b	206.9a	195.3a
	H1.3	314.4a	415.17a	552.8a	269.4ab	243.7c	154.0de	183.1cd	158.5b	130.7ab	147.8b	191.5ab	180.5a
	H1.4	274.51a	179.3ef	337.8b	262.1ab	179.8d	208.9cd	156.9e	163.6b	132.6ab	150.0b	188.9ab	134.4b

H1.5	159.2de	234.7d	349.8b	282.15a	209.1cd	235.2c	152.7e	116.9e	144.3a	174.9a	193.1ab	115.7b

The reported data are means of three replicates. Means followed by different letters within a column indicate significant differences between the treatments at $P < 0.05$.

1.3.3 Soil microbial respiration and metabolic quotient

The values of cumulative soil microbial respiration (presented as cumulative CO_2 emission) in Planosols treatments were generally higher in treatments with low water content (L0.9 to L1.5) than the control (H0.9) and high water content (H1.1 to H1.5) at the end of the RS phase (Figs. 1.3a and 1.3c). In low moisture conditions, the highest and lowest values were observed in L1.5 and L1.1 treatments, respectively, while in high moisture conditions, the high compacted treatments (H1.3, H1.4 and H1.5) had higher cumulative respiration than other treatments at day 35. In contrast, by the end of the RL phase at day 74, the Planosols treatments with low water content (L0.9 to L1.5) generally showed lower cumulative respiration than the control (H0.9) and high water content (H1.1 to H1.5) treatments. In low moisture conditions, the L0.9 treatment showed the lowest cumulative respiration and the values generally increased with increasing the compaction level (Fig. 1.3a). However, in high moisture conditions, the control treatment (H0.9) showed lower cumulative respiration than other treatments (except the H1.5 treatment) by the end of the experiment (Fig. 1.3c).

The values of cumulative soil microbial respiration in Nitisols treatments showed different trends for high (BD $\geq$ 1.3 g cm^{-3}) and low (BD < 1.3 g cm^{-3}) compacted treatments at the RS phase. The cumulative respiration values in treatments with BD $\geq$ 1.3 g cm^{-3} were lower with high water content than low water content conditions, while the adverse trend was observed in treatments with BD < 1.3 g cm^{-3} at day 35 (Figs. 1.3b and 1.3d). In low moisture conditions, the highest and lowest values were observed in L1.5 and L0.9 treatments, respectively, while in high moisture conditions, the control treatment (H0.9) showed the highest cumulative respiration and the values generally decreased with increasing the compaction level. At the end of the RL phase (day 74), the Nitisols treatments with low water content (L0.9 to L1.5) showed significantly ($P < 0.05$) lower cumulative respiration than the control (H0.9) and high water content (H1.1 to H1.5) treatments. In low moisture conditions, the cumulative respiration values were generally increased with increasing the compaction level (Fig. 1.3b). However, in high moisture conditions, the control treatment (H0.9)

showed the highest cumulative respiration and the observed values generally decreased with increasing the compaction level (day 74; Fig. 1.3d).

The changes in metabolic quotient (qCO_2) values did not show a specific pattern among Planosols treatments in the first two weeks of the RS phase (Table 1.3), while the observed values were generally higher than the Nitisols treatments. At the end of the RS phase, all Planosols treatments with high water content (H1.1 to H1.5) showed significantly ($P < 0.05$) lower qCO_2 values than the control treatment (H0.9), while the highest ($P < 0.05$) qCO_2 values were observed in L1.5 followed by L1.4 treatments (under high compaction and low water content). The qCO_2 values significantly ($P < 0.05$) decreased in all Planosols treatments during the first week of the RL phase (except H1.3 treatment). However, by the end of the RL phase, the L1.5 treatment showed the highest ($P < 0.05$) and L0.9 and H1.5 treatments showed the lowest ($P < 0.05$) qCO_2 values. The Nitisols treatments under low compaction and low water content (L0.9 to L1.2) or high compaction and high water content (H1.3 and H1.4, except H1.5) showed significantly ($P < 0.05$) lower qCO_2 values than other treatments at the end of the RS phase. However, by the end of the experiment at day 74, There were no significant differences between Nitisols treatments with high water content (H1.1 to H1.5) and the control treatment (H0.9), while all Nitisols treatments with low water content (L0.9 to L1.5, except L1.4) had significantly ($P < 0.05$) lower qCO_2 values than control treatment.

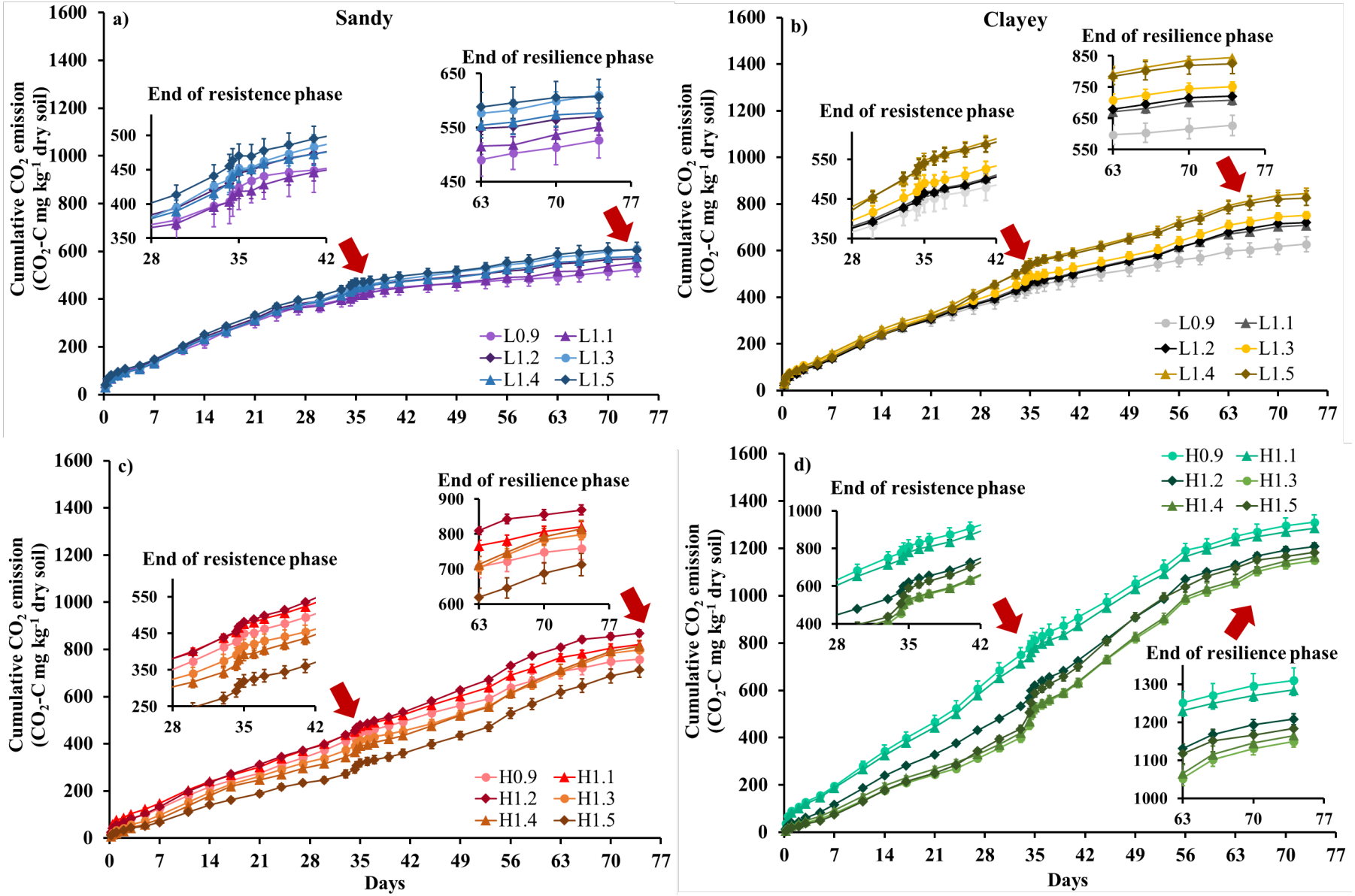

a)
Sandy
End of resistence phase
End of resilience phase
Cumulative CO_2 emission (CO_2-C mg kg^{-1} dry soil)
L0.9 L1.1 L1.2 L1.3 L1.4 L1.5
b)
Clayey
End of resistence phase
End of resilience phase
L0.9 L1.1 L1.2 L1.3 L1.4 L1.5
c)
End of resistence phase
End of resilience phase
H0.9 H1.1 H1.2 H1.3 H1.4 H1.5
Days
d)
End of resistence phase
End of resilience phase
H0.9 H1.1 H1.2 H1.3 H1.4 H1.5
Days

Fig. 1.3 Effect of combined compaction and moisture stresses on cumulative CO_2 emissions in sandy (a and c) and clayey (b and d) sugarcane soils (n = 18). L, low moisture level (15% GWC); H, high moisture level (35% GWC). Numbers followed by the letter L or H indicate bulk density (e.g. 0.9 means 0.9 g cm^{-3}). Vertical bars are standard error of three replicates.

Table 1.3 Metabolic quotient (qCO_2) of sugarcane soils under different moisture and compaction stresses.

Soil types	Treatments	Metabolic quotient (μg CO_2-C h^{-1} mg^{-1} microbial biomass C)					
		day7	day14	day35	day42	day49	day74
Sandy	L0.9	1.29c	4.11b	6.18d	2.87cd	4.96e	4.82f
	L1.1	0.88d	17.17a	12.07c	4.89b	14.50b	11.68b
	L1.2	1.67b	12.94a	12.11c	3.01c	11.79c	13.19b
	L1.3	1.3c	5.82b	7.59d	4.46b	7.05d	9.62cd
	L1.4	2.12a	16.40a	17.31b	4.33b	21.05a	14.10b
	L1.5	1.61b	16.24a	33.75a	3.10c	11.87c	31.45a
	H0.9	1.09cd	6.21b	11.41c	2.99c	17.02a	13.56b
	H1.1	2.31a	5.66b	6.87d	3.92b	15.85b	15.48b
	H1.2	2.63a	6.73b	8.60d	3.54c	12.10c	16.31b
	H1.3	1.26c	3.27c	6.60d	7.93a	7.38d	10.78b
	H1.4	1.62b	5.21b	6.46d	5.13b	11.38c	14.03b
	H1.5	0.89d	2.05c	4.75d	3.42c	6.11e	6.37e
Clayey	L0.9	0.63e	1.12c	1.85e	1.82d	3.23e	4.63f
	L1.1	0.56e	1.77c	2.99e	2.08d	3.00e	4.24f
	L1.2	0.51e	1.94c	3.07e	2.68cd	3.59e	4.86f
	L1.3	0.92d	2.08c	4.63d	2.94c	3.55e	5.01e
	L1.4	0.78d	3.93b	5.52d	3.11c	4.89e	9.12cd
	L1.5	0.69de	1.54c	5.02d	2.35d	3.51e	5.10e
	H0.9	1.03cd	2.80c	5.46d	3.42c	5.48e	7.57d
	H1.1	1.47b	2.49c	4.85d	4.58b	5.19e	6.90d
	H1.2	0.65e	2.15c	4.12d	3.39c	7.09d	9.58cd
	H1.3	0.31f	1.08b	2.24e	2.17d	3.52e	7.29d
	H1.4	0.51e	4.71b	2.87e	2.01d	8.05d	7.75d
	H1.5	0.54e	2.59c	5.40d	2.79cd	7.47d	9.84cd

The reported data are means of three replicates. Means followed by different letters within a column indicate significant differences between the treatments at $P < 0.05$.

Table 1.4 Stepwise multiple linear regression equations between cumulative CO_2 emissions and soil properties at the end of resistance and resilience phases.

Incubation phase	Equation	R^2	P value
Resistance phase (day 35)	Y (CO_2) = −27.04 (Labile C: N Ratio) −3.32 (chitinase) + 484.1	0.57	0.05
Resilience phase (day 74)	Y (CO_2) = 27.21(Labile C: N Ratio) + 36.71(MBC: MBN Ratio) + 1.19 (chitinase) + 345.65	0.81	0.05

1.3.4 Relationship between soil properties under stress, and resistance and resilience indices

The principal component analysis (PCA) between soils (Planosols and Nitisols) physicochemical and biological properties at the end of RS (day 35; Fig. 1.4a) and RL (day 74; Fig. 1.4b) phases indicate that principal component 1 (PC1) and 2 (PC2) explain 37.1–41.2% and 22.2– 27.6% of the observed changes in soil properties, respectively. Soil properties with the highest correlation coefficients (> 0.8) for PC1 are HWEOC, HWETN, MBC, MBN, β-glucosidase activity and cumulative respiration, while the WFPS and BD of soil samples show the highest correlation coefficient (> 0.8) for PC2. The PCA analysis at the end of the RS phase (Fig. 1.4a) clearly separates Planosols treatments from Nitisols treatments, with a similar pattern observed from low to high compaction stress (indicated by arrows) at both soil types. The results also show a higher resistance to moisture stress in the Planosols, compared with the Nitisols, as indicated by lower difference in Planosols treatments under nil and high moisture stress. The PCA analysis at the end of the RL phase (Fig. 1.4b) indicates a different pattern of recovery in soil properties under low and high moisture stresses, while this pattern is very similar between Planosols and Nitisols.

The stepwise multiple linear regression analysis was performed at the end of RS (day 35) and RL (day 74) phases to explore the relationship between cumulative soil microbial respiration (cumulative CO_2 emission) and other soil (Planosols and Nitisols) physicochemical and biological properties (Table 1.4). The results indicate that soil labile C:N ratios and chitinase enzyme activity were the main regulating factors of soil respiration in both soils during the RS phase ($R^2 = 0.57$, $P < 0.05$, $n = 24$). However, in the RL phase, the same parameters (labile C:N ratios and chitinase enzyme activity) as well as soil MBC:MBN ratios were the dominant factors responsible for the recovery of the soil samples under compaction and moisture stresses ($R^2 = 0.81$, $P < 0.05$, $n = 24$).

In Planosols, the highest and lowest RS index of soil microbial C use efficiency (qCO_2) to compaction and moisture stresses (compared with control (H0.9) treatment) were observed in L1.2 and L1.5 treatments, respectively (Fig. 1.5a). Generally, the RS of qCO_2 increased with increasing treatments'

bulk density to 1.2 g cm^{-3} and decreased afterward, indicating the higher RS of the Planosols to moisture stress than compaction stress. The RS of qCO_2 was generally lower in Planosols treatments compared with Nitisols treatments. However, the RS of Nitisols treatments under low compaction and water content (L0.9 to L1.2) and high compaction and water content (H1.3 to H1.5) were significantly ($P < 0.05$) lower than other treatments, indicating the important role of WFPS in regulating the RS of the Nitisols (Fig. 1.5b). In contrast to the RS index, the RL index of qCO_2 in Planosols treatments was higher than in Nitisols treatments. The RL of qCO_2 in Planosols treatments increased with increasing the compaction level, with higher recovery from stress observed in low water content than high water content condition (Fig. 1.5c). This behaviour indicates the higher potential of the Planosols in recovery from low moisture (drought) than high moisture (waterlogging) content. In contrast, the RL of qCO_2 in Nitisols treatments were significantly ($P < 0.05$) lower in low water content than high water content, indicating the lower potential of the Nitisols in recovery from drought stress than waterlogging stress (Fig. 1.5d).

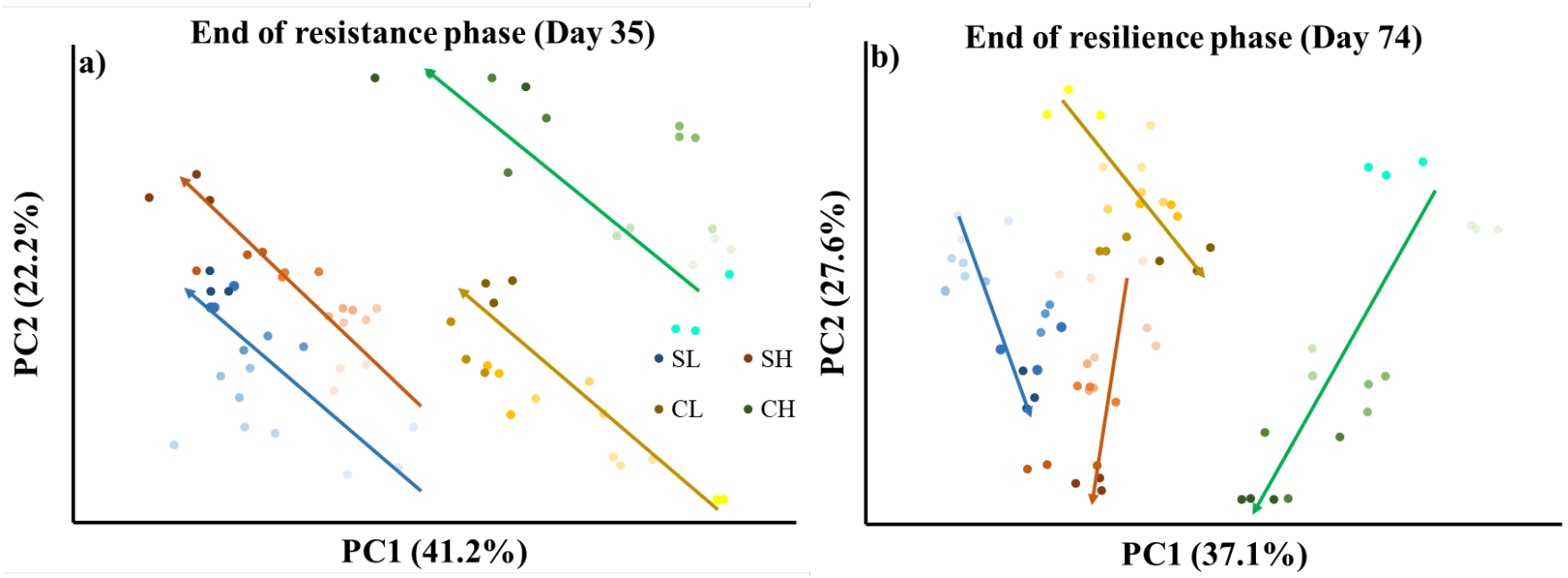


Fig. 1.4 Scores plot of principal component analysis (PCA) showing the separation of different soil samples under compaction and moisture stresses at resistance (a) and resilience (b) phases and loading values of the individual soil parameters (PC1 and PC2) for soil samples (n = 48). SL, sandy soil with low moisture (15%); SH, sandy soil with high moisture (35%); CL, clayey soil with low moisture (15%); and CH, clayey soil with high moisture (35%). Arrows in each plot indicate the distribution of treatments within a moisture group, where treatments with low bulk density (light colours) were close to the tail and treatments with high bulk density (dark colours) were close to the tip of each arrow.

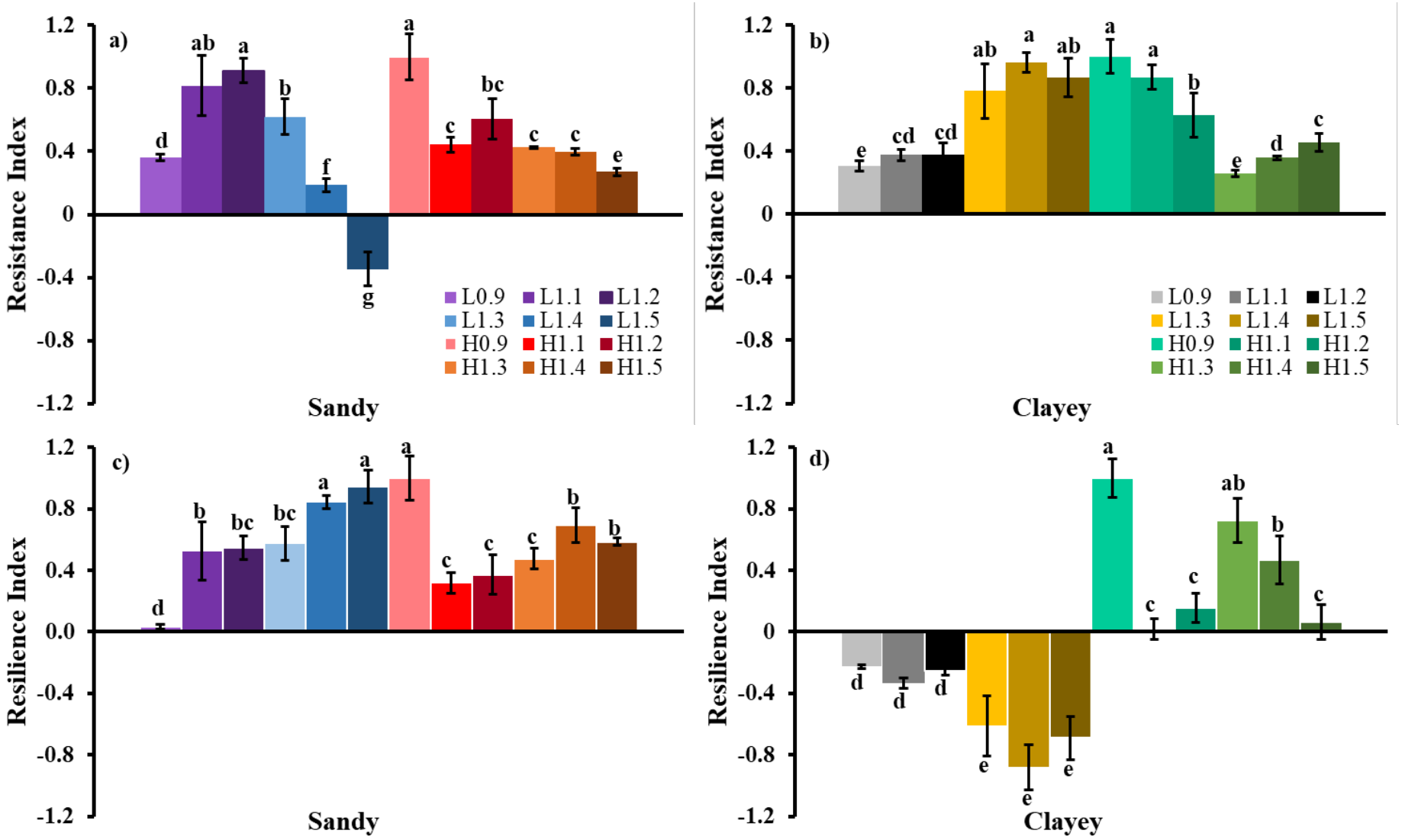

a)
Resistance Index
1.2
0.8
0.4
0
-0.4
-0.8
-1.2
d
ab
a
b
f
g
a
c
bc
c
c
e
L0.9 L1.1 L1.2
L1.3 L1.4 L1.5
H0.9 H1.1 H1.2
H1.3 H1.4 H1.5
Sandy
b)
Resistance Index
e
cd
cd
ab
a
ab
a
a
b
e
d
c
L0.9 L1.1 L1.2
L1.3 L1.4 L1.5
H0.9 H1.1 H1.2
H1.3 H1.4 H1.5
Clayey
c)
Resilience Index
d
b
bc
bc
a
a
a
c
c
c
b
b
Sandy
d)
Resilience Index
d
d
d
e
e
e
a
c
c
ab
b
c
Clayey

Fig. 1.5 The resistance (day 35) and resilience (day 74) indices of soil microbial carbon use efficiency (metabolic quotient) in sandy (a and c) and clayey (b and d) soils. L, low moisture level (15% GWC); H, high moisture level (35% GWC). Numbers follow by the letter L or H indicate bulk density (e.g., 0.9 means 0.9 g cm^{-3}). Vertical bars are standard error of three replicates. Means of the treatments by the same letter are not different at the 5% level of significance.

1.4 DISCUSSION

1.4.1 Response of soil labile organic C and N pools to compaction and moisture stresses

Soil organic matter content and composition can influence various soil physical (e.g. bulk density, aggregation and water holding capacity), chemical (e.g. nutrient stocks, nutrient sorption and exchange capacity) and biological (e.g. microbial biomass, respiration) properties (Guillaume et al., 2016; Kuzyakov et al., 2020). Previous investigations indicate an indirect role of soil organic matter on soil RS and RL to environmental stresses through affecting soil nutrient cycles and microbial functions (Orwin and Wardle, 2004; Yu et al., 2021). Labile C and N pools are the key component of soil organic matter in regulating soil biological health and function as they are bioavailable for both plant uptake and microbial consumption, sensitive to environmental stresses and play an important role in soil nutrient cycling (Hu et al., 1997). The HWEOC and HWETN contents of soil have been widely accepted as the main labile C and N pools of soil for assessment of the effects of changes in environmental conditions and/or field management practices on soil functional biological properties (Chen et al., 2004).

The continuous depletion of HWEOC content in both Planosols and Nitisols (Figs. 1.1a and b) during the RS phase of this experiment (with the lowest values observed in the control treatments at day 35), indicates the dependency of soil microbial functions (i.e. biomass and respiration) on the soil labile C pool as the main source for microbial consumption under limited mineralisation conditions. Similar findings have been reported by Teutscherova et al. (2017), who also observed a significant correlation between soil labile C pool and soil microbial activity. Although the labile C pool in both Planosols and Nitisols showed a similar RS to applied stresses, independent of the compaction and moisture levels, the Nitisols had a higher recovery (RL) in HWEOC content compared with the Planosols during the second phase of the experiment. This behaviour was more obvious in uncompacted Planosols or Planosols with low levels of compaction under moisture stress (L0.9, L1.1 and L1.2) with highest release (mineralisation) and lowest consumption of HWEOC during the RL phase

(possibly due to the highest aerobic condition in these treatments during RS phase). However, the observed peaks of C mineralisation followed ploughing in the RL phase of the experiment, in both Planosols and Nitisols, suggests that availability of soil labile C was not a limiting factor for soil microbial biomass growth and respiration in this study. Removal of environmental stressors, by rewetting dry soils or ploughing compacted soils, has been reported to change the soil microbial community structure and increase the ecosystem C fluxes by increasing the mineralisation of soil organic matter (Banning and Murphy, 2008; Fierer et al., 2001; Guillot et al., 2019; Mikha et al., 2005). In contrast to HWEOC, the HWETN contents in Planosols treatments showed better RS and RL to moisture and compaction stresses compared to Nitisols treatments, with higher sensitivity to changes in compaction than moisture levels. This observation indicates the very low capacity of the Nitisols in recovery of HWETN content following combined waterlogging and compaction stresses. This is in agreement with the findings of previous studies, which indicate the importance of soil moisture status in resilience of soil organic matter to mineralisation process (Hawkes et al., 2017; Hu et al., 2021).

1.4.2 Microbial responses to soil compaction and moisture stresses

Soil biological properties play a critical role in the proper functioning of soil ecosystems, as they are generally more sensitive to environmental stressors than soil physicochemical properties (Graham et al., 2016; Kuzyakov et al., 2020). Microbial stoichiometry (i.e. elemental ratios of soil microbial biomass) has been widely used to assess soil microbial functions (e.g. microbial biomass turnover rate and use efficiency of soil C and N pools) and their energy and nutrient (e.g. resource availability) demands (Cleveland and Liptzin, 2007; Mooshammer et al., 2014). The soil microbial community is also able to shift its stoichiometry, structure and total pool size to respond and adapt to environment stressors (Li et al., 2004). In stressed environments, soil microorganisms would allocate more C and nutrients to survival pathways rather than growth and resource acquisition pathways (Schimel et al., 2007; Tiemann and Billings, 2011).

Soil compaction changes the bioavailability of soil organic C pools for microbial activities. It has been reported that a slight increase in soil compaction level may increase soil MBC and enzyme activities (Deurer et al., 2012), while heavy compaction reduces MBC and microbial activities by increasing soil WFPS and reducing its aeration capacity (Tan et al., 2008). In a similar way, a slight increase in soil moisture content increases soil microbial biomass and activities by increasing soil bioavailable C pools due to the breakdown of soil aggregates, while waterlogging and drought stresses reduce soil microbial biomass and activities by creating anaerobic conditions in the soil profile, or increasing the effect of osmotic stress on stress-sensitive microorganisms, respectively (Butterly et al., 2009; Denef et al., 2001; Guillot et al., 2019; Van Gestel et al., 1993).

Although the Planosols and Nitisols had relatively similar TC contents, the Nitisols had significantly ($P < 0.05$) higher MBC content and microbial respiration in both the RS and RL phases of the experiment (Figs. 1.2 and 1.3). This could be related to the better aggregation in Nitisols compared to Planosols, and the higher protective characteristics of clay-based aggregates for improvement of soil microbial biomass and respiration (Wang et al., 2003). However, the combined effect of compaction and moisture stresses (RS phase) on microbial respiration within the treatments of each soil type was mainly regulated by the WFPS content of each treatment. Therefore, the lowest cumulative microbial respiration was observed in the H1.5 treatment of both Planosols and Nitisols with the highest WFPS (waterlogging condition) content during the RS phase. The regulating effect of soil WFPS on microbial respiration from different treatments could be related to the role of WFPS in determining the dominance of aerobic/anaerobic conditions for microbial growth and activity as: a) treatments with high compaction and low moisture levels had similar WFPS content to treatments with low compaction and high moisture levels in the RS phase; and b) removal of the applied stresses in the RL phase changed the dominant soil environment for microbial activity from anaerobic to aerobic condition in treatments with high water content, while this was not the case in treatments with low water content, as the dominant aerobic condition remained unchanged.

The stepwise multiple linear regression equations between microbial respirations and soil properties indicate that the magnitude of microbial respirations in the soils are mainly regulated by the ratio of soil labile C:N pools in the RS phase and the ratio of soil MBC:MBN contents in the RL phase (Table 1.4). Chuckran et al. (2020) and Deng et al. (2018) also indicate the important role of soil microbial biomass in regulating soil heterotrophic respiration (decomposition of organic matter by soil microorganisms) process under moisture stressed conditions. Regardless of the compaction stress levels, all Nitisols treatments with low water content (L0.9 to L1.5) had lower β-glucosidase and chitinase enzyme activities than control (H0.9) and high water content (H1.1 to H1.5) treatments by the end of the RS phase. The decrease of microbial enzyme activities with decreasing soil moisture status can be attributed to the general reduction in soil microbial biomass content, reduced catalytic capacity and turnover due to the adsorption of enzymes to soil particles under drier conditions, and lower contact of enzymes with substrates due to reduced diffusion rates (Alster et al., 2013; Hueso et al., 2012; Steinweg et al., 2012). In addition, Nitisols treatments generally showed higher C use efficiency than Planosols soil treatments, as evidenced by lower qCO_2 values observed in Nitisols treatments at the end of RS and RL phases (Table 1.3). However, this observation did not lead to higher ecological stability in Nitisols treatments as the effect of compaction and moisture stresses on dynamics of microbial biomass contents (MBC and MBN) and microbial respirations were lower in Planosols treatments than Nitisols treatments.

1.4.3 Potential mechanisms and processes involved in the response of soil microbial system to compaction and moisture stresses

To develop sustainable management practices for improvement of ecological stability, soil fertility and productivity of intensive agricultural systems, it is critical to have a mechanistic understanding of the natural RS and RL of soil biological properties to environmental stressors. However, the mechanisms responsible for the diverse responses of soil processes to environmental stresses are largely unknown and may vary with soil physicochemical properties and stress levels. The RS and RL metrics of soil biological properties can be used to assess the level of improvement of soil

ecosystem towards sustainable states, track thresholds of potential concerns, and help with development of management practices to protect soils that are more sensitive to environmental changes (Corstanje et al., 2015; Quinlan et al., 2016). A resilient ecosystem should be able to withstand the impact of environmental disturbance and fully recover from the impact (Ingrisch and Bahn, 2018). However, different environmental stresses (such as compaction and moisture disturbance) may affect the soil microbial community in different ways. The changes in soil moisture status would alter the decomposition rate of soil organic matter and consequently change the mobility and bioavailability of soil labile C pools for microbial consumption (He et al., 2019; Liu et al., 2016; Wu and Lee, 2011), while the changes in soil compaction level would increase soil WFPS and reduce the aeration capacity of soil profile (Stoessel et al., 2018; Tan et al., 2008).

The findings of this experiment indicate higher RS index of microbial C use efficiency to compaction and moisture stresses in the Nitisols than Planosols. This can be attributed to the greater aggregation potential (associated with more finely textured particles) and higher diversity of microbial community in clayey soils compared with sandy soil (de Andrade Bonetti et al., 2017; Rivest et al., 2013), which may provide higher functional stability in clayey soils following an environmental disturbance. Zhang et al. (2005) and Liu et al. (2007) also illustrate the relationship between soil MBC:MBN ratio and soil microbial community structure, suggesting the dominance of soil microorganisms with higher capacity for C use efficiency in soils with higher MBC:MBN ratios, which agrees with the higher MBC:MBN ratios in the Nitisols treatments, compared with Planosols treatments, observed in the RS phase of this incubation experiment. Soil microbial communities with lower C use efficiency would allocate less labile C to microbial biomass than to aerobic respiration, facilitating soil C loss through significant increase in microbial respiration rates following an environmental stress (Allison et al., 2010; Frey et al., 2013; Manzoni et al., 2012).

These results also show higher RL index of microbial C use efficiency to compaction and moisture stresses in the Planosols treatments in comparison with Nitisols treatments. This observation can be

related to the higher adoption of Planosols' microbial communities to drying and rewetting cycles (due to fast drainage) compared with Nitisols, which may result in their faster recovery from moisture stress. In addition, the rate of microbial turnover and bioavailability of soil labile C pools may also affect the speed of soil microbial recovery after environmental disturbances. This is particularly important during the recovery from drought stress, as rewetting process would physically disrupt soil aggregates and increase the movement and bioavailability of soil organic matter for decomposition (Beare et al., 2009; Schimel, 2018). Pérez-Guzmán et al. (2020) also reports a higher recovery of microbial communities in semiarid agricultural soils with higher sand content, rather than clayey soils, following resource limitations and changes in soil moisture status.

These results indicate that the Nitisols was neither RS nor RL to the drought stress under soil bulk densities ≤ 1.2 g cm^{-3}, while the Planosols treatments were significantly better in recovery from drought stress with higher RL index values than the Nitisols treatments under all applied compaction levels. The Nitisols treatments with low water content and high compaction stresses (L1.4 and L1.5) exhibited the highest RS and lowest RL of microbial C use efficiency. In contrast, Planosols treatments under the same stress levels (L1.4 and L1.5) exhibited the lowest RS and highest RL indices during this incubation experiment. This observation is in agreement with the findings of previous studies that indicate the importance of soil texture, rather than soil organic matter content, in regulating soil RS and RL to compaction and moisture (drying and rewetting) stresses (Corstanje et al., 2015; Gregory et al., 2007; Hu et al., 2021). In this study, the RS index of different treatments was measured as the degree of impairment of their responses relative to a control treatment (H0.9), while their RL index was measured as the rate of recovery following removal of compaction and moisture stresses. Therefore, the negative value of RS index indicates a change of higher than 100% in the microbial C use efficiency of stressed treatment compared to the control treatment. The negative value of the RL index also indicates that the absolute value of difference between the stressed treatment and control treatment was higher at the end of incubation experiment than the end of the RS phase.

1.5 CONCLUSIONS

This study demonstrates that soil microbial activities are directly affected by soil moisture status, whereas compaction may affect soil physiochemical properties and thus affect soil microbial activities indirectly by increasing soil bulk density. Compaction stress increased labile C concentration in both Planosols and Nitisols due to the increase of dissolved organic carbon (DOC) diffusion in high water content condition. Although trends of the shift in labile C pools are similar in both Planosols and Nitisols, their microbial responses towards compaction and moisture content are clearly different. In low compaction and high water content levels (H0.9–H1.2), the microbial respiration increased via the increase in accessibility of substrates, while high compaction stress (H1.3–H1.5) decreased microbial respiration due to the dominance of anaerobic processes. In the Nitisols treatments with high water content (H0.9–H1.5), microbial respiration linearly decreased with the increasing of compaction stress. This observation indicates that the response of soil microorganisms towards compaction is governed by soil texture and moisture status, thus microbial responses manifest a potential robust indicator in soil resilience monitoring. In addition, the Nitisols soil showed higher RS index of microbial C use efficiency, while the Planosols soil had higher RL to compaction and moisture stresses. This may indicate that higher aggregation potential and microbial diversity would build a higher resistance to environmental stresses, while higher adoption of microbial community to frequent disturbance would provide a faster recovery from environmental stresses. The findings imply that the improvement of soil RS and RL to environmental stresses is only achievable through a comprehensive and site-specific approach due to the dominance of different physicochemical and biological processes in different soils within the same cropping system. The assessment of the responses of different soils' microbial community structures and diversity as well as the key functional genes involved in C and N cycling under different stress scenarios may provide more insights into these complicated interactions.

1.6 ACKNOWLEDGMENT

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CHAPTER 2: FIELD MANAGEMENT PRACTICES PLAY A VITAL ROLE IN BUILDING SOIL RESISTANCE AND RESILIENCE AGAINST COMPACTION STRESS

2.1 INTRODUCTION

Soil compaction is a vital factor contributing to global land degradation and has been attracting more interest among researchers in recent years (Shah et al., 2017; Soane and van Ouwerkerk, 1994). Primarily caused by the long-term overuse of machinery in field management practices, soil compaction can result in severe soil health issues, including organic matter loss, root growth restriction, poor root zone aeration, and subsurface soil anaerobic condition (Mirzavand and Moradi-Talebbeigi, 2021; Stoessel et al., 2018). Arvidsson et al. (2014) indicate that soil compaction has a negative effect on crop growth and commonly causes yield loss. Radford et al. (2001) also report that the mean production of wheat reduces by 23% in compacted soil in comparison with uncompacted soil. Research by Cherubin et al., (2016) shows that soil compaction in Brazil resulted in 50% reduction in sugarcane yield potential in the fourth and fifth ratoons, while Bell et al. (2007) conclude that compaction can cause at least 20% reduction in sugarcane yield in the subtropic regions of Australia. The responses of soil nutrient turnover processes to compaction stress and the extent of these responses are governed by the inhabited microbial communities, which have been less explored, particularly in the Australian sugarcane systems. Such investigations provide crucial knowledge in better understanding soil microbial responses and the fate of soil nutrient pools, as well as evaluating soil resistance (RS) and resilience (RL) under different compaction scenarios.

Conservation and organic field management practices are currently considered as mainstream practices to meet the increased demand for sustainable agriculture. Conservation agricultural management includes reducing tillage and using plant residues and/or a trash blanket for permanent ground cover (Chabert and Sarthou, 2020). In addition, incorporation of organic amendments such as

compost show significant advantage in developing sustainable agriculture and delivering higher crop yield with lower levels of pesticide residues in comparison to conventional farming systems (Reganold and Wachter, 2016). Deen and Kataki (2003) report that conservation field management practices reduce 15% of operation costs in comparison with conventional field management. No-tillage field management practices also increase soil organic C by about 5% compared to conventional management practices (Deen and Kataki 2003). However, the reduction in crop yield associated with conservation management practices can be compensated by benefits of sustainable agriculture in the long term. Arvidsson et al. (2014) report that no-tillage practices reduce crop yield by 10% compared to conventional practices. Vyn and Raimbault (1993) also report a significant reduction of corn yield in no-tillage fields compared with other field managements over a 15-year field experiment in southern Ontario, Canada. It has been reported that organic field management can improve crop yield. Kumar et al. (2010) report that composted sugarcane residue can help in maintaining high crop yield and preventing soil degradation. Hernández et al. (2014) also report an increase in crop yield and soil fertility via field application of organic compost. Although there have been many studies conducted to investigate the impacts of organic residue amendment on soil properties, detailed knowledge related to the responses of soil microbial community to different field management practices remain largely unknown. Therefore, more focus is needed on monitoring the shifts in soil physiochemical parameters as well as nutrient cycling processes in agricultural systems under compaction stress. Also, more attention should be paid to the responses of soil microbial communities following application and removal of compaction stress, with and without organic residue amendments, to better predict soil health conditions in the context of increasing crop yield and developing more sustainable and resilient agricultural systems.

Many soil properties have been used as indicators of soil health to assess soil physicochemical processes and their relation to crop yield. However, interpretation of these observations is complicated due to the large number of investigated properties and their interaction with and dependency on each other. The responses of soil microbial properties (Bååth et al., 1995) and enzyme

activities (Sparling, 1997) to environmental stresses are helpful indicators in making accurate decisions regarding soil health status, as these indicators are more sensitive to environmental changes than soil physicochemical properties (Zimmermann and Frey, 2002). Soil labile C pool, which is mainly generated by decomposition of soil organic matter, is considered as the main food source for soil microbial community (Valenzuela-Solano and Crohn, 2006). Moreover, high organic C content and nutrient pools increase soil microbial performance and further improve soil health condition (Flavel and Murphy, 2006).

The concept of soil RS and RL, which is derived from the term 'microbiome RS and RL', is widely used in describing soil microbiomes shifting under environmental disturbances (Allison and Martiny, 2008; Hartmann et al., 2014). These terms are used in this paper to describe the capacity of soil properties to remain unchanged under compaction stress and to recover to their prestress state after the removal of compaction. This study aims to investigate the responses of the soil microbial community following soil compaction stress and estimate soil RS and RL against compaction in two conventional and conservation soil management practices. The underlying hypotheses are: a) soil with improved management practice history has higher RS against compaction stress and higher RL following removal of compaction stress; b) surface application of plant residues does not improve soil RL against compaction stress due to the rapid loss of labile C through the soil microbial respiration process; and c) incorporation of plant residues would improve soil microbial activities and their recovery from compaction stress.

2.2 MATERIAL AND METHOD

2.2.1 Site description and field treatments

The experimental site (two adjacent sugarcane farms with similar silty loam texture and physicochemical properties) was located in a sugarcane production area at Foresthome (18°36′S, 146°12′E), Queensland, Australia. The soil was classified as Red Dermosol in the Australian classification (Isbell, 2016) or Nitisols according to FAO world reference base (WRB, 2014). The

mean annual temperature of the region was 29.3°C and mean annual precipitation was 2262.6 mm. The term 'improved management' was used in this study to represent conservation field management (permanent beds, minimum zonal tillage, mound planting and multiple species legume fallow) compared to conventional field management (full working multiple passes, planting in furrows and grassy fallow). The conventionally managed field had been cropped with sugarcane (Saccharum officinarum, variety Q240) in the past 30 years and the improved management field had been cropped with sugarcane (Saccharum officinarum, variety Q240) in the past 14 years. The legume plantation in the improved management field, during the fallow period (every five years), was a mixed cultivation of Soybean Leichhardt (*Glycine max*), Cowpea Ebony (*Vigna unguiculata*), and Lablab Rongai (*Lablab purpureus*). As a common practice, fine agricultural gypsum (5 tonnes per hectare) was applied to both farms at a depth of ~10 cm using the broadcasting method. Soil sampling from each site was conducted in April 2020 in six replicates. The plots under conventional (1.63 m row space) and improved (1.83 m row space) managements were divided into six subplots. Five soil cores were randomly collected from each subplot, with an auger of 5 cm in diameter and 10 cm in depth, and bulked together to make a composite sample. Fresh soil samples were sieved (< 2 mm) and stored at 4°C prior to the commencement of incubation experiment.

Fresh soils (100 g oven-dry equivalent) were moistened to 55% water holding capacity (WHC) with distilled water. The moistened samples were then gently transferred to 150 mL flat end polypropylene jars, compacted to bulk density of 1.0 g cm^{-3} (no compaction stress) or 1.4 g cm^{-3} (compaction stress) using a modified compactor (the compression process operated in three soil layers to generate a uniform compaction throughout the soil profile), and incubated in dark and aerobic condition at 22 ± 0.5°C for the first 28 days of the experiment (RS phase). At the end of the RS phase, half of the treatments (CP, CCP, IP, ICP, CPO, CCPO, IPO and ICPO) were treated with ploughing practice (bulk density of treatments adjusted to 1.0 g cm^{-3} after ploughing), while the other half (CCK, CC, ICK, IC, CO, CCO, IO and ICO) remained untreated. At the same time, half of treatments (the treatments that end with letter 'O') received legume residue (mixture of Soybean Leichhardt, Cowpea

Ebony and Lablab Rongai at a rate of 5.0 g per jar; dried at 60 °C for two days and then ground into powder for application, with TC of 417 g kg^{-1} and TN of 10.1 g kg^{-1}) as organic amendment (equivalent to 50 t ha^{-1} legume residue). The samples were then incubated in dark and aerobic conditions at 22 ± 1°C until the end of the experiment at day 70 (RL phase). The experiment was conducted in 16 treatments with 15 replicates for each treatment. The treatments were divided into four groups: a) soil with conventional management history (CCK, CC, CP and CCP), where the letter 'C' denotes conventional management, followed by no compaction in the RS phase and no ploughing in RL phase (CK), compaction in RS phase and no-ploughing in RL phase (C), no compaction in RS phase and ploughing in RL phase (P), and compaction in RS phase and ploughing in RL phase (CP); b) soil with conventional management history and legume residue amendment at the end of the RS phase (CO, CCO, CPO and CCPO; the letter 'O' denotes organic amendment; legume residue was surface applied in CO and CCO treatments and incorporated in CPO and CCPO treatments); c) soil with improved management history (ICK, IC, IP and ICP; the letter 'I' denotes improved management); and d) soil with improved management history and legume residue amendment at the end of the RS phase (IO, ICO, IPO and ICPO; legume residue was surface applied in IO and ICO treatments and incorporated in IPO and ICPO treatments). Soil samples were collected at 7, 28, 35 and 70 days after the commencement of the incubation experiment. Three replicates of each treatment were randomly selected and destructively sampled for measurement of mineral N (NH_4^+-N and NO_3^--N), hot water extractable organic C (HWEOC), hot water extractable total N (HWETN), microbial biomass C (MBC), microbial biomass N (MBN), overall microbial activities (which measured via FDA method) and β-glucosidase activities. Soil respiration samples were collected at 26 sampling dates (gas sampling was undertaken every 1–4 days depending on the expected levels of CO_2 emissions) during the incubation experiment, using three randomly selected replicates of each treatment.

2.2.2 Soil physicochemical analysis

Soil bulk density was estimated as the ratio of soil weight to its volume using the method described by (Maynard and Curran, 2007). Soil pH (1:5 soil to water ratio) value was measured with the glass electrode method described by Rayment and Lyons (2011). Soil mineral N (NH_4^+-N and NO_3^--N) was extracted by 2M KCl at a 1:5 ratio of soil to extractant using an end-over-end shaker for one hour, filtered by a Whatman 42 filter paper (Rayment and Lyons, 2011) and concentrations of NH_4^+-N and NO_3^--N were determined by a SEAL AA3 Continuous Segmented Flow Analyzer (SEAL Analytical Limited, USA). Soil total C (TC) and total N (TN) contents were measured by the combustion method using a LECO CNS-2000 analyser (LECO Corporation, MI, USA). The HWEOC and HWETN concentrations of the samples were measured using the method described by Chen et al. (2004). Briefly, 4.0 g (oven-dry equivalent) of fresh soil was incubated with 20 mL of water in a capped falcon-tube at 70°C for 18 hours. After incubation the tubes were shaken on an end-over-end shaker for five minutes and filtered through a Whatman 42 filter paper (Whatman Ltd, Maidstone, UK), followed by a 0.45-µm filter membrane. Concentrations of dissolved organic C and total extractable N in the filtrate were determined using a SHIMADZU TOC-VCPH (Shimazu, Kyoto, Japan) TOCN analyser. The results were expressed on an oven-dry basis.

2.2.3 Soil biological analysis

Soil MBC and MBN contents were measured by fumigation-extraction method using an E*c* conversion factor of 2.64 (Vance et al., 1987) and an E*n* conversion factor of 2.22 (Wilson, 1988). Concentrations of soluble C and N pools of the fumigated and non-fumigated soil samples were determined using a SHIMADZU TOC-VCSH/ CSN TOCN analyser (Shimadzu Scientific Instruments, Japan). Soil respiration was measured using a gas sampling method as described by Rezaei Rashti et al. (2016). At each gas sampling event, three replicates of each treatment were placed into 2 L airtight glass jars that were then continually flushed with ambient compressed air for one minute before closure. Gas samples were collected from the headspace of the glass jars eight hours after closure using a 25 mL gas-tight syringe and immediately transferred to pre-evacuated 12 mL glass vials (Labco, UK). Gas samples were analysed for CO_2 concentration using a gas chromatograph

(Shimadzu GC-2010 Plus). Linearity tests on CO_2 concentration increases were performed on all treatments over a subset of sampling times during the incubation. These samples were taken initially after closure of the glass jars and then every 60 minutes for ten hours. The emissions for days without gas sampling were estimated using the arithmetic mean of the measurements on the two closest days (Rezaei Rashti et al., 2016). The cumulative emissions were calculated by summing the daily emission measurements. The microbial metabolic quotient (qCO_2), defined as the C respired per unit of MBC per day, and calculated from the ratio of CO_2-C emission (μg CO_2-C kg^{-1} soil day^{-1}) to concentration of MBC (mg C kg^{-1} soil) of each treatment. Fluorescein diacetate (FDA) hydrolysis is widely accepted as an accurate and simple method for measuring the overall microbial activity in a range of environmental samples, including soils at pH 7.6 (Green et al., 2006) and was used to measure soil microbial activity in this study. The activities of soil β-glucosidase (hydrolysing cellulose to glucose) was measured at pH 6.0 (Tabatabai, 1994).

2.2.4 Soil microbial resistance and resilience indices

To assess the functional RS and RL of C use efficiency (metabolic quotient) in soil microbial communities under compaction stress, the following indices were used according to Orwin and Wardle (2004):

$$RS = 1 - [(2 \times |D_{28}|) / (C_{28} + |D_{28}|)]$$

D_{28} is the difference between soil metabolic quotient value in the control (CCK in conventional and ICK in improved management practice), treatment (C_{28}), and the stressed treatment (compaction stress) at the end of the RS phase (day 28). The RL index for the microbial C use efficiency of each treatment was calculated at the end of the incubation experiment (day 70), according to:

$$RL = [(2 \times |D_{28}|) / (|D_{28}| + |D_{70}|)] - 1$$

D_{70} is the difference between soil metabolic quotient value in the control (CCK and CO in conventional, and ICK and IO in improved management practice) treatment and the stressed treatment at the end of the RL phase (day 70). 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 C use efficiency to compaction stress. Negative values of the RS index indicate a change greater than 100% in the response variable compared with that in the control treatment. If the absolute value of D_{70} is higher than the absolute value of D_{28}, then the RL index has a negative value.

2.2.5 Statistical analysis

Differences at $P < 0.05$ between treatments were considered statistically significant and all variables were tested for normality of distribution using the Kolmogrov–Smirnov test. Pearson linear correlation was used to describe the relationship between soil properties. In addition, data on all soil properties were subjected to principal component analysis (PCA) to distinguish the effect of compaction stress on the investigated soils, at the end of RS and RL phases, using the IBM SPSS Statistics 26 software package (IBM Corp., released 2020, IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp). The net cumulative CO_2 emissions in the current experiment were calculated as the differences in cumulative emissions between the treatments and their control (no compaction stress) at each sampling time (i.e. CCK and ICK for treatments without legume residue amendment, and CO and IO for treatments with legume residue amendment).

2.3 RESULTS

At the end of incubation, soil samples were well mixed and analysed for changes in chemical properties of each treatment due to the short-term compaction stress and ploughing and legume amendment practices. The soils in all treatments were slightly acidic (pH 5.7–6.3), while there were no significant differences in soil pH values among all treatments. In addition, the initial total C and N contents were significantly ($P < 0.05$) higher in treatments with improved management (1.51% total C and 0.086% total N) than conventional management (1.24% total C and 0.092% total N).

2.3.1 Soil available organic carbon and nitrogen

The treatments with improved management history had significantly ($P < 0.05$) higher concentration of HWEOC in comparison to treatments with conventional management history in the first week of the RS phase (Table 2.1). Regardless of soil management history, compaction stress generally increased labile C concentration compared with noncompacted treatments at day 7. After the first week of incubation, concentration of HWEOC decreased over time in treatments without legume residue amendment, until the end of the experiment. As expected, legume residue application significantly ($P < 0.05$) increased HWEOC concentration in all amended treatments, regardless of soil management history, in the first week of the RL phase (day 35). The incorporation of legume residue significantly ($P < 0.05$) increased and decreased the concentration of HWEOC, in comparison with surface application of legume residue, in treatments with conventional and improved management history at day 35, respectively. This observation indicates that combined ploughing and organic residue amendment practices increased the mineralisation and depolymerisation processes of soil organic compounds and enhanced the release of soil labile organic C in treatments with conventional management history, while this was not the case in treatments with improved management history. At the end of the RL phase, concentration of HWEOC also significantly ($P < 0.05$) decreased in ploughed treatments regardless of compaction status and management history.

The treatments with improved management history had significantly ($P < 0.05$) higher concentration of HWETN than the treatments with conventional management history, in the first week of the RS phase (Table 2.1). Also, like HWEOC, the compaction stress increased concentration of HWETN compared with noncompacted treatments by the end of the RS phase (day 28). At day 28, a slight decrease in concentration of HWETN was observed in treatments with improved management history, while this trend was not clear in conventionally managed treatments. The results also indicate that removal of compaction stress with ploughing practice significantly ($P < 0.05$) increased concentration of HWETN in soil with improved management history (ICP vs IC), while the opposite pattern was observed in soil with conventional management history (CCP vs CC) at day 35. The amendment of legume residue at the end of the RS phase significantly ($P < 0.05$) increased soil

HWETN content in all treatments. Additionally, at day 35, treatments that received surface application of legume residue had a significantly ($P < 0.05$) higher concentration of HWETN than treatments that received legume residue incorporation, regardless of soil management history. By the end of the RL phase (day 70) and in treatments with legume residue amendment, both incorporation and surface application methods showed significantly ($P < 0.05$) lower concentration of HWETN in treatments with improved management history in comparison to treatments with conventional management history.

2.3.2 Soil mineral N pool

Compaction stress generally decreased soil mineral N concentration at day 7, regardless of soil management history, although the observed pattern in conventional treatments was not as clear as treatments with improved management history (Table 2.1). The mineral N content generally increased in treatments with both management history at the end of the RS phase and the first week of the RL phase (day 35). The removal of compaction stress significantly ($P < 0.05$) increased mineral N content in soil with improved management history (ICP), but only slightly increased mineral N in soil with conventional management history (CCP). Surface application of legume residue significantly ($P < 0.05$) increased mineral N content in soil with conventional management history, while compacted treatment (CCO) had lower mineral N content in comparison to control (CO), although there was no significant difference between ploughed treatments (CPO and CCPO) at day 35 (Table 2.1). Similarly, legume residue amendment increased mineral N content in soil with improved management history. However, we found that legume residue incorporation significantly ($P < 0.05$) increased soil mineral N content in compacted treatment (ICPO) in comparison to noncompacted treatment (IPO) at day 35. Soil mineral N content slightly accumulated in ploughed treatments (CP and CCP) but consumed in compacted treatment (CC) and control treatment (CCK) with conventional management history, at the end of the RL phase. Although this pattern was similar in the treatments with improved management history, the changes were less clear compared with conventionally managed treatments. At the end of the RL phase, soil mineral N content was slightly cumulated in

treatments with surface application of legume residue (CO and CCO), but significantly ($P < 0.05$) increased in treatments with legume residue incorporation (CPO and CCPO) under conventional management history (Table 2.1). The concentration of soil mineral N in all of legume residue applied treatments with improved management history was lower than conventional management treatments by the end of the experiment. In addition, a sharp decrease in mineral N concentration was observed in IO and ICO treatments between day 35 and day 70 of the experiment.

Table 2.1 Dynamics of soil hot water extractable C and N and mineral N concentrations during the incubation period, in treatments under different management practices, with and without organic residue amendment.

Treatment		HWEOC (mg kg^{-1} dry soil)				HWETN (mg kg^{-1} dry soil)				Mineral N (mg NH_4^+-N + NO_3^--N kg^{-1} dry soil)			
		Day 7	Day 28	Day 35	Day 70	Day 7	Day 28	Day 35	Day 70	Day 7	Day 28	Day 35	Day 70
Without organic residue amendment	CCK	217.7c	213.1abc	189.5a	98.2b	9.3b	9.4a	9.9a	6.7b	84.8b	111.9a	125.7b	104.9d
	CC	222.3c	196.9c	189.1a	115.6abc	9.4b	9.6a	10.4a	6.6b	79.3b	97.6b	126.5b	120.9c
	CP	206.2c	210.5bc	181.8a	107.3bc	8.5b	8.3bc	9.5ab	9.2a	83.6b	121.4a	122.3b	152.1a
	CCP	222.3c	210.2bc	183.3a	102.4cb	8.7b	9.2ab	9.0b	8.3a	78.6b	94.3b	112.7b	139.2b
	ICK	299.8b	202.2c	178.2a	112.8abc	13.3a	7.5cd	7.3cd	8.8a	110.8a	124.1a	130.4b	117.8c
	IC	326.2a	227.2ab	185.5a	129.0ab	13.3a	8.0c	6.8d	7.9a	103.8a	97.9b	139.5ab	121.1c
	IP	298.0b	230.4a	174.1a	138.2a	12.6a	6.5d	7.1cd	11.6a	110.4a	102.9a	124.9b	125.1c
	ICP	309.6ab	214.1abc	183.6a	119.1abc	12.9a	7.3cd	7.8c	9.1a	101.4a	92.5b	179.8a	154.6a
With organic residue amendment	CO	225.6d	223.6a	642.8e	563.3b	9.2c	9.6a	53.3a	38.4b	79.3c	91.8c	510.2b	515.5ab
	CCO	242.4c	194.9bc	558.0f	519.8c	9.6c	8.6b	51.3a	48.2a	82.5c	85.5c	434.7c	606.0a
	CPO	222.3d	205.3b	892.1b	450.4d	9.2c	7.4cd	30.2d	35.6b	80.4c	88.1c	191.3e	542.8ab
	CCPO	238.4c	189.8c	723.4d	467.5d	9.6c	7.4cd	25.2d	37.4b	81.7c	83.9c	204.9e	474.9bc
	IO	323.9a	223.6a	1047.7a	798.2a	14.1a	6.9d	57.2a	10.1d	114.2a	128.2a	534.1b	288.5d
	ICO	296.9b	221.8a	1082.1a	777.1a	11.7b	8.1bc	61.7a	18.8c	98.1b	112.2b	618.8a	352.2d
	IPO	317.2a	201.9bc	832.1c	574.3b	13.7a	7.1cd	38.7c	14.1cd	113.5a	123.7a	264.4d	296.3d
	ICPO	296.9b	200.8bc	917.3b	473.1d	10.6b	8.1bc	39.3c	12.8d	97.2b	107.1b	361.1c	386.8d

The treatment names that start with the letter C refer to soil under conventional practice management, including: CCK, control (bulk density of 1.0 g cm^{-3}); CC, compaction only (bulk density of 1.4 g cm^{-3}); CP, ploughing only; CCP, compaction and ploughing; CO, control with organic residue amendment; CCO, compaction and organic residue amendment; CPO, ploughing and organic residue amendment; CCPO, compaction, ploughing and organic residue amendment. The treatment names that start with letter I refer to soil under improved practice management, with the same experimental definitions as the above treatments, including: ICK, IC, IP, IO, ICO, IPO, and ICPO treatments. The reported data are means of three replicates. Means followed by different letters within a column indicate significant differences between the treatments at $P < 0.05$.

2.3.3 Soil microbial pools

In general, the present study indicates that compaction stress increased concentration of MBC in treatments with conventional management history (CC and CCP), while it decreased concentration of MBC in treatments with improved management history (IC and ICP) compared to control treatments (CCK and ICK) at day 7 (Table 2.2). However, the concentration of MBC reached a stable level at day 28 in all treatments, regardless of the management history and compaction status. In the first week of the RL phase, there were no significant changes in concentration of MBC in control treatments (CCK and ICK), while the MBC contents slightly decreased in noncompacted and ploughed treatments (CP and IP) and sharply decreased in compacted and ploughed treatments (CCP and ICP) without legume residue application. At the end of the experiment (day 70), concentration of MBC had increased in noncompacted and ploughed treatments (CP and IP), while it had decreased in control treatments (CCK and ICK), regardless of soil management history (Table 2.2). In addition, the increase in MBC content was lower in the compacted treatment with improved management history (IC), compared to the conventional management history (CC), by the end of the experiment. Comparison of compacted and ploughed treatments at the end of the RL phase also indicated that the concentration of MBC was significantly ($P < 0.05$) higher in the treatment with improved management history (ICP) than the treatment with conventional management history (CCP). This observation indicates that the improved management history may provide a higher potential for the soil microbial community to recover after removal of compaction stress, without an organic residue amendment. After the legume residue amendment, the concentration of MBC boosted in all treatments regardless of soil management history and residue application method. The incorporation of legume residue (CPO, CCPO, IPO and ICPO) resulted in a generally higher concentration of MBC in comparison to its surface application (CO, CCO, IO and ICO) method in the first week of the RL phase. However, at the end of the experiment (day 70), concentration of MBC decreased in treatments with residue incorporation method (CPO, CCPO, IPO and ICPO), while it remained unchanged or slightly increased in surface applied treatments (CO, CCO, IO and ICO).

Compaction stress showed no significant impact on concentration of MBN in treatments with conventional management history (CC and CCP), compared with the control treatment, while decreased MBN concentration in treatments with improved management history (IC and ICP), during the RS phase of the experiment (Table 2.2). After removal of compaction stress at the end of the RS phase, the concentration of MBN significantly ($P < 0.05$) increased in ICP treatment with improved management history, while it significantly ($P < 0.05$) decreased in CCP treatment with conventional management history, at day 35. At the end of the experiment (day 70), concentration of MBN slightly decreased in all treatments without legume residue amendment, regardless of soil compaction status and management history. The legume residue amendment significantly ($P < 0.05$) increased concentration of MBN in all treatments at day 35, regardless of the application method. In addition, the removal of compaction stress along with the incorporation of legume residue significantly ($P < 0.05$) increased the MBN content in ICPO treatment with improved management history, compared with the control treatment, while this pattern was not clear in CCPO treatment with conventional management history. At the end of the RL phase (day 70), the MBN content decreased in all treatments (except CCO and IPO treatments), regardless of residue application method and soil management history.

The β-Glucosidase activity was not significantly different among treatments at the end of the RS phase (day 28), regardless of soil management history, while the β-Glucosidase activity significantly ($P < 0.05$) decreased at day 28 in comparison to the first week of the experiment (Table 2.2). In the first week of the RL phase (day 35), ploughed treatments (CP, CCP, IP and ICP) showed higher β-Glucosidase activity than compacted-only treatments (CC and IC), while treatments with improved management history generally had higher β-Glucosidase activities. However, the β-Glucosidase activity slightly increased in all treatments without a legume residue amendment at the end of the experiment. With the legume residue amendment at the end of the RS phase, we observed a significant ($P < 0.05$) increase in β-Glucosidase activity in all treatments regardless of soil management history at day 35. In addition, ploughed treatments (CPO, CCPO, IPO and ICPO) had higher β-Glucosidase

activity than compacted-only treatments (CCO and ICO), while treatments with conventional management history had significantly ($P < 0.05$) higher β-Glucosidase activities. At the end of the experiment (day 70), the β-Glucosidase activity generally increased in treatments with surface legume residue application, except IO treatment, while generally decreased in treatments with legume residue incorporation, except CCPO treatment.

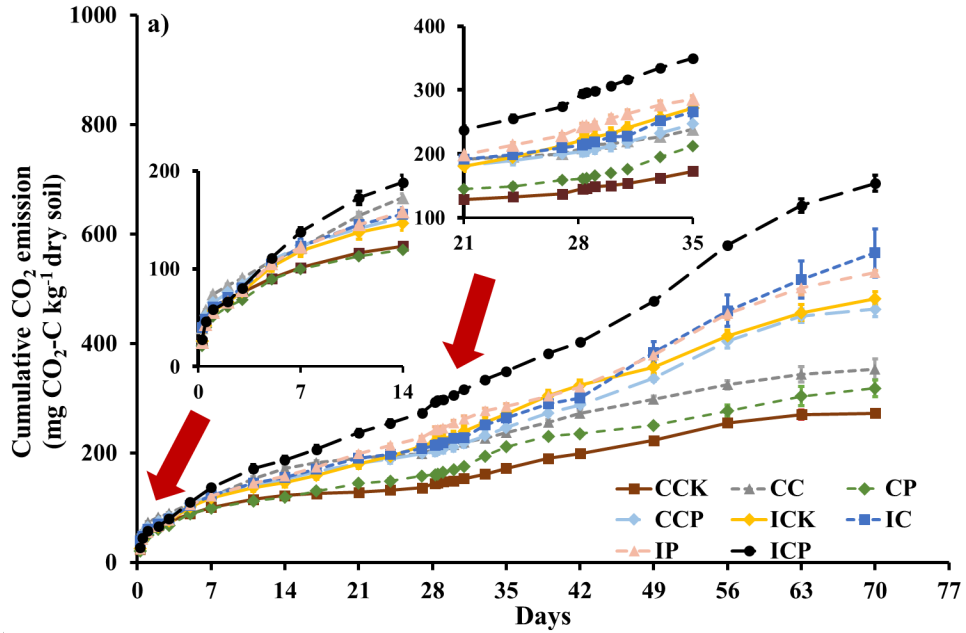
Table 2.2 Dynamics of soil microbial biomass and β-Glucosidase activity during the incubation period, in treatments under different management practices, with and without organic residue amendment.

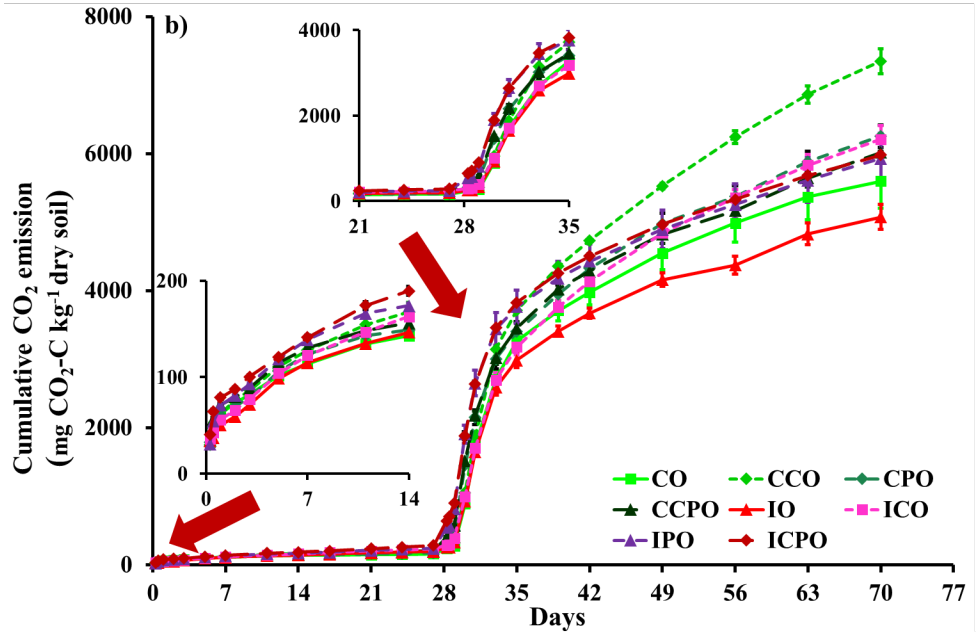
Treatment		MBC (mg kg^{-1} dry soil)				MBN (mg kg^{-1} dry soil)				β-Glucosidase (μg p-nitrophenol h^{-1} g^{-1} dry soil)			
		Day 7	Day 28	Day 35	Day 70	Day 7	Day 28	Day 35	Day 70	Day 7	Day 28	Day 35	Day 70
Without organic residue amendment	CCK	151.9b	159.6a	164.9a	139.1bc	47.0b	54.6b	47.5a	27.1b	68.3a	24.4a	13.1bc	16.13abcd
	CC	215.9a	162.8a	127.7bc	160.4ab	40.4bc	37.3c	33.9b	27.6b	72.8a	24.4a	4.6d	11.6e
	CP	158.4b	154.7a	141.3b	165.9ab	43.2b	49.5b	47.2a	26.6b	70.0a	25.8a	9.4c	14.74bcde
	CCP	211.1a	160.2a	113.2c	114.0cd	41.6bc	52.4b	26.7c	20.7b	64.2a	23.1a	12.8bc	16.6abc
	ICK	198.6a	169.1a	157.4a	93.9d	74.9a	65.4a	31.5b	23.6a	62.1a	25.0a	11.6bc	12.8de
	IC	145.7b	160.1a	112.9c	137.4bc	25.6d	35.2c	34.7b	27.0b	69.2a	23.4a	7.2c	13.3cde
	IP	185.9a	174.9a	130.1bc	144.5bc	71.1a	58.4a	34.0b	20.8b	64.1a	23.2a	21.3a	18.6a
	ICP	144.7b	163.9a	109.7c	186.8a	27.8d	32.5c	50.1a	41.0a	69.2a	23.0a	17.8ab	18.2ab
With organic residue amendment	CO	161.6b	150.9a	476.5d	499.6d	42.0b	46.7bc	242.6c	121.7d	71.4a	21.6a	72.2f	85.4d
	CCO	202.4a	157.6a	787.7b	749.5b	47.0b	55.3b	144.5de	364.7a	69.5a	26.1a	86.1ef	134.8b
	CPO	159.6b	161.1a	738.2c	540.4c	43.7b	52.7b	155.2de	140.4d	68.7a	22.5a	151.4a	130.4bc
	CCPO	199.1a	164.2a	899.2ab	466.6c	45.7b	43.1cd	123.0e	90.9d	62.5a	23.3a	152.4a	155.6a
	IO	182.2a	175.9a	723.0c	769.0b	78.5a	73.7a	323.5b	220.2c	63.3a	25.1a	131.4b	81.6d
	ICO	148.0b	161.7a	837.2b	886.4a	37.7b	45.2bc	464.3a	283.3b	68.3a	24.2a	101.3de	117.2c
	IPO	180.5a	174.2a	986.7a	741.8b	76.2a	70.6a	198.7cd	338.7a	64.3a	23.9a	109.2cd	64.3e
	ICPO	155.3b	166.3a	1019.4a	520.3c	35.0b	21.7d	412.6a	196.4c	65.3a	23.8a	124.8bc	83.6d

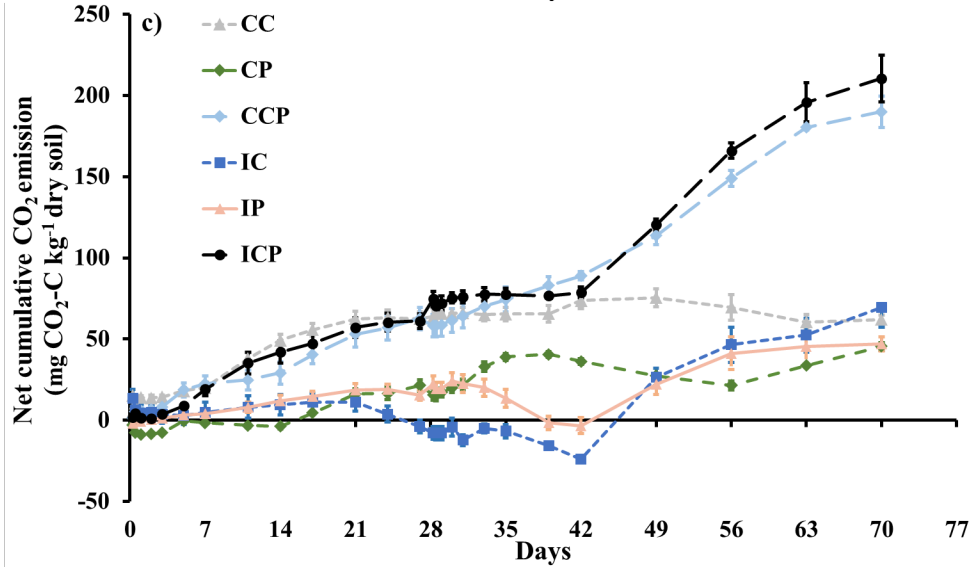
The reported data are means of three replicates. Means followed by different letters within a column indicate significant differences between the treatments at $P < 0.05$. Description of treatments same as Table 2.1.

2.3.4 Cumulative CO_2 respiration and net cumulative CO_2 respiration

Treatments with improved management history had higher cumulative CO_2 emissions than treatments with conventional management history, while there were no significant differences in net cumulative CO_2 emissions between treatments with improved and conventional management history by the end of the RL phase. The compacted and ploughed treatments (CCP and ICP) had the highest cumulative CO_2 respiration followed by compacted-only treatments (CC and IC), while control treatments (CCK and ICK) had the lowest cumulative CO_2 respiration among treatments with the same management history (Figs. 2.1a and 2.1c). This observation indicates that the differences between treatments with the same compaction stress only resulted from their management history as the responses to RS and RL processes over entire incubation period were similar in both soils. Surface application of the legume residue resulted in higher cumulative CO_2 emissions in treatments with conventional management history in comparison with improved management treatments (CO vs IO, and CCO vs ICO), while significant differences were observed between legume residue incorporated treatments (CPO, CCPO, IPO and ICPO), regardless of their management history (Fig. 2.1b). In addition, the surface application of legume residue showed higher net cumulative CO_2 emissions in treatment with conventional management history (CCO) in comparison to treatment with improved management history (ICO), while the incorporation method of the legume residue amendment resulted in lower net cumulative CO_2 emissions in conventional management treatments compared to improved management treatments (Fig. 2.1d). This observation indicates that surface application of legume residue to treatments with conventional management would result in higher loss of applied organic C via CO_2 emissions. In addition, legume residue incorporation to soil with improved management history minimised the fluctuation of net cumulative CO_2 emissions between compacted and noncompacted treatments, by the end of the RL phase, while this was not the case in treatments with conventional management history (Fig. 2.1d).


a)
Cumulative CO_2 emission (mg CO_2-C kg^{-1} dry soil)
Days
CCK
CC
CP
CCP
ICK
IC
IP
ICP


b)
Cumulative CO_2 emission (mg CO_2-C kg^{-1} dry soil)
Days
CO
CCO
CPO
CCPO
IO
ICO
IPO
ICPO


c)
Net cumulative CO_2 emission (mg CO_2-C kg^{-1} dry soil)
Days
CC
CP
CCP
IC
IP
ICP

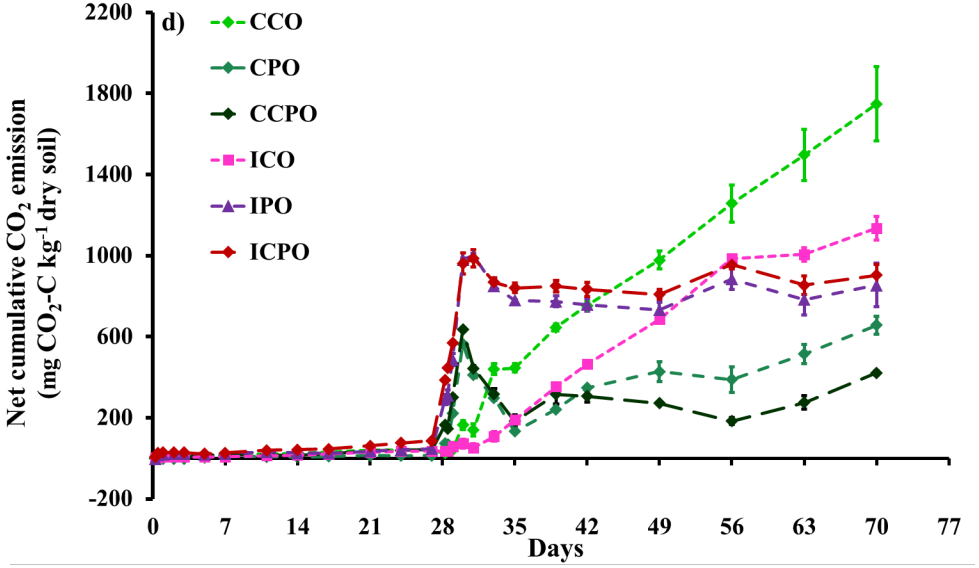

d)
Net cumulative CO_2 emission (mg CO_2-C kg^{-1} dry soil)
Days
CCO
CPO
CCPO
ICO
IPO
ICPO

Fig. 2.1 Cumulative (a and b) and net cumulative (c and d) CO_2 emissions from different treatments during the incubation period. The values of net cumulative emission for each treatment were calculated based on their differences from their control treatment at each sampling time (i.e. CCK and ICK for treatments without organic residue amendment, and CO and IO for treatments with organic residue amendment). Description of treatments are the same as Table 2.1.

2.3.5 Microbial activity

The FDA method is a commonly used tool to investigate soil overall microbial activity. Generally, microbial activity was higher in treatments with conventional management compared to treatments with improved management by the end of the RS phase (day 28), while there was no significant difference among treatments in the first seven days of incubation (Fig. 2.2a). The microbial activity significantly ($P < 0.05$) increased in all treatments at day 28, regardless of soil management history and compaction status. In the first week of the RL phase, soil microbial activity significantly ($P < 0.05$) decreased in all treatments without legume residue amendment, except the ploughed treatments with improved management history (IP and ICP), which showed a significant ($P < 0.05$) increase in their microbial activity (Fig. 2.2a). However, at the end of the experiment, soil microbial activity significantly ($P < 0.05$) decreased in all treatments regardless of their management history and compaction stress status. The legume residue amendment significantly ($P < 0.05$) increased microbial activity in the first week of the RL phase (day 35) in all treatments (Fig. 2.2b). However, at the end of the RL phase the observed values significantly ($P < 0.05$) increased in non-ploughed treatments, while they generally decreased in ploughed treatments.

The compaction stress significantly ($P < 0.05$) increased qCO_2 values in treatments with conventional management history, compared with noncompacted treatments, while significantly ($P < 0.05$) decreased qCO_2 values in treatments with improved management history, at day 7 (Table 2.3). However, at the end of the RS phase (day 28) the compacted treatments had generally higher qCO_2 values than noncompacted treatments, regardless of their management history. The removal of compaction in treatments without legume residue amendment (CCP and ICP) resulted in a significant ($P < 0.05$) increase of qCO_2 value compared with other treatments of the same soil at day 35, while there was no significant difference between compaction-only (CC and IC) and ploughing-only (CP and IP) treatments with the same management history. At the end of the RL phase (Day 70), the compacted and ploughed treatment (CCP) showed the highest qCO_2 value in treatments with conventional management history, while similar treatment with improved management history (ICP)

showed the lowest qCO_2 value. The legume residue amendment significantly ($P < 0.05$) increased qCO_2 value in all treatments regardless of their management history. However, the soil with improved management history generally had lower qCO_2 values following legume residue amendment. In addition, the compacted and ploughed treatments (CCPO and ICPO) showed the highest qCO_2 values within treatments with the same management history.

Table 2.3 Dynamics of soil metabolic quotient during the incubation period, in treatments under different management practices, with and without organic residue amendment.

Treatment		qCO_2 (μg CO_2-C h^{-1} mg^{-1} MBC)			
		Day 7	Day 28	Day 35	Day 70
Without organic residue amendment	CCK	0.55c	0.83b	1.05d	1.97c
	CC	0.76b	1.33ab	1.64c	2.21c
	CP	0.59c	1.03b	1.54c	1.94c
	CCP	0.78b	1.33ab	2.13b	4.08b
	ICK	1.21a	1.24ab	1.99b	5.37a
	IC	0.75b	1.73a	2.22b	5.98a
	IP	1.53a	1.98a	2.14b	4.50ab
	ICP	0.80b	2.05a	2.74a	4.03b
With organic residue amendment	CO	0.51c	1.08b	6.23a	13.13a
	CCO	0.81b	1.34b	6.39a	9.83b
	CPO	0.52c	1.10b	3.92b	10.46b
	CCPO	0.84b	1.24b	4.38b	14.53a
	IO	1.21a	1.71a	4.29b	6.64c
	ICO	0.81b	1.96a	3.80b	7.59c
	IPO	1.33a	1.70a	3.83b	8.02c

	ICPO	0.82b	1.78a	3.81b	10.80b

The reported data are means of three replicates. Means followed by different letters within a column indicate significant differences between the treatments at $P < 0.05$. Description of treatments same as Table 2.1.

2.3.6 Statistical analysis

The PCA results show that principal components (PC) PC1 and PC2 explained 27.2–46.1% and 22.6–29.5% of the data variance, respectively (Fig. 2.3). The PCA analysis also clearly separates treatments from each other based on the PC1 and PC2 components. The parameters with the highest correlation coefficients (> 0.85) for PC1 were HWEOC, HWETN, mineral N and MBC, while the parameters with the highest correlation coefficient (> 0.85) for PC2 were microbial activity, β-glucosidase, and CO_2 emissions. The treatments with conventional and improved management histories were clearly separated based on PC1 and PC2 at days 7 and 28 of the experiment (RS phase; Figs. 2.3a and 2.3b). In the RL phase of the experiment without legume residue amendment, the treatments with ploughing practice and improved management history (IP and ICP) were clearly separated from other treatment at days 35 and 70, while the same pattern was not observed in treatments (CP and CCP) with conventional management history (Figs. 2.3c and 2.3d). Interestingly, in the first week following legume residue amendment (day 35), treatments with conventional and improved management histories were clearly separated from each other based on PC1 and PC2 components (Fig. 2.3e). In addition, treatments with conventional management history were clearly separated based on PC2, while soils with different management history were clearly separated based on PC1 at the end of the RL phase in day 70 (Fig. 2.3f).

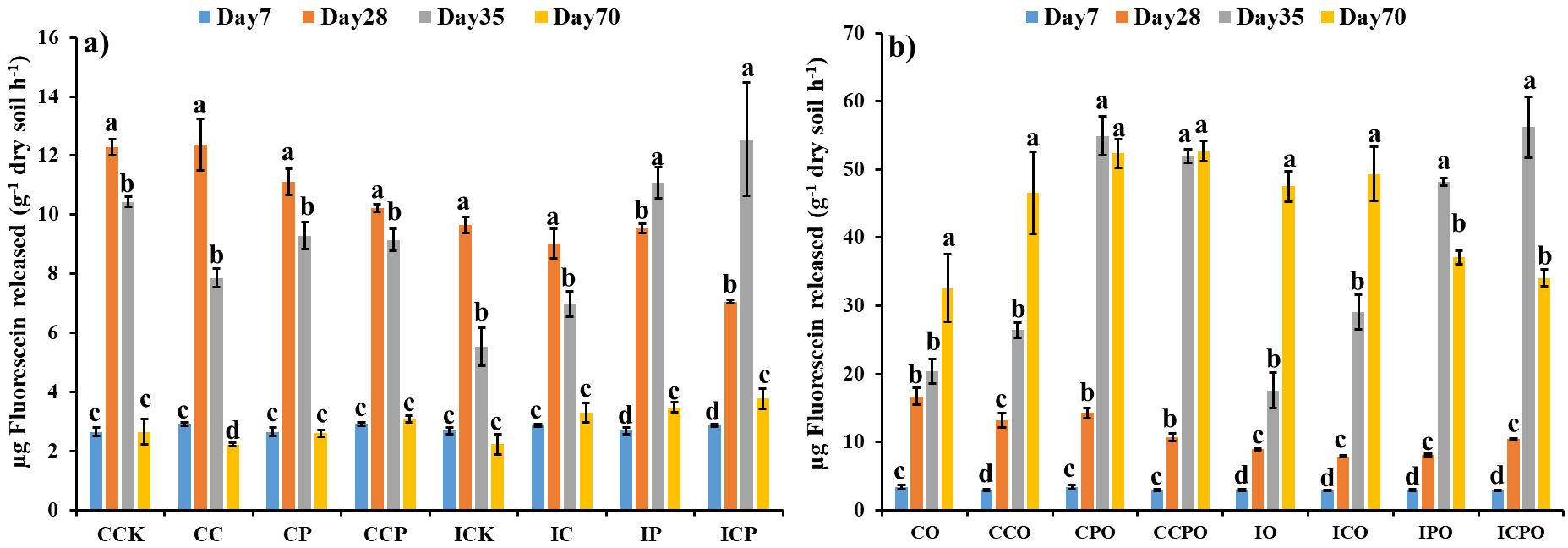


Fig. 2.2 Dynamics of soil microbial activity during the incubation period in treatments under different management practices, without (a) and with (b) organic residue amendment. Description of treatments are the same as Table 2.1. Vertical bars are standard error of three replicates. Means of the values for different sampling events, within a treatment, with the same letter are not different at the 5% level of significance.

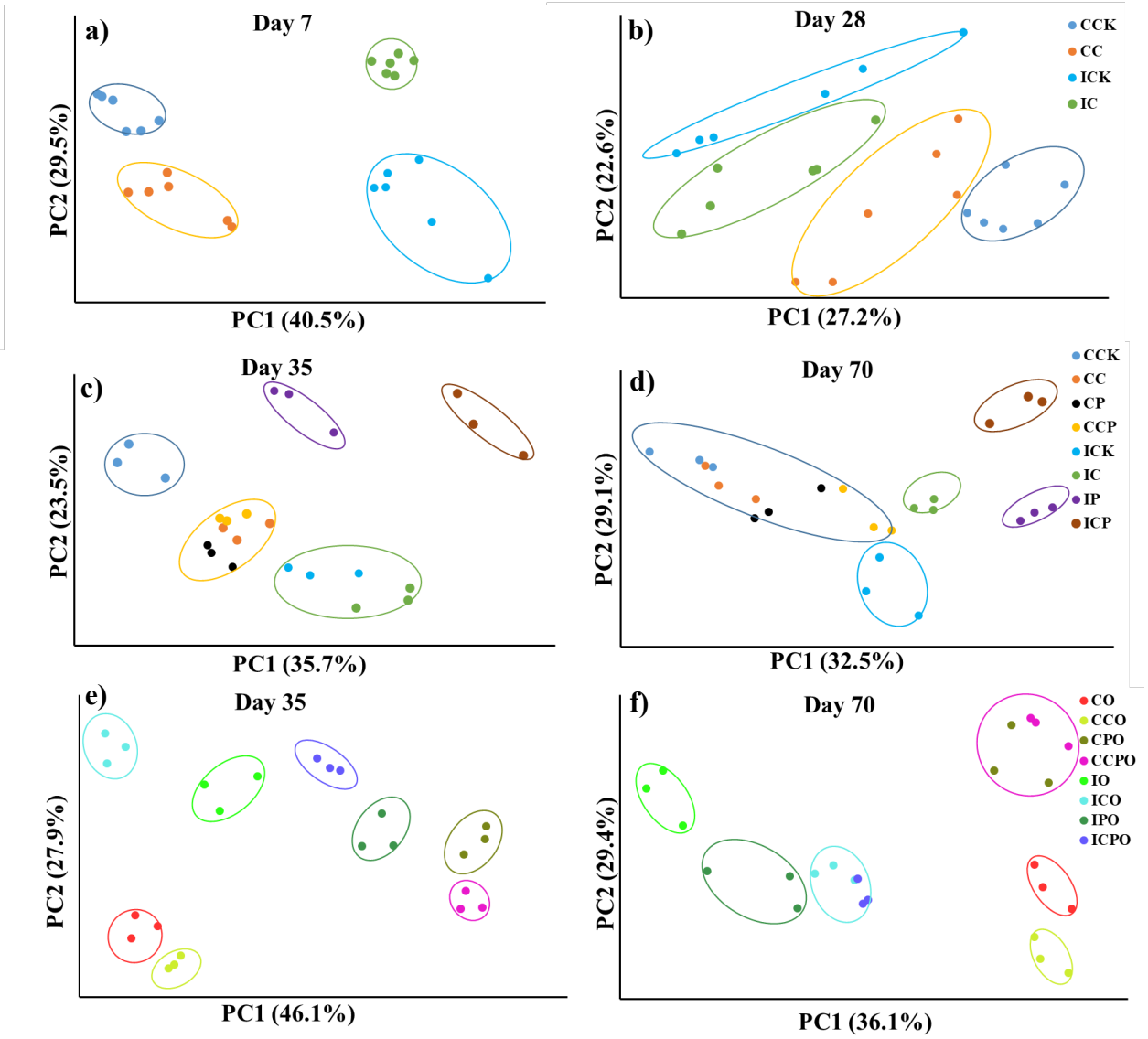

a)
Day 7
PC2 (29.5%)
PC1 (40.5%)
b)
Day 28
PC2 (22.6%)
PC1 (27.2%)
CCK
CC
ICK
IC
c)
Day 35
PC2 (23.5%)
PC1 (35.7%)
d)
Day 70
PC2 (29.1%)
PC1 (32.5%)
CCK
CC
CP
CCP
ICK
IC
IP
ICP
e)
Day 35
PC2 (27.9%)
PC1 (46.1%)
f)
Day 70
PC2 (29.4%)
PC1 (36.1%)
CO
CCO
CPO
CCPO
IO
ICO
IPO
ICPO

Fig. 2.3 Scores plot of principal component analysis (PCA) showing the separation of treatments under different management practices, with and without organic residue amendment, and loading values of the individual soil parameters (PC1 and PC2) for each treatment. Description of treatments are the same as Table 2.1.

The compaction stress resulted in a significantly ($P < 0.05$) lower RS index of C use efficiency (qCO_2) in both soils in comparison to their control treatments (CCK and ICK; Fig. 2.4a). However, no significant difference was observed in the RS index of compacted treatments with different management history. This may indicate that the impacts of compaction stress were similar on the microbial C use efficiency of both soils regardless of their management history. The RL index of qCO_2 in compacted-only treatments (CC and IC) was significantly ($P < 0.05$) lower than their control treatments (CCK and ICK), while the CC treatment showed a significantly higher recovery from compaction stress than the IC treatment (Fig. 2.4b). In addition, the ploughing-only treatment with conventional management (CP) showed a significantly ($P < 0.05$) higher recovery rate of qCO_2 compared with the similar treatment with improved management history (IP), while an opposite response was observed in compacted and ploughed treatments (CCP vs ICP). After the legume residue amendment, all treatments showed very low recovery rate of qCO_2 in both surface application and incorporation methods. Generally, surface application of legume residue resulted in higher RL index of qCO_2 compared with the incorporation method. The results also indicate no significant difference between RL index of qCO_2 in legume residue incorporated treatments with improved management history (IPO and ICPO), while this was not the case in treatment with conventional management history as the CCPO treatment showed a significantly ($P < 0.05$) higher RL index of qCO_2 compared with CPO treatment (Fig. 2.4c).

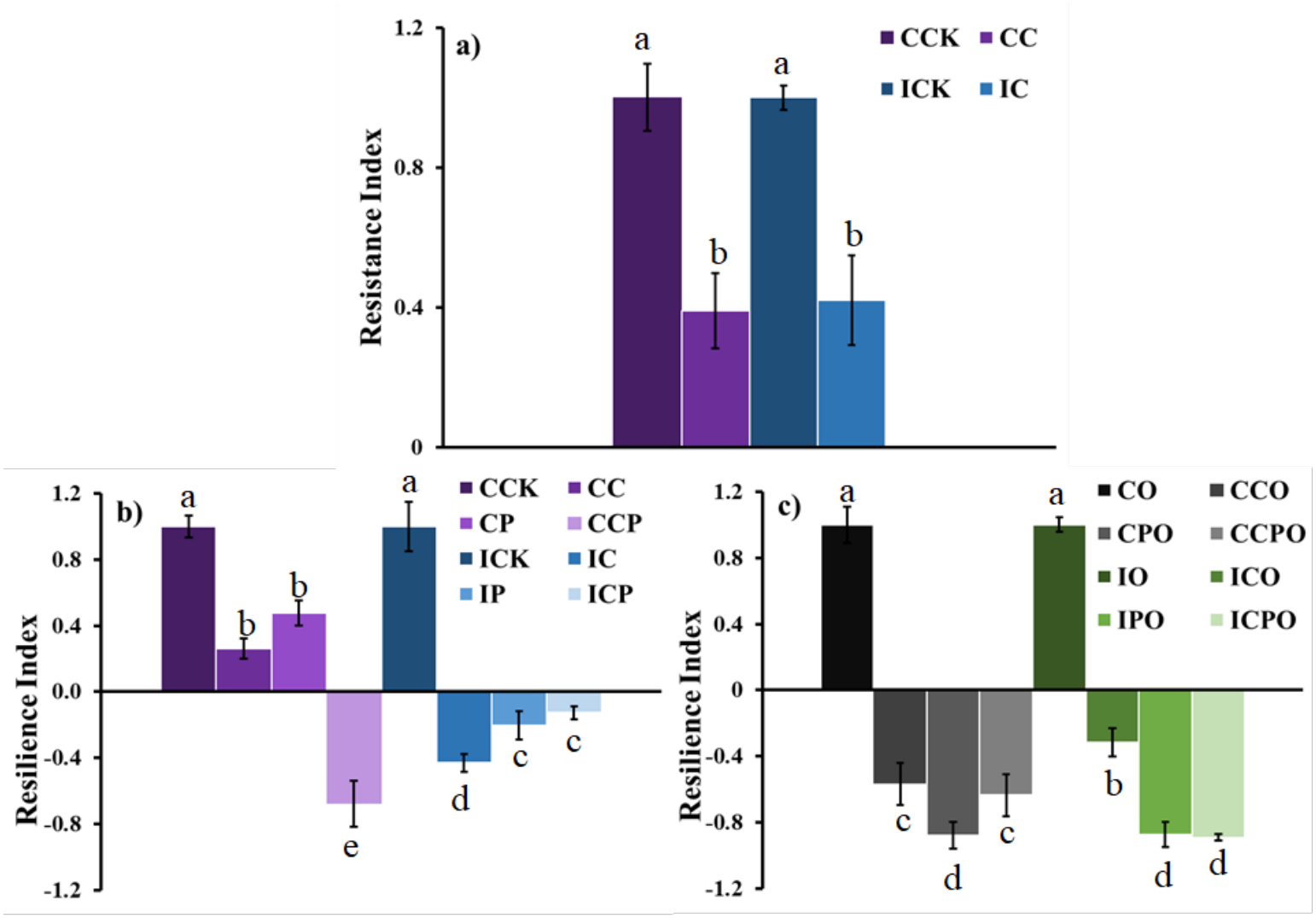

a)
Resistance Index
1.2
0.8
0.4
0
CCK
CC
ICK
IC
a
b
a
b
b)
Resilience Index
1.2
0.8
0.4
0.0
-0.4
-0.8
-1.2
CCK
CC
CP
CCP
ICK
IC
IP
ICP
a
b
b
e
a
d
c
c
c)
Resilience Index
1.2
0.8
0.4
0
-0.4
-0.8
-1.2
CO
CCO
CPO
CCPO
IO
ICO
IPO
ICPO
a
c
d
c
a
b
d
d

Fig. 2.4 The resistance (a; day 28) and resilience (b and c; day 70) indices of soil microbial carbon use efficiency (metabolic quotient) in treatments under different management practices, with (b) and without (c) organic residue amendment. Description of treatments are the same as Table 2.1. Vertical bars are standard error of three replicates. Means of the treatments by the same letter are not different at the 5% level of significance.

2.4 DISCUSSION

In order to tackle issues related to the effects of compaction stress on soil health decline and crop production, various soil management practices, such as organic residue amendment with different methods (e.g. surface (Sirousmehr et al., 2014) or deep layer (Liu et al., 2018) application), have been developed that are able to alleviate soil compaction in the field and improve soil health and fertility (Yang et al., 2019). It has been suggested that the application of organic amendments can ameliorate soil compaction and improve soil health through reducing bulk density (Diacono and Montemurro, 2011), increasing accessibility of soil organic matter for microorganisms and increasing soil microbial activities (Ninh et al., 2015). Agricultural field management practices, such as ploughing, may have positive effects on crop yield through the improvement of organic matter mineralisation (Rogers and Burns, 1994) and soil microbial activity (Schneider et al., 2017). It has also been reported that ploughing practice can improve plant root growth and sugarcane yield (Garside et al., 2005). However, the responses of soil nutrient pools and microbial biomass and activity to compaction stress and their recovery after removal of this stress may vary with soil management history. The mechanisms behind diverse responses of soil nutrient pools and microbial processes to compaction stress and its removal also remained largely unknown.

2.4.1 Responses of soil labile carbon and nitrogen pools to compaction and its removal

As a key component of soil health, labile pools of soil organic matter are bioavailable for both plant uptake and microbial consumption, sensitive to shifts in environmental conditions and play an important role in nutrient cycling (Hu et al., 1997). The HWEOC and HWETN contents of soil have been recommended as a good representative of soil labile C and N pools and are considered as some of the best indicators for assessment of the impacts of field management practices on soil health status (Chen et al., 2004).

The presented study shows that labile C was the main C source for microbial consumption, which is supported by Teutscherova et al. (2017) who indicate that labile C decreased during the incubation

period possibly due to the consumption by soil microbial community. In the present study, soil labile C concentration decreased with time and this downward trend was observed in all of treatments, regardless of their management history. In addition, concentration of labile C was generally higher in treatments with improved management, when there was no legume residue amendment. This observation can be related to the improvement of soil labile pools and microbial activity due to long-term changes in soil management practices. This is consistent with the findings of previous studies that indicate the significant effect of legume plant rotation and organic amendments on improvement of soil organic matter content in agricultural systems (Berthrong et al., 2013; Buckley and Schmidt, 2001; Fraser et al., 1988; Stark et al., 2007). The legume residue amendment increased soil labile C content in the present study, which is supported by previous studies that also report that concentration of labile C increased with the input of organic soil amendment (Lima et al., 2009; Zhou et al., 2021). The boosted labile C further increased soil microbial activities to improve soil nutrient cycling processes via higher decomposition rate of soil organic matter (Guenet et al., 2012). Interestingly, the present research shows that the concentration of labile C increased in compacted treatments, while this pattern was not clear after ploughing practice at the end of the RS phase. This indicates that compaction stress could increase accessibility of soil native organic C for the local microbial activity. Another potential explanation could be related to the effect of compaction stress on increasing 'hotspot' (aggregate surfaces) numbers within the soil profile, which can consequently accelerate soil microbial activities (Kuzyakov and Blagodatskaya, 2015). As expected, legume residue amendment significantly ($P < 0.05$) increased labile C content in both soils. However, treatments with a surface application of the legume residue had higher labile C content compared with legume residue incorporated treatments, within the same management history. Additionally, there were no significant changes in concentration of labile C in surface applied treatments, while labile C content significantly decreased in incorporated treatments at the end of the experiment. This indicates that the legume residue incorporation method would increase labile C turnover and soil microbe C use efficiency compared with the surface application method.

The concentration of HWETN generally decreased over time in all treatments at the RS phase of this study. Also, ploughing practice did not show any significant impact on the concentration of HWETN among treatments. After the legume residue amendment, labile N significantly increased in all treatments, which could be due to the acceleration of the N mineralisation process by the soil microbial community under stress relieved condition. In addition, concentration of HWETN was lower in ploughed treatments at the end of the RL phase, regardless of their management history, which may be attributed to the higher N immobilisation rate by soil microbial community in these treatments following the ploughing process. Most importantly, we found that the concentration of HWETN was significantly lower in treatments with improved management in comparison to treatments with conventional management, at the end of the RL phase and without any legume residue amendment. Linking this observation to higher labile C and N in treatments with improved management at day 70, it can be suggested that a high concentration of labile C triggers soil catabolic responses in the resident microbial community and consequently shift the mining of soil organic matter to N-rich components (Rousk et al., 2016) which decreased labile C and N content in soil with improved management history.

Soil mineral N pools are regulated by various biogeochemical processes, including N transformation (mineralisation, nitrification and immobilisation), plant uptake and leaching, and their dynamics are highly affected by soil properties, environmental factors and management practices (Burton et al., 2007). The present study shows a cumulation in concentration of soil mineral N at the end of the RS phase. Findings also suggest that ploughing practice increases soil mineral N content, although the observed pattern in treatments with conventional management was not as clear as in treatments with improved management history. This is consistent with a study by Myrbeck et al. (2012), which indicates that ploughing practice results in accumulation of mineral N in soil profile (Myrbeck et al., 2012). As expected, concentration of soil mineral N increased among all treatments following legume residue amendment. The increase of soil mineral N concentrations, following organic amendments,

has also been reported in previous studies (Eghball et al., 2004). In general, we found that soil mineral N content was lower in treatments with improved management than treatments with conventional management history. This could possibly be attributed to the better functioning of microbial N cycling system in soil with improved management history in comparison to soil with conventional management history, which did not have legume crop rotation in the past 30 years.

2.4.2 Microbial responses to soil compaction and its removal

Soil moisture content is considered an important factor in regulating microbial activity and diversity (Pérez Castro et al., 2019). The present study suggests that compaction stress increased the size of microbial C pool in soil with conventional management history but decreased it in soil with improved management history. It is reported by previous studies that compaction stress decreases soil microbial pools (Beylich et al., 2010; Dick et al., 1988; Tan et al., 2005); however, in some cases, compaction results in an increase of soil microbial C pool by enhancing substrate availability via increasing soil water fill pore space (Pengthamkeerati et al., 2011). This may indicate that substrate availability was limited for soil microbe uptake in soil with conventional management history. In addition, the decrease of soil microbial biomass C indicates a change in soil microbial structure in treatments with compaction and ploughing practices, which may illustrate that soil disturbance favours specific groups of organisms over others (Ponder and Tadros, 2002). At the end of the RL phase, the content of microbial biomass C increased back to its initial level in soil with improved management history, in comparison to soil with a conventional management history, which indicates that improved field management helps to build a higher RL against compaction stress. With legume residue amendment, results suggest a sharp increase in soil microbial pools in all treatments, while the legume residue incorporation method resulted in a higher MBC content compared with surface application method, regardless of management history. This further proves that organic amendment can increase nutrient pools for soil microbial consumption as soluble organic C and N pools can provide an available energy source for utilisation by soil microbial communities, leading to an increase in soil microbial activity (Mendham et al., 2003). However, this process may not alter soil microbial responses (RS

and RL) to compaction stress. The soil with improved management had higher concentrations of MBN in comparison to conventionally managed soil, but compaction stress showed no significant impact on MBN content in soils with conventional management history. Also, compaction stress significantly ($P < 0.05$) decreased the concentration of MBN in soil with improved management, which was similar to the observed MBC contents. As the soil bacterial community is reported to be sensitive to compaction stress (Li et al., 2004), our results would indicate that the soil with improved management history had higher RS to maintain microbial community structure unchanged. With the legume residue amendment, results suggest an overall increase of microbial pools in all treatments, which may indicate a shift in soil microbial community structure following the availability of new food source for soil microorganisms (Chen et al., 2016).

In the present study, β–glucosidase activity showed no significant difference among treatments in the RS phase of the experiment. In the RL phase, β–glucosidase activity was higher in soil with improved management, while the observed values were lower in compacted treatments than noncompacted treatments regardless of their management history. Siczek and Frąc (2012) also report that severe compaction stress decreases soil enzyme activity mainly by decreasing soil porosity, which restricts microbial community growth. Application of organic amendments to agricultural lands has been reported to increase soil enzyme activities (Albiach et al., 2000), and reduce the undesirable impacts of compaction stress (Pengthamkeerati et al., 2011). The legume residue amendment in this study, significantly ($P < 0.05$) increased the activity of β-glucosidase, regardless of soil management history and the compaction status of treatments. This was likely due to the higher bioavailability of organic C and N pools derived from the amended legume residue, as Phalke et al. (2016) showed that soil organic C content had a significant correlation with soil β-glucosidase activity after organic amendment to a soybean-maize cropping system. Although microbial activity decreased over time, the observed values were much higher in treatments with improved management in response to the removal of compaction stress in the RL phase. This indicates that the soil microbial community was more sensitive in treatments with improved management history compared to treatments with

conventional management history. The legume residue incorporation method increased soil microbial activity at the start of the RL phase, regardless of soil management history. However, the observed values slightly decreased in treatments with conventional management, but sharply decreased in treatments with improved management, which was consistent with the observed changes in the activity of β-glucosidase and cumulative CO_2 emissions. This also indicates that the soil with improved management history was able to provide a better physicochemical structure for a higher RL and more stabilised microbial activity after removal of compaction stress and amendment of legume residue. Griffiths et al. (2008) also reported that soil physicochemical structure governs soil RL to environmental stresses via regulating soil microbial community composition and microbial physiology.

Soil microbial respiration has been recognised as an indicator of soil fertility and health in relation to field management practices (Bastida et al., 2008). Treatments with improved management had higher cumulative CO_2 respiration, and this may be attributable to the higher labile C content provided by improved management history. Interestingly, treatments with improved management history had higher cumulative CO_2 emission in comparison with conventionally managed treatments, but there was no significant difference in net cumulative CO_2 emissions between improved and conventionally managed treatments, by the end of the RL phase. This observation indicates that the difference between treatments under the same compaction stress only results from their management history. Within treatments with the same management history, the treatments with ploughing practice following compaction stress generated the highest cumulative and net cumulative CO_2 emission, followed by compaction-only and ploughing-only treatments. As expected, a significant increase in CO_2 emissions was observed in all residue amended treatments, which can be attributed to the increase in soil labile C pools in the RL phase of the experiment.

Treatments with conventional management had higher cumulative CO_2 emissions in comparison with treatments with improved management following legume residue amendment, which was opposite to

the observed pattern in treatments without legume residue amendment. Surface application of legume residue showed higher net cumulative CO_2 emissions in treatments with conventional management in comparison to treatments with improved management, while the incorporation method resulted in lower net cumulative CO_2 emissions in conventionally managed treatments in comparison to treatments with improved management. This observation indicates that surface application of legume residues to conventionally managed soils would result in a significant loss of applied organic C via gaseous emissions. Interestingly, legume residue incorporation along with improved management minimised fluctuation of net cumulative CO_2 emissions between compacted and noncompacted treatments by the end of the RL phase, while this was not the case in treatments with conventional management history. This may indicate that soil with conventional management history had lower RL towards recovery from compaction stress following legume residue amendment. A possible explanation could be the ecological concept that the simple ecosystem which builds via conventional management practices had lower RS and RL to environmental stresses, which further confirmed that improved management would increase soil microbial complexity and its RS and RL to compaction stress.

The results indicate a similar response of the investigated soils to compaction stress regardless of their management history. The results also show a higher RL in soil with improved management history, as soil with conventional management was more sensitive to compaction stress compared to soil with improved management history without legume residue amendment. In the present study, the RS index of different treatments was measured as the degree of impairment of their responses relative to a control treatment and their RL index was measured as the rate of recovery following the removal of applied stress. Therefore, the negative value of the RS index indicates a change of higher than 100% in the microbial C use efficiency of stressed treatments compared to the control treatment. The negative value of the RL index also indicates that the absolute value of difference between the stressed treatments and control treatment was higher at the end of the incubation experiment than the end of the RS phase.

2.5 CONCLUSION

The present study shows that different field management practices may not alter soil microbial response patterns to compaction stress as microbial activity is mainly governed by water content and water fill pore space. Improved field management is able to increase soil RS and RL to compaction stress, which is shown as a faster stabilisation of microbial properties after compaction stress and its removal due to their higher organic matter content and complexity of microbial community. The legume residue amendment increased soil labile organic C and N contents. In addition, the legume residue amendment also increased the microbial biomass content and enzyme activity of treatments regardless of soil management history. However, surface application of the legume residue did not improve soil RL against compaction stress due to the rapid loss of produced labile C through respiration process. The HWEOC pool was highly related to the cumulative CO_2 emissions and soil microbial activity. This confirmed that the application of legume residue improved soil chemical conditions, increased the supply of organic C and N for soil microbial community, enhanced the nutrient cycling processes and improved soil health status. Future research should focus on determination of soil microbial community composition and key functional genes involved in C and N cycling for better understanding of the mechanisms regulating soil microbial responses and nutrient dynamics in the compaction affected agricultural lands.

2.6 ACKNOWLEDGMENT

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CHAPTER 3: MANAGEMENT HISTORY GOVERNS SOIL MICROBIAL RESPONSES TO DROUGHT IN GRAIN CROPPING SYSTEMS

3.1 INTRODUCTION

An estimation has been made on the World Economic Forum that drought costs $6–8 billion per year globally, due to agricultural and related businesses losses (Botterill and Cockfield, 2013). The socioeconomic impacts of drought are particularly severe in Australia. For example, recent prolonged dry conditions associated with the 1997–2010 Millennium drought (Gallant et al., 2012; Kiem and Verdon-Kidd, 2011) led to water restrictions in major cities and significantly reduced irrigation allocations across the Murray-Darling Basin, the largest agricultural region in Australia, resulting in major socioeconomic and environmental impacts (van Dijk et al., 2013).

Drought may happen in virtually all regions of the world, regardless of precipitation or temperature regimes, but its impact can vary markedly based on different climate and environmental conditions. The simplest definition of drought is a deficit of water to support soil ecological functions (Sheffield et al., 2012). Drought may result in severe soil health decline as soil nutrients in the drying soil profile become less bioavailable at the root surface when soil water deficit restricts nutrient transport via mass flow and diffusion processes, particularly in the topsoil (Ma et al., 2015). Drought can also decrease the uptake of nutrients due to root shrinking and reduction of soil–root contact area (North and Nobel, 1997), which could further result in a significant yield loss. A greenhouse experiment conducted at Irbid, Jordan, reported that moderate drought reduced about 50% and severe drought reduced about 57% of barley grain yield (Samarah, 2005). A meta-analysis on drought stress also reviewed 55 published rice studies and 60 published wheat studies and concluded that drought decreases wheat and rice yields by 27% and 25%, respectively (Zhang et al., 2018).

While different soils are expected to react differently to water deficit, it is generally accepted that drought stress can impact soil biogeochemical characteristics and performance (Kimball et al., 2001;

Sardans and Peñuelas, 2005). Therefore, it is crucial to investigate how agricultural soils react to prolonged periods of drought, and how they recover from stress when it does rain again. It is also critical to study the sustainability of soil ecosystems under drought stress and provide effective and site-specific soil management practices that farmers can apply to optimise the performance of their soils and improve crop yield when drought stress is removed. It is expected from a sustainable ecosystem to minimise the impact of a disturbance and to rapidly adopt and recover from the impact (Ingrisch and Bahn, 2018). However, soil biological properties are generally more sensitive to environmental stresses compared with soil physical and chemical properties (Kuzyakov et al., 2020), and the relationships between soil biological properties and their response to stressors are remarkably complex. Several indicators have been used to assess the biological responses of soils to environmental and anthropogenic stresses (Guillot et al., 2019). Soil respiration and its relation to changes in soil microbial biomass and enzyme activities are considered the most widely used and robust biological indicators of soil health and its resistance (RS; ability of a soil to maintain its functional stability after a disturbance) and resilience (RL; speed with which a soil system can return to its pre-disturbance condition) to stressors (Orwin and Wardle, 2004; Yu et al., 2021). Previous studies indicate that drought stress can regulate soil respiration (Wang et al., 2014; Zhang et al., 2015), by changing the microbially-mediated soil C and nutrients cycling processes (Zhou et al., 2019). The shift in soil enzyme activities, following a disturbance, can also be considered as an early indicator of changes in soil metabolic capacity (Alhameid et al., 2019).

To address conventional field management caused soil health decline, widely adopted field management practices are generally divided into two main managements, namely conservation and organic management practices. Conservation agricultural management includes not only reducing tillage and lengthening and diversifying crop rotations, often by including legume crops, but also using crop residues and/or cover cropping for permanent ground cover (Chabert and Sarthou, 2020). Organic field management has also proved to be more profitable and environmentally friendly and delivers equally or more yield that contains less (or no) pesticide residues, compared with

conventional farming (Reganold and Wachter, 2016). Deen and Kataki (2003) indicate that conservation field management would have 15% less cost in comparison with conventional field management. This study also reports that no-tillage field management increases by about 5% of soil organic carbon in comparison to conventional management. However, Arvidsson et al. (2014) report that no-tillage practice management results in 10% lower crop yield in comparison with conventional practice management in a 30-year field experiment in Sweden. Vyn and Raimbault (1993) also report a similar result that corn yield was significantly lower in a no-tillage field in comparison to other treatments over a 15-year experiment in southern Ontario, Canada. Organic field management is widely adopted to improve crop yield. Kumar et al. (2010) reported that soil organic amendments such as composted sugarcane residue are useful in maintaining high crop yield and reducing soil organic matter and nutrient depletion. Also, crop yield and soil fertility has been reported to increase via field application of organic compost incorporating cow manure, alperujo (solid olive mill by-product) and olive prunings (Hernández et al., 2014). Calcino et al. (2009) indicate that Bedminster compost (compost of garbage and sewage sludge) increases sugarcane yields as well as concentration of soil nitrogen (N) and other soil nutrients like calcium (Ca), magnesium (Mg), potassium (K) and copper (Cu). Although many studies have investigated the impacts of organic residue amendments on soil properties, scientific knowledge regarding soil microbial responses to different field management history remains largely uncertain. Hence, the assessment of shifting in soil physicochemical parameters is fundamental in monitoring nutrients' cycling under drought stress. Evaluation of soil microbial responses could further explore the underlying mechanisms of the effects of field management history on microbial mediated nutrient cycling processes under drought stress and following the rewetting of dry soils. Also, limited information is available regarding the responses of soil microbial communities and changes of soil nutrient pools following organic residue amendments and during the recovery from drought stress. This information would be critical for better prediction of soil health condition in the context of boosting crop yield and developing sustainable agriculture.

Despite widespread interest in assessment of the detrimental impacts of drought and rewetting cycles in agricultural systems, the response of the belowground nutrient turnover processes to drought stress and how such responses are mediated by the microbial communities are less explored, particularly in grain systems. Such investigations may provide a mechanistic understanding that is crucial for improved predictions of soil microbial responses (resistance and resilience to stressors) and fate of soil nutrient pools under different drought stress scenarios. Therefore, the main objective of this study is to assess the mechanisms responsible for grain farming soils' microbial functional responses to drought stress and field management history, using a soil resistance and resilience approach. The underlying hypotheses are: a) soil biological functions such as microbial respiration and microbial biomass and activity are reliable indices for the assessment of soil health and resilience to drought stress; b) the response pattern of the soil microbial community to drought stress is highly related to the applied stress levels rather than the history of field management practices; and c) soils under crop rotation practices are more resistant and resilient to drought stress than soils under monoculture practices, due to their higher organic matter content and nutrients bioavailability.

3.2 MATERIALS AND METHODS

3.2.1 Sample preparation and experimental design

Fresh soils with an initial water content of 5% (w/w) were collected from 0–10 cm depth of two adjacent wheat (*T. aestivum*) farms (with similar loamy texture and physicochemical properties) under conventional practice (wheat monoculture) and improved practice (crop rotation) managements at Wickepin (32°47'07"S, 117°38'12"E), Western Australia in 2019. At each site, the soil samples were randomly collected from five locations on the farm and bulked as a composite sample. The soil was classified as Arenosols according to Australian soil classification (Isbell, 2016) or Planosols according to FAO world reference base (WRB, 2014), with an initial pH (1:5 water) of 6.2 and water holding capacity (WHC) of 22%. The total C (TC) and total N (TN) contents were 7.1 g kg^{-1} and 0.5 g kg^{-1} for soil under conventional management, and 11.1 g kg^{-1} and 0.8 g kg^{-1} for soil under improved practice management, respectively. Crop rotation of wheat (*T. aestivum*; 2018), Lupin (*L.*

angustifolius; 2017), Barley (*Hordeum vulgare*; 2016), and wheat (*T. aestivum*; 2015) were used in the improved practice management, while a monoculture of wheat (*T. aestivum*) was used in the conventional management in the last five years before soil sampling.

Fresh soils (100 g oven-dry equivalent) were moistened to 15% WHC (severe drought stress), 30% WHC (moderate drought stress) and 55% WHC (no drought stress) with distilled water. The moistened samples then gently transferred to 150 mL flat end polypropylene jars, compacted to bulk density of 1.2 g cm^{-3} (field bulk density) using a modified compactor (the compression process operated in three soil layers to generate a uniform compaction throughout the soil profile), and incubated in dark and aerobic condition at 22 ± 0.5 °C for the first 28 days of the experiment (RS phase). At the end of the RS phase, the drought stress was removed by rewetting dry soil samples and increasing the moisture content in all treatments to 55% WHC. At the same time, half of the treatments were incorporated with wheat residue (3.0 g per jar; dried at 60 °C for two days and then ground for application with TC of 459 g kg^{-1} and TN of 4.8 g kg^{-1}) as organic amendment (equivalent to 36 t ha^{-1} wheat residue). The samples were then incubated in dark and aerobic condition at 22 ± 1°C till the end of the experiment at day 70 (RL phase). The experiment was conducted in 12 treatments with 15 replicates for each treatment. The treatments were divided into four groups: a) soil with conventional management history (CC, CM and CS; the letter 'C' denotes conventional management, followed by the drought stress level of control (55% WHC), moderate stress (30% WHC), and severe stress (15% WHC) in the treatments); b) soil with conventional management history and wheat residue amendment at the end of the RS phase (CO, CMO and CSO; the letter 'O' denotes organic amendment); c) soil with improved practice management history (IC, IM and IS; the letter 'I' denotes improved practice management); and d) soil with improved practice management history and wheat residue amendment at the end of the RS phase (ICO, IMO and ISO).

Soil samples were collected at 7, 28, 35 and 70 days after the commencement of the incubation experiment. Three replicates of each treatment were randomly selected and destructively sampled for

measurement of mineral N (NH_4^+-N and NO_3^--N), hot water extractable organic C (HWEOC), hot water extractable total N (HWETN), microbial biomass C (MBC), microbial biomass N (MBN), overall microbial activities (which measured via FDA method), and β-glucosidase activities. Soil respiration samples were collected at 26 sampling dates (gas sampling was undertaken every one-to-four days depending on the expected levels of CO_2 emissions) during the incubation experiment, using three randomly selected replicates of each treatment.

3.2.2 Analysis of soil chemical properties

Soil pH (1:5 soil to water ratio) value was measured using the glass electrode method described by Rayment and Lyons (2011). The TC and TN contents were measured by combustion method via a LECO CNS-2000 analyser (LECO Corporation, MI, USA). The HWEOC and HWETN concentration of the samples were measured using the method described by Chen et al. (2000). In brief, 5.0g (oven-dry equivalent) of fresh soil was incubated with 25 mL of water in a capped falcon-tube at 70°C for 18 hours. Tubes were shaken on an end-over-end shaker for five minutes and filtered through a Whatman 42 filter paper (Whatman Ltd, Maidstone, UK), followed by a 0.45-μm filter membrane after incubation. Concentrations of extractable organic C and total N in the filtrates were measured using a SHIMADZU TOC-VCPH (Shimazu, Kyoto, Japan) TOCN analyser. Soil mineral N was extracted by 2M KCl at a 1:4 ratio of soil to extractant using an end-over-end shaker, filtered by a Whatman 42 filter paper (Rayment and Lyons, 2011) after one hour shaking and concentrations of NH_4^+-N and NO_3^--N were measured by a SEAL AA3 Continuous Segmented Flow Analyzer (SEAL Analytical Limited, USA). The results were expressed on an oven-dry basis.

3.2.3 Analysis of soil biological properties

Soil microbial biomass C (MBC) and N (MBN) contents were measured by fumigation-extraction method using an Ec conversion factor of 2.64 (Vance et al., 1987) and an En conversion factor of 2.22 (Wilson, 1988) as described by Liu et al. (2018). Dissolved organic C and total N contents in fumigated and unfumigated samples were determined using a SHIMADZU TOC-VCSH/CSN total organic carbon and N analyser (Shimadzu Scientific Instruments, Japan). Soil respiration was

measured using a gas sampling method, as described by Rezaei Rashti et al. (2016). At each gas sampling event, three replicates of each treatment were placed into 2 L airtight glass jars that were then continually flushed with ambient compressed air for one minute. Gas samples were collected from the headspace of the glass jars eight hours after closure using a 25 mL gas-tight syringe and immediately transferred to pre-vacuumed 12 mL glass vials (Labco, UK). Gas samples were measured for CO_2 concentration using a gas chromatograph (Shimadzu GC-2010 Plus) method. Linearity tests on CO_2 concentration increases were conducted on all treatments over a subset of sampling times during the incubation. These samples were taken initially after closure of the glass jars and then every 60 minutes for ten hours. The emissions for days without gas sampling were estimated using the arithmetic mean of the measurements on the two closest days (Rezaei Rashti et al., 2016). The cumulative emissions were calculated by summing the daily emission measurements. The microbial metabolic quotient (qCO_2), defined as the C respired per unit of MBC per day, and calculated from the ratio of CO_2-C emission (μg CO_2-C kg^{-1} soil day^{-1}) to concentration of MBC (mg C kg^{-1} soil) of each treatment. The activities of β-glucosidase (hydrolysing cellulose to glucose) at pH 6.0 in soil samples were measured according to enzyme activity method for acidic soils (Tabatabai, 1994). Fluorescein diacetate (FDA) hydrolysis is accepted worldwide as an accurate and simple method for measuring the overall microbial activity in a range of environmental samples, including soils at pH 7.6 (Green et al., 2006) and was used to measure soil microbial activity in this study.

3.2.4 Soil microbial resistance and resilience indices

To assess the functional RS and RL of C use efficiency (metabolic quotient) in soil microbial communities under drought stress, the following indices were used according to Orwin and Wardle (2004):

$$RS = 1 - [(2 \times |D_{28}|) / (C_{28} + |D_{28}|)]$$

D_{28} is the difference between soil metabolic quotient value in the control (CC in conventional and IC in improved management practice) treatment (C_{28}) and the stressed treatment (drought stress) at the end of the RS phase (day 28). The RL index for the microbial C use efficiency of each treatment was calculated at the end of the incubation experiment (day 70), according to:

$$RL = [(2 \times |D_{28}|) / (|D_{28}| + |D_{70}|)] - 1$$

D_{70} is the difference between soil metabolic quotient value in the control (CC and CCO in conventional, and IC and ICO in improved management practice) treatment and the stressed treatment at the end of the RL phase (day 70). 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 C use efficiency 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 treatment. If the absolute value of D_{70} becomes higher than the absolute value of D_{28}, then the RL index will have a negative value.

3.2.5 3 Statistical analysis

Differences at $P < 0.05$ between treatments were considered significant and all variables were tested for normality of distribution using the Kolmogrov–Smirnov test. Pearson linear correlation was used to describe the relationship between soil properties. In addition, data on all soil properties were subjected to principal component analysis (PCA) to distinguish the effect of drought levels on the investigated soils, at the end of RS and RL phases, using the IBM SPSS Statistics 26 software package (IBM Corp., released 2020, IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp). The net cumulative CO_2 emissions in the current experiment were calculated as the differences in cumulative emissions between the treatments and their control (no drought stress) at each sampling time (i.e. CC and IC for treatments without wheat residue amendment, and CCO and ICO for treatments with wheat residue amendment at the end of the RS phase).

3.3 RESULTS

The basic properties (pH, TC and TN) of the investigated soils were measured in all treatments to assess their changes due to the short-term drought and rewetting cycle. The soil samples in all treatments were slightly acidic (pH values were around 6.2). However, there were no significant differences in soil pH values among treatments. In addition, the initial TC and TN contents were found to be significantly ($P < 0.05$) higher in treatments with improved management than treatments with conventional management history.

3.3.1 Labile organic C and N pools

Table 3.1 shows that concentration of hot water extractable organic C (HWEOC) was significantly ($P < 0.05$) higher in treatments with improved management history (IC, IM, and IS) than treatments with conventional management history (CC, CM and CS), while there were no significant differences observed between treatments with the same management history at day 7. At the end of the RS phase (day 28), there was no significant difference between concentration of HWEOC among treatments with conventional management history, while improved management treatments without drought stress (IC and ICO) showed the lowest concentration of HWEOC in their treatment group (Table 3.1). This may indicate the higher sensitivity of the soil with improved management history to drought stress. In the first week of the RL phase (day 35) and among the treatments without wheat residue amendment, the IS treatment had the highest concentration of HWEOC, while conventional management treatments showed significantly ($P < 0.05$) lower concentrations. However, no significant differences were found within treatments from the same management history, while concentration of HWEOC was higher in the improved management treatments than conventional management treatments at the end of the experiment (day 70). As expected, wheat residue application significantly ($P < 0.05$) increased HWEOC concentrations at day 35. It is important to point out that treatments under severe drought stress (CSO and ISO) showed the highest concentration of HWEOC within treatments with the same management history at day 70.

The HWETN concentration was significantly ($P < 0.05$) higher in treatments with improved management than conventional management history at the RS phase of the experiment regardless of the drought stress level (Table 3.1). After the removal of drought stress at day 28, no significant difference was observed among all treatments without wheat residue amendment until the end of the experiment. However, in treatments with the wheat residue amendment, the concentration of HWETN was significantly ($P < 0.05$) higher in severe drought treatment (ISO) than the control treatment (ICO) in improved management history, while there was no significant difference among conventional management treatments regardless of drought stress level at day 35 (Table 3.1). At the end of the RL phase (day 70), improved management treatment with severe drought stress (ISO) showed the highest concentration of HWETN in comparison with other treatments.

Table 3.1 Dynamics of soil hot water extractable C and N and mineral N concentrations during the incubation period, in treatments under different management practices, with and without organic residue amendment.

Treatment		HWEOC (mg kg^{-1} dry soil)				HWETN (mg kg^{-1} dry soil)				NH_4^+-N (mg kg^{-1} dry soil)				NO_3^--N (mg kg^{-1} dry soil)			
		Day7	Day28	Day35	Day70	Day7	Day28	Day35	Day70	Day7	Day28	Day35	Day70	Day7	Day28	Day35	Day70
Without organic residue amendment	CC	353.1b	371.7c	314.9c	264.6b	11.5b	10.8b	11.8a	1.4a	9.4b	9.3b	9.3bc	6.5c	60.1b	90.9b	85.4bc	138.9c
	CM	383.9b	367.2c	302.6c	260.1b	13.2b	10.8b	11.9a	2.9a	9.5b	9.6b	8.8c	6.5c	64.5b	78.9bc	95.3b	147.7c
	CS	382.3b	347.6c	296.4c	259.4b	13.3b	10.2b	10.7a	3.2a	9.4b	8.7b	11.1ab	6.1c	68.8b	64.0c	68.6c	128.4c
	IC	470.4a	416.5b	341.0b	303.5a	17.1a	14.2a	12.9a	2.4a	10.0b	8.8b	8.8c	7.0bc	85.2a	110.4a	125.7a	188.9a
	IM	474.2a	450.3ab	360.4ab	300.6a	16.4a	14.5a	12.4a	2.3a	9.7b	8.4b	9.2bc	7.3ab	85.8a	92.0b	122.1a	176.3ab
	IS	499.4a	481.8a	387.4a	309.6a	15.8a	14.2a	12.0a	1.5a	13.7a	15.6a	12.9a	7.8a	71.3b	76.1bc	94.3b	152.0b
With organic residue amendment	CCO	359.8b	365.1c	728.4a	446.9a	12.9b	12.1b	12.0ab	1.7b	9.5b	9.7b	9.7ab	6.8c	60.1b	90.6ab	3.1a	3.4b
	CMO	373.9b	367.2c	624.3a	511.7a	13.2b	10.8b	11.0ab	2.9ab	9.6b	9.7b	9.9ab	6.9c	64.6b	79.0bc	0.8a	3.6b
	CSO	372.3b	345.3c	674.8a	583.7a	13.3b	10.3b	11.2ab	0.9b	9.4b	8.8b	9.3b	7.7ab	68.6b	64.0bc	2.1a	3.3b
	ICO	476.5a	426.6b	681.2a	520.9a	17.1a	14.2a	9.6b	1.4b	10.0b	8.9b	9.5ab	8.1b	85.2a	107.1a	3.0a	5.0a
	IMO	476.2a	440.8ab	689.1a	509.4a	16.4a	14.6a	11.3ab	1.3b	9.7b	8.1b	10.5a	7.3ab	86.8a	92.1ab	2.9a	5.1a
	ISO	489.4a	471.9a	695.3a	564.1a	15.8a	14.3a	13.0a	4.5a	13.7a	15.5a	9.9ab	9.3a	71.4b	74.1bc	3.3a	4.4a

The treatment names starting with the letter C refer to soil under conventional practice management, including: CC, control (55% WHC); CM, moderate drought (30% WHC) and rewetting; CS, severe drought (15% WHC) and rewetting; CCO, control with organic residue amendment; CMO, moderate drought and rewetting with organic residue amendment; CSO, severe drought and rewetting with organic residue amendment. The treatment names starting with the letter I refer to soil under improved practice management, with the same experimental definitions as the above treatments, including IC, IM, IS, ICO, IMO, and ISO treatments. The reported data are means of three replicates. Means followed by different letters within a column indicate significant differences between the treatments at $P < 0.05$.

3.3.2 Mineral N pool

The concentration of NH_4^+-N was generally higher in treatments with improved management history. However, the concentration of NH_4^+-N was significantly ($P < 0.05$) higher in severe drought stress of improved management treatment (IS) than other treatments, while there was no significant difference among the rest of treatments, regardless soil management history and drought stress level, from day 7 to day 28 of the experiment (RS phase). At day 35, the present study shows that removal of severe drought stress (CS and IS treatments) without a wheat residue amendment resulted in the highest concentration of NH_4^+-N but there was not a significant difference between the control and moderate drought stress treatments regardless of the management history. Interestingly, significant ($P < 0.05$) differences were observed between severe (CS) and moderate (CM) drought stress treatments with a conventional management history, while in treatments with improved management history severe drought stress (IS) and control (IC) treatments showed a significant ($P < 0.05$) difference in NH_4^+-N concentration. At the end of the experiment, the concentration of NH_4^+-N was higher in the IS treatment, which recovered from severe drought stress, in comparison to other treatments with the same management history, while there were no significant differences among treatments with conventional management history. With wheat residue application, the present study shows that the IMO treatment had the highest concentration of NH_4^+-N, while the CSO treatment had the lowest concentration at day 35. At the end of the experiment, the removal of severe drought stress (CSO and ISO) resulted in the highest concentration of NH_4^+-N within treatments with the same soil management history.

In general, concentration of NO_3^--N was higher in treatments with improved management history throughout the entire incubation experiment. However, there was a sharp decrease in NO_3^--N concentration after wheat residue application to both investigated soils, regardless of drought stress levels. In treatments without wheat residue application, concentration of NO_3^--N increased over time from day 7 to day 70. The treatments under severe drought stress (CS and IS) tended to have the lowest concentration of NO_3^--N within the same management history at the end of the RS phase (day

28). After removal of drought stress, the treatments under severe drought (CS and IS) showed the lowest concentrations of NO_3^--N among the treatments with the same management history until the end of the experiment (day 70). As mentioned above, there was a sharp decrease in concentration of NO_3^--N after wheat residue application, regardless of drought stress levels and management history (day 35). After that, no significant differences were observed among treatments with the same management history, although improved management treatments tended to have higher concentrations of NO_3^--N in comparison with conventional management treatments.

3.3.3 Soil microbial properties

In general, the concentration of microbial biomass C (MBC) was higher in control treatments (CC, IC, CCO and ICO) throughout the experimental period, regardless of management history (Table 3.2). In treatments without wheat residue application, drought stress significantly ($P < 0.05$) decreased concentration of MBC, although there was no significant difference between drought levels at day 7, regardless of soil management history. There was a significant ($P < 0.05$) increase in concentration of MBC in treatments with improved management history at day 28, while severe drought stress to conventionally managed soil (CS) decreased the concentration of MBC at the end of the RS phase. After removal of drought stress, the concentration of MBC increased in treatments with conventional management, while slightly decreased in treatments with improved management history. This may indicate the slower recovery of soil microbial community in treatments with improved management history than conventionally managed treatments. The concentration of MBC continued increasing in treatments with conventional management, while it stabilised in treatments with improved management history. In treatments with wheat residue amendment, it was clear that organic matter input significantly ($P < 0.05$) increased concentration of MBC in all treatments. It should be mentioned that, at day 35, the concentration of MBC decreased when increasing the drought stress level in both soils regardless of their management history, although the concentration of MBC was higher in treatments with improved management practices. At the end of the RL phase (day 70), the concentration of MBC decreased in control and moderate drought treatments (CCO and CMO), but

slightly increased in severe drought-treated soil (CSO) with conventional management. However, the concentration of MBC in treatments with improved management history slightly decreased by the end of the experiment (day 70).

In general, the present study shows that drought stress significantly ($P < 0.05$) decreased concentration of microbial biomass N (MBN) in treatments that received conventional management, while there was no significant difference in soil that received improved management until the end of the RS phase (day 28). After the removal of drought stress, there were no significant changes in the soil with conventional management by day 35, but a sharp increase occurred in stressed treatments (CM and CS) at day 70 (Table 3.2). Interestingly, improved management treatments showed a different pattern after removal of drought stress. The results suggest that drought stress did not show a significant impact on the concentration of MBN in soils with improved management at day 35. However, the stressed treatments (IM and IS) showed significantly ($P < 0.05$) higher concentrations of MBN than the control (IC) by the end of the experiment (day 70). Table 3.2 shows that severe drought stress (IS) had the highest concentration of MBN, while the control treatment (IC) had the lowest concentration. At day 35 there was a significant ($P < 0.05$) increase in the concentration of MBN after the wheat residue application, regardless of management history, while the concentration of MBN decreased with the increase of drought stress level. Interestingly, at day 70, the concentration of MBN showed the highest value in severely stressed conventional management treatment (CSO) but had the lowest value in severely stressed improved management treatment (ISO) compared to other treatments.

3.3.4 Soil *β*-glucosidase activity

Overall, activity of β-glucosidase increased over time until the end of incubation (Table 3.2). The wheat residue application also significantly ($P < 0.05$) increased the activity of β-glucosidase regardless of soil management history and drought stress levels. The increase of drought stress levels resulted in the reduction of the activity of β-glucosidase at the end of the RS phase, regardless of soil

management history. After removal of drought stress, no significant differences were observed among treatments with conventional management history, while significantly ($P < 0.05$) higher activity of β-glucosidase was observed in the severe drought treatment (IS) with improved management history at day 35. At the end of the experiment, recovery from drought stress increased activity of β-glucosidase regardless of stress levels and soil management history. With the wheat residue application, there was no significant difference among treatments at day 35. This may indicate that the addition of organic matter boosts β-glucosidase activity and masks the impacts caused by drought stress. At day 70, no significant differences were found among treatments with improved management history, while β-glucosidase activity was higher in the severe drought-stressed treatment (CSO) than the control treatment (CCO) in conventionally managed treatments.

Fig. 3.1 showed the total microbial activity under drought stress in soils with two types of field management history. The total microbial activity decreased from day 7 to day 28 in control (CC and IC) and moderate drought (CM and IM) treatments, while no clear pattern observed in severe drought treatments (CS and IS). After removal of drought stress, total microbial activity increased over time, till the end of the experiment, while this pattern was not clear in severe drought-stressed treatment (CS) with conventional management (Fig. 3.1a). Additionally, total microbial activity increased in a short period after removing the stress in improved management treatments, but decreased at the end of the experiment, although this pattern was not clear in severe drought stresses treatment (IS). With the wheat residue application, total microbial activity significantly ($P < 0.05$) increased following removal of drought stress at day 35, and then significantly ($P < 0.05$) decreased by the end of the experiment (Fig. 3.1b). This pattern was observed in all treatments regardless of soil management history and drought stress levels.

3.3.5 CO_2 and net CO_2 respirations

In general, treatments with improved management had significantly ($P < 0.05$) higher cumulative CO_2 respiration in comparison to conventionally managed treatments throughout the incubation

period, when there was no wheat residue amendment in the RL phase. In addition, drought stress significantly ($P < 0.05$) decreased cumulative CO_2 respiration regardless of soil management history (Fig. 3.2a). Also, there was a significant ($P < 0.05$) increase of cumulative CO_2 respiration in severe drought-stressed treatments at the end of the RS phase (day 28), while this pattern was not clear in the control (CC and IC) and moderate stressed treatments (CM and IM), regardless of soil management history. Additionally, the differences in cumulative CO_2 respiration were smaller between drought-stressed treatments and control in soil with conventional management than soil with improved management history (Fig. 3.2c). This may indicate that soil with improved management was more sensitive to changes in soil moisture levels, which confirms the observed higher microbial property levels, such as higher MBC and enzyme activities, when there were no external organic matter inputs. According to Fig. 3.2b, the wheat residue amendment significantly ($P < 0.05$) increased cumulative CO_2 respiration, regardless of soil management history and drought stress levels during the RL phase of the experiment. The cumulative CO_2 respiration of the control (CCO) and moderate drought (CMO) treatments had similar values in soil with conventional management history, while moderate and severe drought affected treatments (IMO and ISO) in soil with improved management had similar cumulative CO_2 respiration by the end of the experiment. This may indicate that severe drought may decrease C use efficiency in soils with a conventional management history, while soils with an improved management history could tolerate moderate and severe drought, which further indicate a higher drought resilience in comparison to soil with conventional management. Interestingly, the differences of cumulative CO_2 decreased at day 56 but increased at the end of the experiment (day 70) in both drought-stressed treatments (CMO and CSO) with conventional management. However, the net cumulative CO_2 emissions of soil with improved management history decreased throughout the experiment after wheat residue amendment (Fig. 3.2d). This may indicate that soil with a conventional management history tends to have lower resistance and higher recovery towards drought following wheat residue application.

Table 3.2 Dynamics of soil microbial biomass, β-Glucosidase activity and metabolic quotient during the incubation period, in treatments under different management practices, with and without organic residue amendment.

Treatment		MBC (mg kg^{-1} dry soil)				MBN (mg kg^{-1} dry soil)				β-Glucosidase (µg p-nitrophenol h^{-1} g^{-1} dry soil)				qCO_2 (µg CO_2-C h^{-1} mg^{-1} MBC)			
		Day7	Day28	Day35	Day70	Day7	Day28	Day35	Day70	Day7	Day28	Day35	Day70	Day7	Day28	Day35	Day70
Without organic residue amendment	CC	104.7a	111.4a	154.4a	170.4a	72.3a	76.6a	81.9a	90.6d	29.6ab	35.6ab	49.6c	37.5d	1.8b	5.1a	3.1a	3.2b
	CM	54.9b	74.9b	128.9b	143.4b	33.8b	30.1b	21.5b	64.5e	29.0ab	34.3ab	45.7c	58.3c	3.4a	4.6a	2.8a	4.3b
	CS	62.8b	48.7c	111.3c	169.8a	11.5c	23.6b	33.8b	103.1c	23.6b	24.2b	46.6c	58.2c	1.6b	4.0a	2.7a	2.6b
	IC	57.3b	124.1a	107.3c	115.2c	6.9c	11.7c	90.3a	109.7c	32.1ab	57.8a	61.4b	72.9b	1.8b	4.6a	1.5b	6.6a
	IM	35.2c	110.0a	85.1d	66.0d	8.3c	10.7c	82.4a	135.2b	35.6a	44.1ab	66.8b	89.6a	4.2a	3.9a	1.5b	9.8a
	IS	36.3c	86.0b	76.5d	84.3d	10.9c	14.4c	81.9a	195.5a	28.2ab	26.1ab	85.4a	86.9a	1.4b	3.7a	1.1b	7.4a
With organic residue amendment	CCO	128.1a	114.4a	343.3a	269.1a	62.0a	63.2a	75.7ab	93.9c	29.7ab	35.6ab	99.2a	65.6b	1.5b	5.1a	16.0a	13.2c
	CMO	45.0b	91.6a	282.5b	245.0b	30.4b	20.1b	81.0ab	93.1c	29.0ab	34.3ab	102.8a	80.2ab	3.0a	4.6a	17.1a	15.1b
	CSO	56.2b	45.4b	221.1d	227.9b	10.2c	16.9b	66.1b	145.9a	23.7b	24.2b	101.7a	118.5a	1.3b	4.3a	15.4a	12.2c
	ICO	60.7b	116.6a	362.7a	204.9c	5.3c	6.4c	117.2a	144.5a	32.2ab	57.9a	107.2a	121.5a	1.6b	4.4a	5.5b	16.8a
	IMO	35.2c	83.3a	306.5b	170.6d	6.3c	8.7c	104.3ab	132.8b	35.6a	44.1ab	107.7a	115.8a	3.7a	3.6a	3.9b	16.9a
	ISO	36.3c	82.6a	265.9c	155.9d	11.7c	9.4c	60.9b	91.5c	28.2ab	26.1ab	110.1a	122.6a	1.2b	3.2a	5.6b	18.1a

The reported data are means of three replicates. Means followed by different letters within a column indicate significant differences between the treatments at $P < 0.05$. Description of treatments same as Table 3.1.

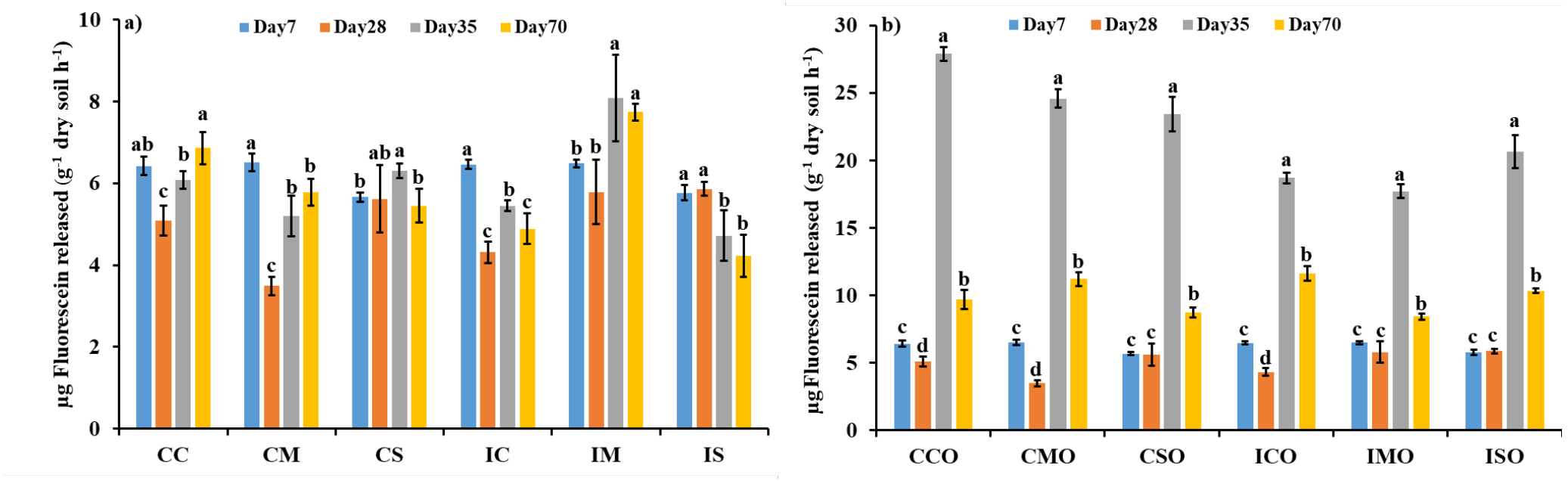


Fig. 3.1 Dynamics of soil microbial activity during the incubation period, in treatments under different management practices, without (a) and with (b) organic residue amendment. Description of treatments are the same as Table 3.1. Vertical bars are standard error of three replicates. Means of the values for different sampling events, within a treatment, with the same letter are not different at the 5% level of significance.

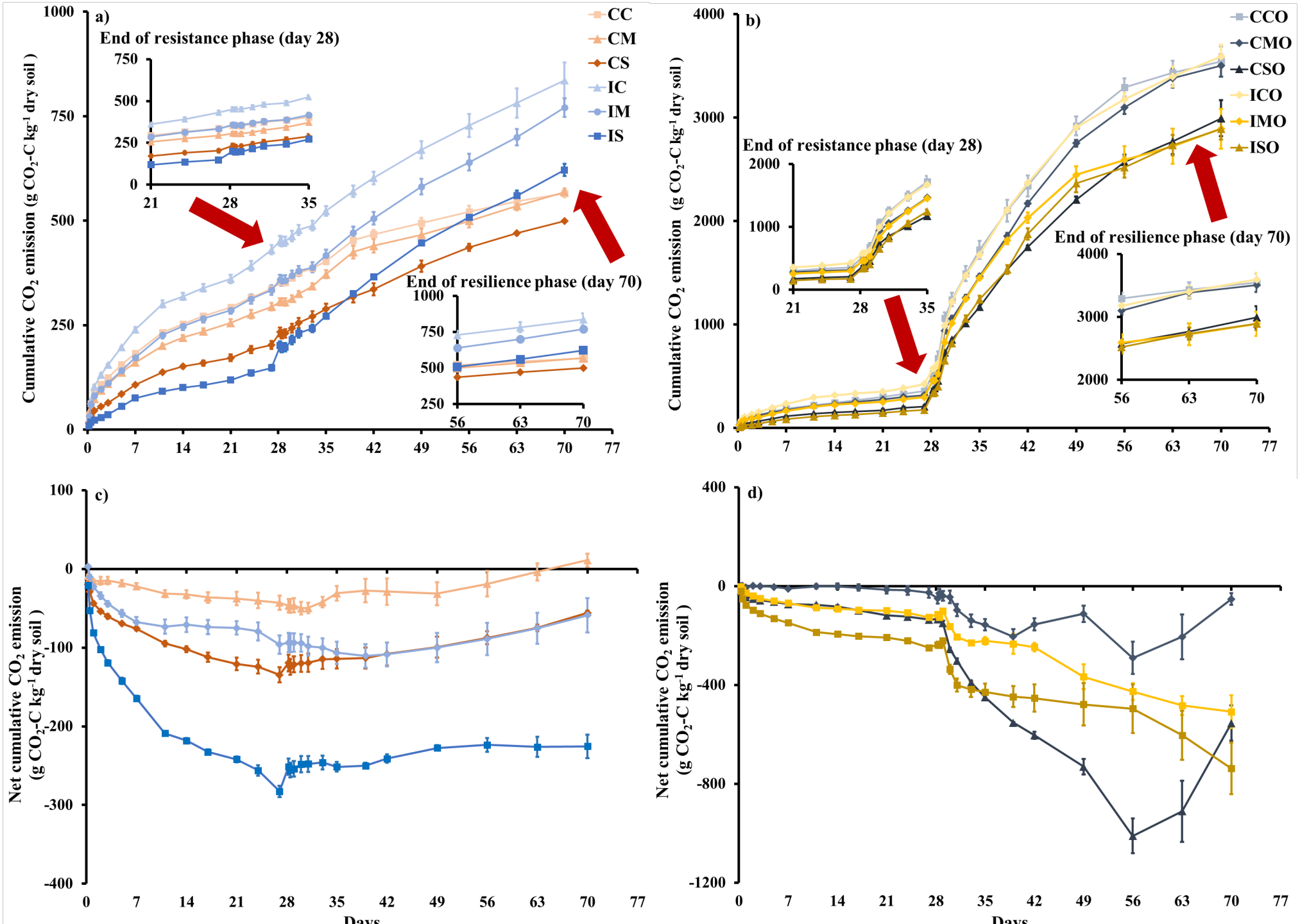

a)
End of resistance phase (day 28)
End of resilience phase (day 70)
CC
CM
CS
IC
IM
IS
Cumulative CO2 emission (g CO2-C kg-1 dry soil)
b)
CCO
CMO
CSO
ICO
IMO
ISO
c)
Net cumulative CO2 emission (g CO2-C kg-1 dry soil)
Days
d)

Fig. 3.2 Cumulative (a and b) and net cumulative (c and d) CO_2 emissions from different treatments during the incubation period. The values of net cumulative emission for each treatment were calculated based on their differences from their control treatment at each sampling time (i.e. CC and IC for treatments without organic residue amendment, and CCO and ICO for treatments with organic residue amendment). Description of treatments are the same as Table 3.1. Vertical bars are standard error of three replicates.

3.3.6 qCO_2 values

Table 3.2 shows clearly that the qCO_2 value increased in the moderate drought level regardless of management history at day 7. There was an increase of qCO_2 at day 28, regardless of soil drought level and management history. Without the wheat residue application, soil with the improved management history had a significantly lower qCO_2 level in comparison to soil with a conventional management history at day 35, but a higher qCO_2 level at the end of the experiment (day 70). It is important to mention that the qCO_2 level tended to be higher in moderate drought affected treatments regardless of soil management history. This may indicate that soil responses to drought would not be affected by soil management history. With the wheat residue application, there was an increase of qCO_2 found in all treatments after drought removal, while there was no significant difference found among treatments within the same soil management history, although the qCO_2 level in conventionally managed soil was significantly ($P < 0.05$) higher than in soils with an improved management history. Similarly, treatments with no wheat residue applied and improved management history had a significantly higher qCO_2 level in comparison to soil with a conventional management history at the end of the experiment (day 70). This may indicate that the application of wheat residue increases qCO_2 values but has no impact on soil response process to drought stress.

3.3.7 Relationships between soil physiochemical and microbial properties and the PCA analysis

The correlation coefficients (r) between soil physicochemical and biological properties (n = 192) are shown in Table 3.3. The results indicate that soil MBC was highly related to soil labile C content and soil MBN was highly related to soil labile N and mineral N ($P < 0.01$) contents. In addition, Table 3.3 confirms that soil total microbial activity (FDA), β-glucosidase activity and cumulative CO_2 respiration were highly related to soil labile pools and mineral N contents ($P < 0.01$).

The PCA results show that principal components (PC) PC1 and PC2 explained 24.6–57.6% and 16.8–28.7% of the data variance, respectively (Fig. 3.3). The treatments were clearly separated from each other based on PC1 and PC2 components. The parameters with the highest correlation coefficients

(> 0.85) for PC1 were labile C, labile N, mineral N and MBC, while the parameters with highest correlation coefficient (> 0.85) for PC2 were total microbial activities, β-glucosidase, and CO_2 respiration. The treatments with conventional management were clearly separated based on PC1, while treatments with improved management were clearly separated based on PC2 at day 7 (Fig. 3.3a). At day 28, soils under with management histories were clearly separated based on PC1 (Fig. 3.3b). After the removal of drought stress, soils with both management histories were clearly separated based on PC2 at day 35 and day 70 (Figs. 3.3c and 3.3d). Interestingly, with the wheat residue application, treatments with conventional management history were clearly separated based on PC1, while treatments with improved management history were clearly separated based on PC2 at day 35. However, the treatments in both soils were clearly separated based on PC1 and PC2 at day 70 (Figs. 3.3e and 3.3f). Fig. 3.4a shows that the RS index of C use efficiency decreased by increasing drought stress, regardless of soil management history. This may indicate that the response of soil microbes to drought stress is governed by water content rather than soil management history. This is also demonstrated in Fig. 3.4b; without wheat residue input, the soil with a conventional management history and the soil with improved management history had similar patterns in response to drought stress levels. With the presence of wheat residue, the soil with improved management history had significantly higher RS index of C use efficiency in comparison to soil with conventional management history (Fig. 3.4c).

Table 3.3 Correlation coefficients (r) between treatments' physicochemical and biological properties during the incubation period (n = 192).

	HWEOC	HWETN	NH_4^+-N	NO_3-N	MBC	MBN	β-Glucosidase	FDA	Cumulative CO_2 emissions	qCO_2
HWEOC	1									
HWETN	0.17*	1								
NH_4^+	0.24**	0.607**	1							
NO_3^-	−0.76**	0.00	−0.10	1						
MBC	0.60**	−0.39**	−0.26**	−0.59**	1					
MBN	0.10	−0.78**	−0.43**	−0.10	0.52**	1				
β-Glucosidase	0.57**	−0.49**	−0.27**	−0.43**	0.71**	0.69**	1			
FDA	0.78**	−0.10	0.00	−0.64**	0.78**	0.28**	0.63**	1		
Cumulative CO_2	0.56**	−0.59**	−0.33**	−0.68**	0.75**	0.59**	0.75**	0.57**	1	
qCO_2	0.27**	−0.67**	−0.41**	−0.38**	0.34*	0.60**	0.64*	0.20**	0.83**	1

* Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)

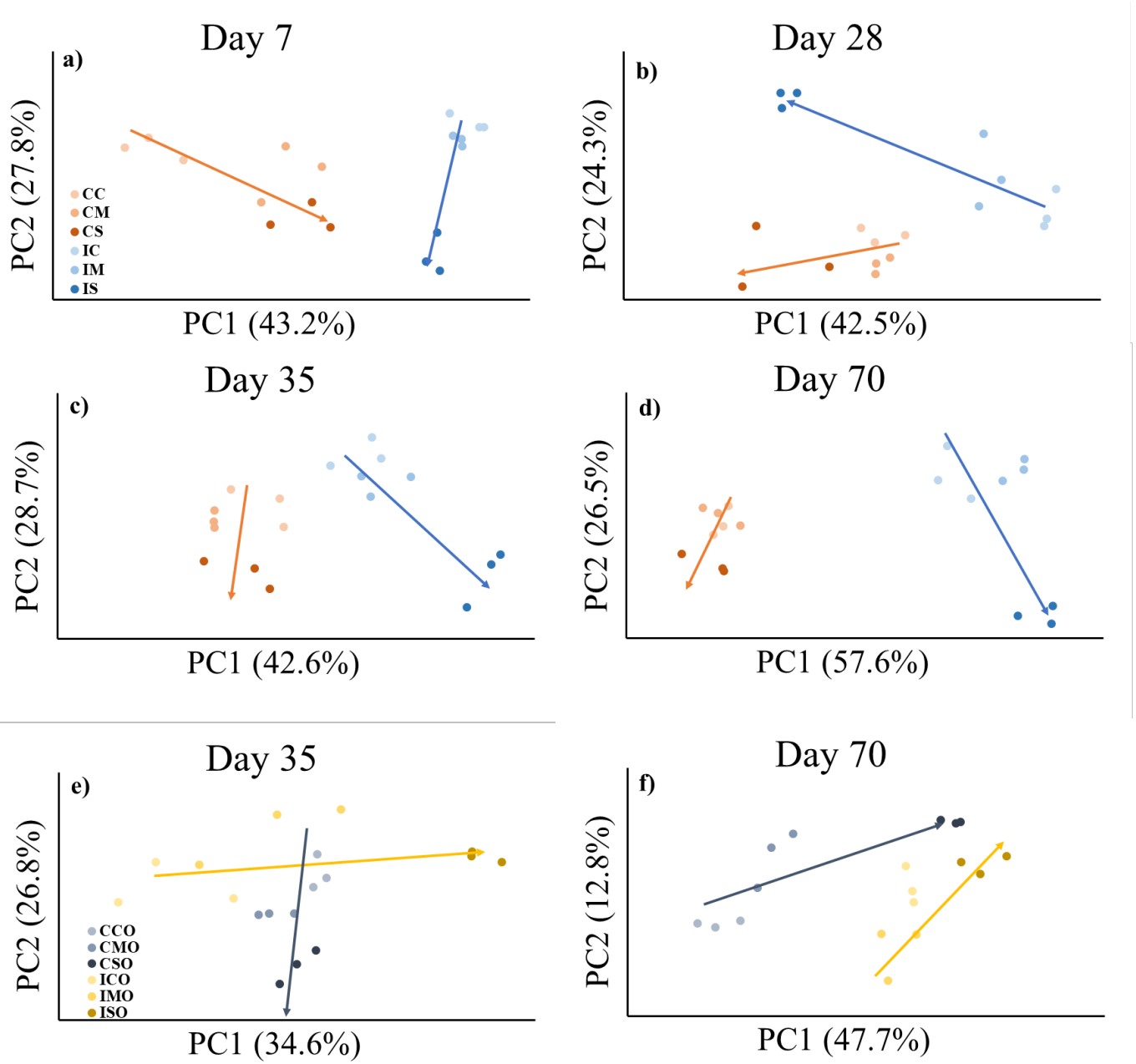

Day 7
a)
PC2 (27.8%)
PC1 (43.2%)
CC
CM
CS
IC
IM
IS
Day 28
b)
PC2 (24.3%)
PC1 (42.5%)
Day 35
c)
PC2 (28.7%)
PC1 (42.6%)
Day 70
d)
PC2 (26.5%)
PC1 (57.6%)
Day 35
e)
PC2 (26.8%)
PC1 (34.6%)
CCO
CMO
CSO
ICO
IMO
ISO
Day 70
f)
PC2 (12.8%)
PC1 (47.7%)

Fig. 3.3 Scores plot of principal component analysis (PCA) showing the separation of treatments under different management practices, with and without organic residue amendment, and loading values of the individual soil parameters (PC1 and PC2) for each treatment. Description of treatments are the same as Table 3.1. Arrows in each plot indicate the distribution of treatments within a field management practice, where control treatments (no stress; light colours) were close to the tail and treatments with severe drought and rewetting cycle (dark colours) were close to the tip of each arrow.

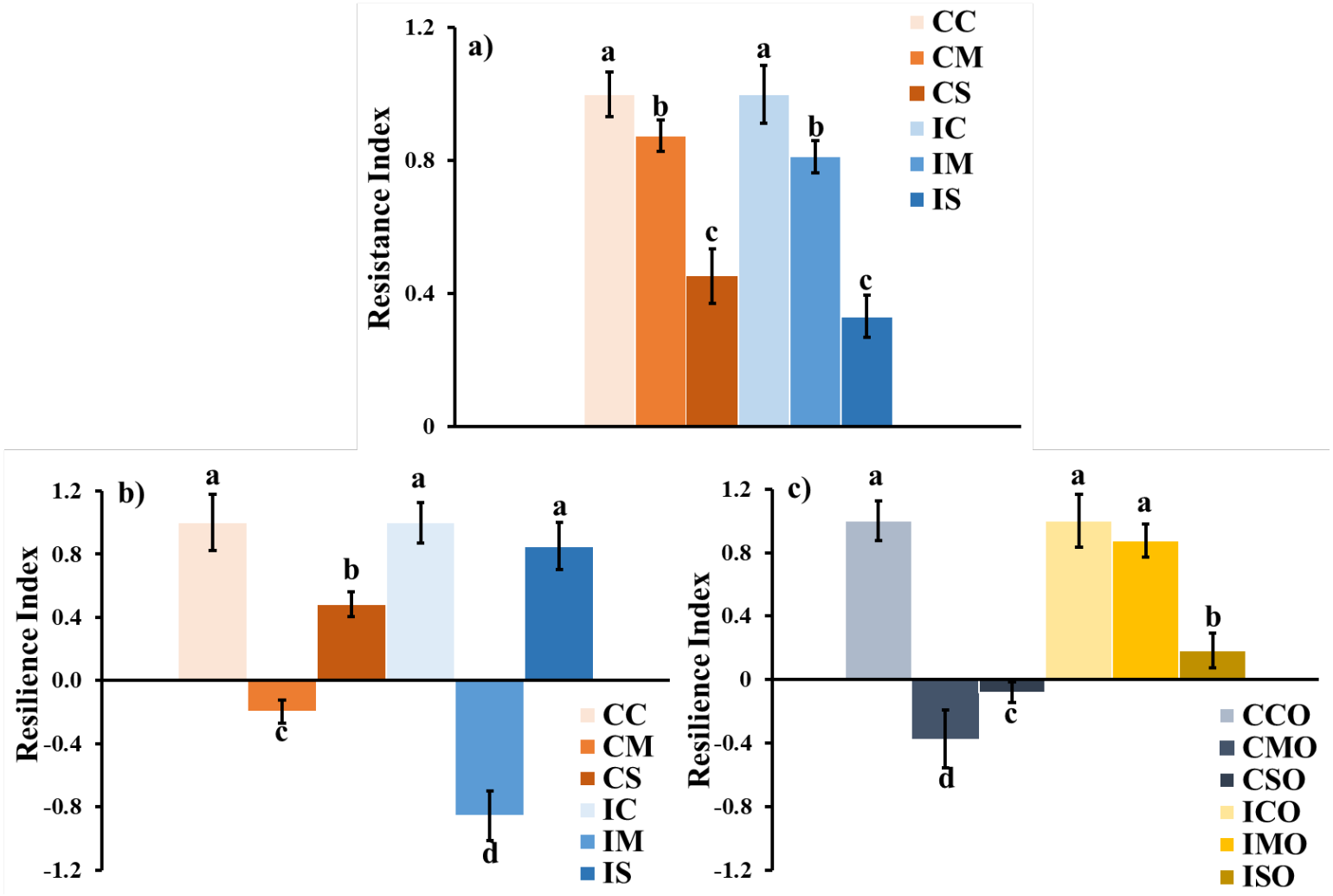


Fig. 3.4 The resistance (a; day 28) and resilience (b and c; day 70) indices of soil microbial carbon use efficiency (metabolic quotient) in treatments under different management practices, with (b) and without (c) organic residue amendment. Description of treatments are the same as Table 3.1. Vertical bars are standard error of three replicates. Means of the treatments by the same letter are not different at the 5% level of significance.

3.4 DISCUSSION

In order to address issues related to the effects of drought stress on decline of soil health and crop production, various soil management practices such as organic residue incorporation with different methods (e.g. surface application (Sirousmehr et al., 2014) or deep trench application (Liu et al., 2018)) have been developed to alleviate drought stress in the field and improve soil health and fertility. It has been suggested that the organic residue amendment can ameliorate drought stress and improve soil health by increasing water retention, reducing soil acidity, increasing soil microbial activity and increasing bioavailability of soil organic matter (Jeffery et al., 2011). Agricultural field management practices such as ploughing may increase crop yield through the improvement of labile C bioavailability, microbial activity and general soil health conditions (Schneider et al., 2017). It has been reported that ploughing can stimulate plant root growth and increased crop yield (Garside et al., 2005). However, the responses of soil nutrient pools and microbial biomass and activity to drought stress and their recovery from drought may vary with soil management history and drought stress levels. The mechanisms responsible for the diverse responses of soil nutrient dynamics and microbial processes to drought stress and its removal are largely unknown.

3.4.1 Responses of soil labile organic carbon and nitrogen pools to drought stress and its removal

Labile pools of soil organic matter are key fractions in assessment of soil health as they are bioavailable for plant uptake and microbial consumption, sensitive to changes in environmental conditions and play an important role in soil nutrient cycling (Hu et al., 1997). The HWEOC and HWETN components of soil organic matter have been recognised as soil labile C and N pools and are considered as the best indicators for the effects of field management practices on soil health conditions (Chen et al., 2004). The presented investigation demonstrates that labile C could be the main C source for microbial consumption. This is supported by a study conducted by Teutscherova et al. (2017) that labile C decreased during incubation period due

to the soil microbial community consumption. A study by Liu et al. (2018) also suggests that labile C was consumed by the soil microbial community as the main C source in soil C cycling. In the present study, soil labile C content decreased during the experiment and this downward trend was found in all soils and drought stress levels, although the concentration of labile C was higher in the improved management soil with no wheat residue input. This may be due to the enlargement of the soil labile pool from the improved management practice, which could provide a better environment for the soil microbial community. Application of the wheat residue also boosted soil labile C content in the present study, which is consistent with previous investigations (Lima et al., 2009; Zhou et al., 2021). The increase in soil labile C would further increase soil microbial activities and boost soil nutrient cycling processes via priming of soil organic matter decomposition (Guenet et al., 2012). Interestingly, the present research shows that labile C accumulated in severe drought-stressed treatments after the input of the wheat residue. This observation indicates that severe drought stress could cause a significant inhibition in C consumption and decrease the size and activity of the soil microbial biomass. A 180-day incubation study conducted by Hueso et al. (2012) shows higher organic matter content in severe drought-stressed soil and indicates that organic amendments can improve the size and activity of a soil microbial community under stress conditions. In addition, moderate drought stress showed no significant impact on concentration of labile C in soil that received improved management practices in comparison to soil that received conventional management practices, indicating that proper management practices along with the application of organic residues could help to improve soil resilience against moderate drought stress.

In terms of HWETN, an overall downward trend was observed in all treatments (Table 3.1) before removal of drought stress at the end of the RS phase (day 28). After removal of drought stress, no significant difference was observed among all treatments without the wheat residue amendment until the end of the experiment. However, after the wheat residue application, labile

N content was significantly ($P < 0.05$) higher in the severe drought treatment compared with the control treatment with improved management history, while there was no significant difference among the conventionally managed treatments regardless of drought stress level (Table 3.1). The labile N content was relatively higher in soil that received conventional management in comparison with soil that received improved management, although there was no difference in labile N contents among treatments with conventional management history. This confirms that proper management practices may help to improve soil resilience against drought stress in intensive grain cropping systems.

This study shows an increase in concentration of soil mineral N during the RS phase. In addition, concentrations of NH_4^+-N showed a slight increase after the wheat residue application at the start of the RL phase. It has been reported that soil mineral N concentration would increase following organic amendment to soil (Eghball et al., 2004). Interestingly, the concentration of NO_3^--N slightly increased in treatments without the wheat residue amendment while it sharply decreased in wheat residue amended treatments. This sharp decrease in NO_3^--N content could be due to increased microbial consumption and N_2O emissions contributed by mineralisation of soil organic matter, which is consistent with previous observations reported in a field study conducted in Hunan of China (Wu et al., 2017).

3.4.2 Soil microbial responses to drought stress and its removal

Soil moisture content is an important factor in governing soil microbial activity and diversity (Pérez Castro et al., 2019). In the present study, drought stress decreased the size of the soil microbial C pool regardless of management history. This observation indicates that proper field management practices would increase nutrient pools for soil microorganisms, while they may not alter the soil microbial responses to drought stress. In addition, following the removal of drought stress at the start of the RL phase, the MBC content increased in conventional practice treatments and increased with drought stress level, while this was not the case in improved

management practice treatments without wheat residue input. Considering this observation along with the cumulative CO_2 respiration data, the results suggest that conventionally managed soil had higher microbial pools and lower cumulative CO_2 respiration than improved practice managed soil. This indicates that C use efficiency was higher in soil with conventional management than soil using improved management practices. With the wheat residue application, a sharp increase in soil microbial pools was observed in all treatments, while MBC content decreased with the increasing of drought stress levels. This observation indicates that plant residue input would increase nutrient pools for the soil microbial community as soluble organic C and N in soil would provide an available energy source for utilisation by soil microorganisms. This may further result in an increase in soil microbial activity (Mendham et al., 2003), while it may not alter soil microbial responses to drought stress. It is also supported by the positive correlations between soil MBC and the HWEOC and HWETN contents in the present study. The investigated soil with improved management had lower MBN content in comparison to conventionally managed soil. In addition, drought stress showed no significant impact on MBN content in treatments with improved management practices, while significantly decreased MBN content in treatments with conventional practices. After the removal of drought stress, MBN content immediately increased in treatments with improved management history when there was no external wheat residue input. With the wheat residue application, a sharp increase of microbial pools was observed in all treatments, while MBN contents decreased with increasing of drought stress level. Interestingly, by the end of the RL phase, severe drought stress increased MBN content in the conventional management treatment but decreased its content in the improved management treatment.

Field application of soil organic amendments has been reported to significantly increase soil enzyme activity (Albiach et al., 2000). In the present study, the β–glucosidase activity tended to be higher in treatments with improved management history in comparison to the treatments

with conventional management at the beginning of the RS phase. Also, the wheat residue application significantly increased the activity of β-glucosidase regardless of soil management history and drought stress levels. This observation can be related to the organic inputs from the wheat residue application, as shown by the significant and positive correlations between enzyme activities and soil MBC and MBN as well as soil HWEOC and HWETN contents. Phalke et al. (2016) show that soil organic C content had a significant correlation with soil β-glucosidase activity after organic amendment to a soybean-maize cropping system. It is necessary to point out that there was no significant difference among treatments after the wheat residue application, which indicates that organic matter input could boost β-glucosidase activity and mask the impacts caused by drought stress.

The soil microbial activity decreased in control and moderate drought-stressed treatments during the RS phase of the experiment, while microbial activity did not show a clear pattern in severe drought-stressed treatments regardless of soil management history. After removal of drought stress in the RL phase, soil microbial activity increased until the end of the experiment, but this pattern was not clear in severe drought-stressed treatment (CS) under conventional management without wheat residue application (Fig. 3.1a). This indicates that severe drought stress would significantly impact soil microbial activity regardless of soil management history, while the treatments with improved management history would have higher RL to severe drought stress. With the wheat residue application, microbial activity significantly boosted at day 35 and then decreased by the end of the experiment (Fig. 3.1b). This pattern was observed in all treatments regardless of soil management history and drought stress levels. In this study, microbial activity showed a significant and positive correlation with labile C and the improvement of enzyme activities in the improved management practice and wheat residue applied treatments, suggesting a partial alleviation of constraint during the RL phase of the experiment.

Soil microbial respiration has been increasingly recognised as an indicator of soil fertility and health in relation to field management practices (Bastida et al., 2008). In the present study, by the end of the RL phase, significant differences in cumulative CO_2 respiration were observed among all treatments. Interestingly, cumulative CO_2 respiration decreased with increasing drought stress levels regardless of soil management history, which can be attributed to the decrease in the soil MBC pool and microbial activity by increasing drought stress levels. In addition, the present study showed that treatments with conventional management history had similar net CO_2 respiration patterns to treatments with improved management history, indicating that soil moisture content played a key role in governing soil microbial respiration rather than soil management history. The investigated soil with improved management showed higher cumulative CO_2 respiration, and this may be attributable to the presence of higher labile C content in this soil. It is important to mention that there was a significant increase in cumulative CO_2 respiration in all treatments after wheat residue application, which can be attributed to the increase of labile C following residue amendment. The present study also shows that cumulative CO_2 respiration of control and moderate drought stress treatments had similar values in soil with a conventional management history, while moderate and severe drought stress treatments in the soil with improved management had similar cumulative CO_2 respiration (Fig. 3.2b). This may indicate that severe drought stress decreased C use efficiency in the soil with a conventional management history, while the soil with an improved management history could better recover from moderate and severe drought stress, which further indicates a higher drought resilience in comparison to soil with conventional managements. Interestingly, we found that the difference of cumulative CO_2 decreased at the beginning but increased at the end of the experiment in both drought-stressed treatments with conventional management. However, the net cumulative respiration of soil with improved management history was different from the soil with conventional field management as it was

decreasing throughout the RL phase after the wheat residue application. This may indicate that the soil with the conventional management history had slightly higher resistance but significantly lower recovery from drought stress following the wheat residue application. A possible explanation could be an ecological concept that more complex ecosystems have lower resistance but higher recovery from environmental stresses, which further confirms that proper field management practices would increase soil microbial complexity and resilience to drought stress.

3.5 CONCLUSION

The present study has shown that different field management practices may not change soil microbial response patterns to drought as microbial activity is mainly governed by soil water content. Improved field management could help soil build resilience to droughts, which is shown as the tolerance to moderate drought and resistance to severe drought, due to high organic matter content and complexity of microbial community. However, soil under conventional field management would have a lower resistance but higher recovery to drought stress. Also, it is strongly demonstrated that the organic application increased inputs of labile organic C and N (HWEOC, HWETN). On the other hand, the organic residue application also increased the concentration of microbial biomass and enzyme activity regardless of soil management history. The HWEOC pool was highly related to the cumulative CO_2 respiration and microbial activity. This confirms that the application of organic residue improved soil chemical conditions, increased the supply of organic C and N for the soil microbial community, enhanced nutrient cycling processes, and improved soil health conditions. Future research should focus on investigation on the shifting of the microbial community structure and key functional genes involved in C and nutrients cycling in response to drought stress, to better understand the mechanisms of microbial responses and nutrient dynamics in the drought affected grain cropping system.

3.6 ACKNOWLEDGMENT

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APPENDIX A: SUPPLEMENTARY INFORMATION FOR CHAPTER 1

Supplementary Table 1 Three-way ANOVA analysis of the effects of soil type, moisture stress and compaction stress on HWEOC contents.

Factor	HWEOC											
	day7		day14		day35		day42		day49		day74	
	F	p	F	p	F	p	F	p	F	p	F	p
Soil Type	260.1	***	178.6	***	171.0	***	8.8	***	3.4	ns	29.8	***
Moisture Stress	104.1	***	96.9	***	20.6	***	26.7	***	27.5	***	96.8	***
Compaction Stress	1.4	ns	18.1	***	5.7	***	1.4	ns	1.8	ns	1.9	ns
Soil Type * Moisture Stress	1.1	ns	8.2	***	4.6	*	0.2	ns	1.4	ns	0.2	ns
Soil Type * Compaction Stress	0.7	ns	13.5	***	2.1	ns	1.9	ns	0.7	ns	2.7	*
Moisture Stress * Compaction Stress	9.3	***	5.5	***	5.7	***	9.8	***	11.3	***	11.9	***
Soil Type * Moisture Stress * Compaction Stress	0.6	ns	11.7	***	2.5	*	6.1	***	4.3	***	7.7	***

*: $p < 0.05$; ***: $p < 0.01$; ns: not significant

Supplementary Table 2 Three-way ANOVA analysis of the effects of soil type, moisture stress and compaction stress on HWETN contents.

Factor	HWETN											
	day7		day14		day35		day42		day49		day74	
	F	p	F	p	F	p	F	p	F	p	F	p
Soil Type	293.5	***	1092.1	***	586.2	***	748.5	***	349.4	***	253.4	***
Moisture Stress	21.1	***	54.6	***	141.1	***	111.8	***	186.2	***	418.8	***
Compaction Stress	19.2	***	6.7	***	10.6	***	46.2	***	33.4	***	24.5	***
Soil Type * Moisture Stress	3.2	ns	25.1	***	25.1	***	45.0	***	57.3	***	71.4	***
Soil Type * Compaction Stress	8.7	***	1.9	ns	6.9	***	19.3	***	11.6	***	8.5	***
Moisture Stress * Compaction Stress	11.4	***	7.2	***	3.4	***	30.8	***	31.3	***	22.0	***
Soil Type * Moisture Stress * Compaction Stress	2.9	*	7.6	***	1.8	ns	6.5	***	9.6	***	5.5	***

*: $p < 0.05$; ***: $p < 0.01$; ns: not significant

Supplementary Table 3 Three-way ANOVA analysis of the effects of soil type, moisture stress and compaction stress on NH_4^+-N contents.

Factor	NH_4^+-N											
	day7		day14		day35		day42		day49		day74	
	F	p	F	p	F	p	F	p	F	p	F	p
Soil Type	49.4	***	0.2	ns	119.6	***	354.8	***	6.9	***	119.2	***
Moisture Stress	72.8	***	35.9	***	82.3	***	13.8	***	0.1	ns	87.1	***
Compaction Stress	4.0	***	6.4	***	14.8	***	2.8	ns	3.8	***	2.6	*
Soil Type * Moisture Stress	2.6	ns	16.7	***	3.3	ns	6.7	***	4.4	*	27.3	***
Soil Type * Compaction Stress	5.8	***	4.2	***	1.7	ns	4.1	***	5.2	***	14.7	***
Moisture Stress * Compaction Stress	5.5	***	8.4	***	7.9	***	3.9	***	5.3	***	6.6	***
Soil Type * Moisture Stress * Compaction Stress	0.7	ns	2.3	ns	2.0	ns	3.8	***	19.0	***	2.7	*

*: $p < 0.05$; ***: $p < 0.01$; ns: not significant

Supplementary Table 4 Three-way ANOVA analysis of the effects of soil type, moisture stress and compaction stress on NO_3^--N contents.

Factor	NO_3^--N											
	day7		day14		day35		day42		day49		day74	
	F	p	F	p	F	p	F	p	F	p	F	p
Soil Type	1530.6	***	906.2	***	812.8	***	1463.4	***	818.7	***	588.1	***
Moisture Stress	152.2	***	209.1	***	110.2	***	712.2	***	1310.6	***	0.3	ns
Compaction Stress	13.4	***	11.7	***	29.7	***	71.1	***	138.5	***	113.7	***
Soil Type * Moisture Stress	85.7	***	45.0	***	14.3	***	368.8	***	250.0	***	178.5	***
Soil Type * Compaction Stress	11.6	***	8.3	***	6.6	***	28.9	***	58.8	***	104.8	***
Moisture Stress * Compaction Stress	4.9	***	10.4	***	61.4	***	131.5	***	68.8	***	114.6	***
Soil Type * Moisture Stress * Compaction Stress	2.1	ns	6.6	***	4.6	***	37.7	***	21.1	***	48.2	***

*: $p < 0.05$; ***: $p < 0.01$; ns: not significant

Supplementary Table 5 Three-way ANOVA analysis of the effects of soil type, moisture stress and compaction stress on cumulative microbial respiration.

Factor	cumulative microbial respiration											
	day7		day14		day35		day42		day49		day74	
	F	p	F	p	F	p	F	p	F	p	F	p
Soil Type	6.7	*	14.7	***	76.6	***	239.6	***	439.1	***	823.2	***
Moisture Stress	84.6	***	25.5	***	3.5	ns	91.1	***	302.1	***	1068.8	***
Compaction Stress	33.1	***	22.2	***	11.1	***	5.4	***	3.2	*	1.6	ns
Soil Type * Moisture Stress	1.9	ns	16.9	***	42.2	***	124.4	***	193.8	***	268.3	***
Soil Type * Compaction Stress	3.0	*	2.7	*	3.7	*	3.3	*	3.9	***	4.3	***
Moisture Stress * Compaction Stress	28.6	***	16.9	***	17.9	***	12.5	***	9.8	***	5.6	***
Soil Type * Moisture Stress * Compaction Stress	10.4	***	11.3	***	9.2	***	6.1	***	4.7	***	5.5	***

*: $p < 0.05$; ***: $p < 0.01$; ns: not significant

Supplementary Table 6 Three-way ANOVA analysis of the effects of soil type, moisture stress and compaction stress on MBC contents.

Factor	MBC											
	day7		day14		day35		day42		day49		day74	
	F	p	F	p	F	p	F	p	F	p	F	p
Soil Type	743.9	***	2112.6	***	4038.1	***	794.9	***	2498.1	***	2127.9	***
Moisture Stress	71.1	***	7.3	***	49.4	***	6.0	*	0.1	ns	40.6	***
Compaction Stress	7.4	***	102.3	***	106.6	***	16.4	***	53.2	***	29.1	***
Soil Type * Moisture Stress	2.6	*	177.9	***	16.1	***	38.5	***	0.2	ns	2.0	ns
Soil Type * Compaction Stress	8.9	***	52.1	***	50.4	***	17.0	***	24.0	***	21.8	***
Moisture Stress * Compaction Stress	21.4	***	42.0	***	109.8	***	7.0	***	12.1	***	15.0	***
Soil Type * Moisture Stress * Compaction Stress	11.0	***	35.6	***	62.5	***	23.7	***	42.9	***	68.2	***

*: $p < 0.05$; ***: $p < 0.01$; ns: not significant

Supplementary Table 7 Three-way ANOVA analysis of the effects of soil type, moisture stress and compaction stress on MBN contents.

Factor	MBN											
	day7		day14		day35		day42		day49		day74	
	F	p	F	p	F	p	F	p	F	p	F	p
Soil Type	129.7	***	494.7	***	287.9	***	845.7	***	746.5	***	202.4	***
Moisture Stress	0.0	ns	22.1	***	223.5	***	181.3	***	34.2	***	49.3	***
Compaction Stress	1.8	ns	43.3	***	44.5	***	33.5	***	27.7	***	32.9	***
Soil Type * Moisture Stress	0.7	ns	11.9	***	0.7	ns	265.6	***	21.6	***	4.5	*
Soil Type * Compaction Stress	1.4	ns	33.1	***	7.8	***	31.1	***	16.0	***	10.5	***
Moisture Stress * Compaction Stress	1.3	ns	34.0	***	64.3	***	19.6	***	14.1	***	17.7	***
Soil Type * Moisture Stress * Compaction Stress	1.7	ns	22.9	***	29.3	***	41.7	***	10.2	***	3.0	*

*: $p < 0.05$; ***: $p < 0.01$; ns: not significant

Supplementary Table 8 Three-way ANOVA analysis of the effects of soil type, moisture stress and compaction stress on β-glucosidase enzyme activity.

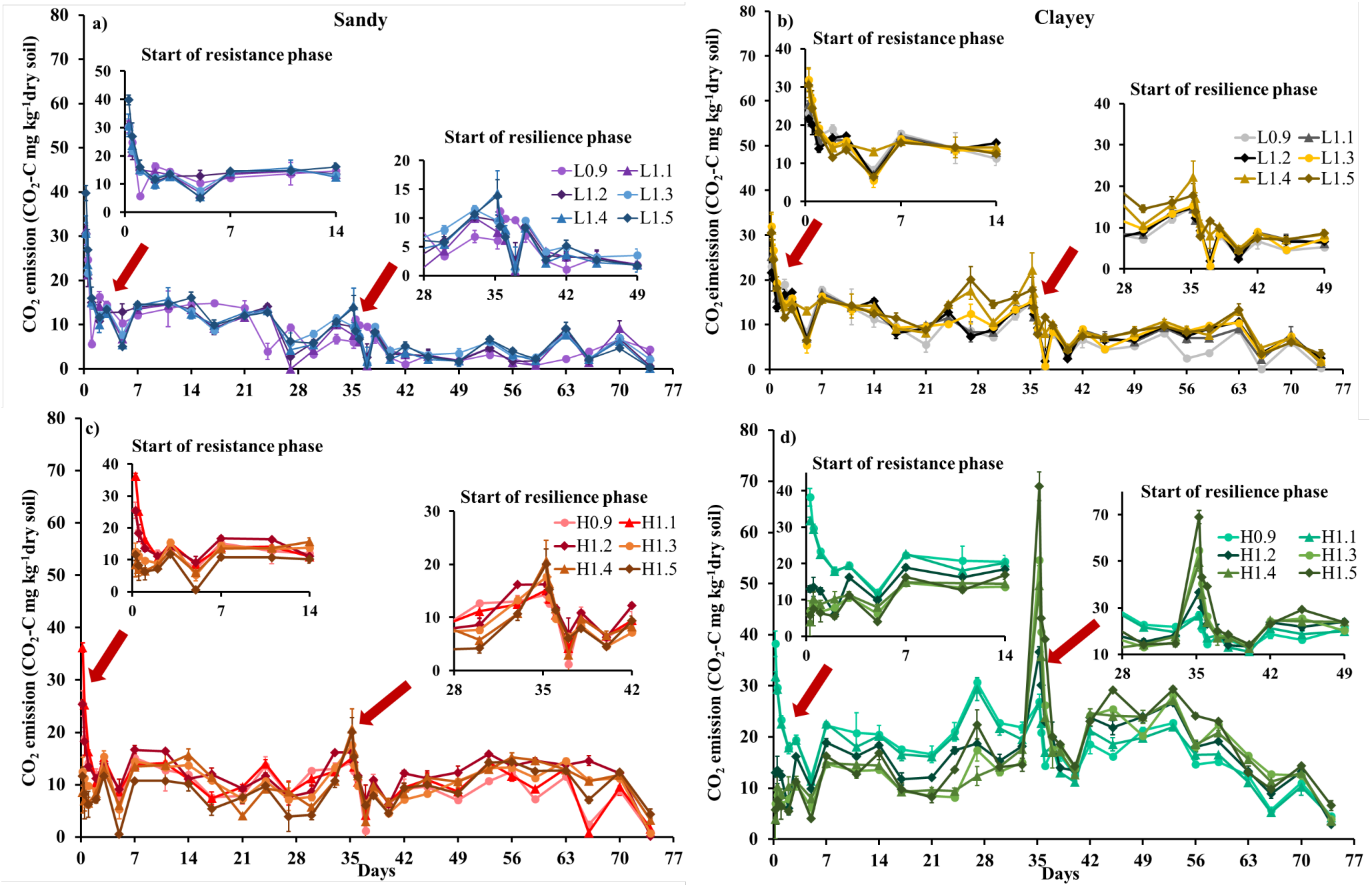
Factor	β-glucosidase enzyme activity											
	day7		day14		day35		day42		day49		day74	
	F	p	F	p	F	p	F	p	F	p	F	p
Soil Type	85.1	***	33.2	***	1320.1	***	21.7	***	331.3	***	475.1	***
Moisture Stress	12.6	***	6.7	***	305.0	***	15.2	***	177.2	***	36.8	***
Compaction Stress	13.1	***	17.9	***	17.2	***	6.0	***	4.4	***	21.8	***
Soil Type * Moisture Stress	23.1	***	12.3	***	120.6	***	0.1	ns	18.3	***	29.1	***
Soil Type * Compaction Stress	5.7	***	3.2	***	10.6	***	2.9	*	14.7	***	29.0	***
Moisture Stress * Compaction Stress	5.0	***	38.1	***	8.1	***	6.7	***	6.9	***	98.8	***
Soil Type * Moisture Stress * Compaction Stress	3.6	***	5.9	***	7.7	***	2.1	***	6.6	***	22.4	***

*: $p < 0.05$; ***: $p < 0.01$; ns: not significant

Supplementary Table 9 Three-way ANOVA analysis of the effects of soil type, moisture stress and compaction stress on chitinase enzyme activity.

Factor	Chitinase enzyme activity											
	day7		day14		day35		day42		day49		day74	
	F	p	F	p	F	p	F	p	F	p	F	p
Soil Type	721.7	***	221.9	***	49.7	***	0.1	ns	32.3	***	34.1	***
Moisture Stress	0.9	ns	6.8	***	48.9	***	117.3	***	117.8	***	58.9	***
Compaction Stress	6.3	***	15.1	***	6.8	***	5.7	***	2.7	*	7.0	***
Soil Type * Moisture Stress	10.9	***	47.5	***	289.5	***	220.4	***	469.9	***	285.8	***
Soil Type * Compaction Stress	8.7	***	2.8	*	3.1	*	2.4	*	2.2	ns	6.0	***
Moisture Stress * Compaction Stress	7.5	***	2.9	*	19.7	***	5.8	***	2.6	*	14.6	***
Soil Type * Moisture Stress * Compaction Stress	7.5	***	5.2	***	1.3	ns	2.2	ns	3.0	*	11.1	***

*: $p < 0.05$; ***: $p < 0.01$; ns: not significant


a)
Sandy
Start of resistance phase
Start of resilience phase
L0.9
L1.1
L1.2
L1.3
L1.4
L1.5
b)
Clayey
c)
H0.9
H1.1
H1.2
H1.3
H1.4
H1.5
d)
Days

Supplementary Fig. 1 Effect of combined compaction and moisture stresses on daily CO_2 emissions in sandy (a and c) and clayey (b and d) sugarcane soils (n=18). L, low moisture level (15% WHC); H, high moisture level (35% WHC). Numbers follow by letter L or H indicates bulk density (e.g. 0.9 means 0.9 g cm^{-3}). Vertical bars are standard error of three replicates.