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in sugarcane and grain systems**

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**Microbial responses to soil constraints as a measure of soil
health in sugarcane and grain systems**

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Abstract

Environmental or human-induced disturbances, such as compaction and drought commonly caused by modern agricultural machinery and extreme climate events, have drawn great attention towards their impacts on soil microbial activities and associated declines in soil health and crop yield. To be specific, soil compaction combined with local extreme weather events, such as intensive rainfall or prolonged drought, may cause a significant decrease in soil microbial activity and threaten sustainable crop production. The use of organic amendment and crop rotation are commonly adopted as improved field management strategies to mitigate the disturbance-induced (compaction and drought) declines in crop yield, soil carbon, and soil health. Although widespread interest exists in the assessment of the detrimental impacts of environmental disturbances on agricultural systems, the response of belowground nutrient turnover processes to compaction and/or drought and how such responses are mediated by the microbial communities are less explored. In addition, there is a paucity of information on soil microbial responses to compaction and drought stresses and their recovery from the stressed conditions, particularly the differences in soils with conventional and improved management history. Furthermore, a robust, cheap, and easy-to-understand index needs to be developed in monitoring soil microbial community responses to compaction and drought stresses as current mainstream soil health indicators are expensive and complicated for end-users.

This thesis aimed to investigate microbial responses to soil constraints as a measure of soil health in sugarcane and grain systems. The specific objectives of this thesis were to a) determine the mechanisms responsible for soils' microbial functional response and

its thresholds to environmental disturbance (compaction and drought); b) adopt this mechanism of the responses of soil microbial community following soil compaction (or drought) stress into both conventional and improved soil management practices contexts; c) generate soil resistance and resilience index based on the responses of soil microbes to compaction and/or drought ; d) enrich the knowledge of soil microbial activity and nutrient pools responses to different practices of removing compaction stress, such as deep treading mill-mud application and shallow furrow application, in fields condition. Three incubation experiments and a field trial were designed to test following hypotheses: 1) Soil biological functions such as respiration and microbial biomass and activity are reliable indices for assessment of soil health and resilience to compaction and moisture (waterlogging or drought) stresses; 2) Soils with improved management practice history have higher resistance against compaction stress and higher resilience following removal of the stress; 3) Response pattern of soil microbial community to drought stress is highly related to the applied stress levels rather than the history of field management practices; 4) Application of organic amendments (e.g., crop residues, mill-mud) and removal of soil compaction stress would increase the size of soil microbial community and microbial activity (indicating soil resistance and resilience), which further improves soil health condition and increases crop yield.

In the first incubation experiment, two contrasting sugarcane soils (Planosols at Rocky Point and Nitisols at Ingham) were exposed to a combination of compaction and drought stresses, and microbial functions were investigated going into the stress as well as their response coming out of the stress. We artificially applied a gradient of bulk densities ($0.9 - 1.5 \text{ g cm}^{-3}$) and water fill pore space (WFPS; 21% to 100%). Under high water content (i.e., WFPS of 47% to 100%), low compaction levels (1.1 and 1.2 g cm^{-3}) increased soil cumulative microbial respiration in Planosols treatments by 18% in

comparison to nil compaction treatment (0.9 g cm^{-3}), while high compaction levels ($1.3 - 1.5 \text{ g cm}^{-3}$) decreased soil cumulative microbial respiration by 25% with increasing of compaction stress in 74 days incubation experiments. In contrast, in Nitisols with high water content, the highest compaction treatment (1.5 g cm^{-3}) significantly increased soil cumulative microbial respiration by 12% in comparison to nil treatment (0.9 g cm^{-3}). Our data revealed that the Nitisols had a higher resistance index of microbial C use efficiency, while the Planosols had a higher resilience index of microbial C use efficiency to compaction and moisture stresses. This could be attributed to greater aggregation potential (associated with more finely textured particles), and higher diversity of the microbial community in the Nitisols, which provide higher functional stability to resist environmental disturbance, while fast drainage and higher adaptation of microbial communities in the Planosols would provide a faster recovery from the applied stresses. This also indicates that soil microbial responses to compaction stress are governed by soil texture and moisture status.

In the second incubation experiment, Nitisols with conventional and improved management history from adjacent sugarcane farms at Foresthome, North Queensland, Australia, were exposed to compaction stress (1.4 g cm^{-3}), and microbial functions were investigated during compaction and ploughing cycle as well as their response to surface and mixing plant residue application methods over 70 days. The improved management block was managed with minimum tillage, mound planting, and legume plantation in comparison to conventional furrow management. Compaction was applied at the commencement of the experiment and removed at day 28 via plant residue application combined with ploughing. Overall, the concentration of labile C 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 CO₂ emissions, followed by compaction-only and ploughing-only treatments. Interestingly, we found that legume residue incorporation along with improved land management minimized fluctuation of net cumulative CO₂ emissions between compacted and non-compacted treatments by the end of experiment; however, we did not find the same pattern in treatments with conventional land management. Our results revealed that different field management practices might 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 practices can improve soil resistance and resilience to compaction stress, which is shown as a faster stabilization of microbial properties after compaction stress and its removal due to their higher organic matter content and complexity of microbial community. The present study confirmed that application of legume residue increased the supply of organic C and N for soil microbial community, enhanced the nutrient cycling processes, and improved soil health status.

In the third incubation experiment, Planosols collected from Wickepin, Western Australia (one soil with conventional management history and one soil with improved management history) were exposed to severe, moderate and nil drought stress and microbial functions were investigated going into stress as well as their response coming out of stress via rewetting and plant residue input. In general, treatments with improved management history had higher (15% - 53%) cumulative CO₂ respiration in comparison to treatments with conventional management throughout the incubation period without plant residue amendment. In addition, drought stress significantly decreased cumulative CO₂ respiration regardless of soil management history. We found that the differences of cumulative CO₂ emissions decreased at day 56 but increased at the end of experiment (day 70) in both drought stressed treatments with conventional management history.

The hot water extractable organic C pool was highly related ($r = 0.56 - 0.78$) to the cumulative CO₂ respiration and microbial activity. The results showed that different field management practices may not change soil microbial response patterns to drought, as microbial activity is mainly governed by soil texture when water content is limited. Improved field management could help to build soil resilience to drought, which is shown as the tolerance to moderate drought and resistance to severe drought due to their 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. The plant residue application increased the concentration of microbial biomass and enzyme activity via increasing labile C and nutrients contents regardless of soil management history.

A field trial was conducted at Burdekin, Australia, to investigate the effects of different decompaction field managements on soil nutrient cycling, associated biological activities, and sugarcane yield. This experiment included four treatments comprising: control (CK, without mill-mud), mill-mud shallow furrow (MS), deep trench without mill-mud (DT), and deep trench mill-mud application (MD). Overall, the application of mill-mud significantly improved soil organic matter and nutrient content. Surface mill-mud application increased the concentration of soil Colwell P six-fold in comparison to the control. Deep trench application of mill-mud increased concentrations of hot water extractable organic C by 30% - 70% and hot water extractable total N by 30% - 90% at the application depth (ca. 20 cm depth). Soil microbial biomass C and N were also higher in mill-mud applied layers (ca. 20 cm depth). As expected, in comparison to the control, mill-mud applied treatments increased plant cane yield by 7% (MS treatment) and 14% (MD treatment). The deep trench without mill-mud (DT) practice also increased the plant cane yield by 11% compared to the control, which may be due

to the release of native organic C and improved soil health. Deep trench combined with the mill-mud application (MD) increased the supply of organic C and N and nutrients to the microbial community within the entire soil profile, enhanced nutrient cycling processes and soil health for sugarcane growth, and thus increased sugarcane productivity.

In the presented thesis, soil microbial responses to two main soil constraints (compaction and drought) under different soil textures, vegetation type and field management history were investigated. Based on the presented result, soil texture and WFPS played vital roles in regulating microbial responses to compaction and drought. The finer soil texture provided a better and stable microbial habitat and WFPS governed the diffusion of soil labile C and nutrients for microbial growth. In addition, field managements such as organic matter amendment and/or ploughing would stimulate microbial responses to soil constraints as organic amendment would directly increase soil organic matter content and ploughing would adjust soil WFPS. Also, these two factors are intensively linked to crop yield as increased sugarcane yield was found in organic matter applied and ploughed treatments, particularly in ploughed only treatment. Additionally, the presented study found that the soil microbial community had higher tolerance to soil disturbances under improved field management history than conventional management history, which could be attributed to the complexity of microbial community structure established through long term improved field management. However, microbial community structure and key functional gene analysis are needed to confirm this statement. In conclusion, application of molecular biology approaches would further increase the potential of using microbial responses to soil constraints as a measure of soil health and generate a comprehensive index in monitoring soil health in the long run.

Statement of Originality

Statement of Originality

This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

(Signed) 

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Parts of this thesis that have been submitted to peer-reviewed journals in advance of submission for examination are listed below:

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2. **Liu, X.**, Rezaei Rashti, M., Van.Zwieten, L., Esfandbod, Rose, M.T., Chen, C.R., Field management practices play a vital role in building soil resistance and resilience against compaction stress. (In preparation for Soil Tillage& Research) (Chapter 4)
3. **Liu, X.**, Rezaei Rashti, M., Van.Zwieten, L., Esfandbod, M., Rose, M.T., Chen, C.R., Management history governs soil microbial responses to drought in grain cropping systems. (In preparation for Soil Tillage& Research) (Chapter 5)
4. **Liu, X.**, Milla, R., Granshaw, T., Van.Zwieten, L., Rezaei Rashti, M., Esfandbod, M., Chen, C.R., Responses of soil nutrients and microbial activity to different mill-mud applications in a compaction-affected sugarcane field. (Submitted to Soil Research) (Chapter 6).

Chapter 1 Introduction

1.1 Current soil constraints in Australia

Development of Australian agriculture has a relatively short history in comparison to major agricultural countries over the world (Larder et al., 2018). Aborigines commonly adopted 'fire-stick' farming approach to survive. This farming practice help aborigines in signalling, clearing the ground, hunting, regeneration of plant and entertaining (Bliege Bird et al., 2008; Jones, 2012). This practice might have improved soil fertility by regenerating nutrients and removing soil 'diseases' via burning at that time when chemical fertilisers were not available. However, burning might lead to soil erosion and nutrient loss when the rain came immediately after fire. After European settlers arrived in Australia in 1788, agricultural lands slowly expanded and reached 2 million hectares at the end of 1900. In the following years, the area of agricultural lands has significantly increased to 23.5 million hectares, due to the developments in farming technology and policy changes (Bellotti and Rochecouste, 2014; Larder et al., 2018). Gradually, Australia becomes an agricultural country which mainly exports agricultural products (wheat, barley, sugar, beef and wine) to other countries (Bellotti and Rochecouste, 2014).

Australia is an ancient continent with highly weathered and infertile soils (Smith and Morton, 1990). Drought is a common environmental issue in Australian agriculture, and many land management strategies are currently developed to reduce the impacts caused by low and irregular rainfall. Thus, conservation agriculture is adopted by a large number of Australian farmers to improve soil water holding capacity and water use efficiency. In addition to drought, soil acidification due to inappropriate agricultural

managements, such as over application of ammonia-based fertilizers, becomes another Australian land degradation issue (Moody and Aitken, 1997). Moreover, salinization which is caused by accumulation of water-soluble salt in soil profile affected 30% of Australian lands and more than 16% of Australian agricultural soils suffering from salinization (Rengasamy, 2006). Additionally, compaction is another soil constraint which is induced by heavy machinery and caused severe crop yield decline in most of Australian agricultural lands (Ghadim et al., 1991). Most importantly, carbon decline has been observed country-wide in Australia. For example, soil organic C stock reduced by 12.5% from 1690 till now in New South Wales, Australia (Gray et al., 2016).

1.1.1 Soil drought

Drought happens globally but its impact can vary significantly due to local climate and environmental conditions differences regardless of precipitation or temperature patterns. The World Economic Forum that reported \$ 6–8 billion cost per year worldwide due to drought caused agricultural and related business losses (Botterill and Cockfield, 2013). In Australia, these socioeconomic impacts of drought are particularly tremendous. It is reported that Australia is particularly prone to drought (Khan and Gilani, 2021). By the year 2070, it is predicted that Australia may face 40% increase in drought events in the eastern parts and the sky-high greenhouse gas emission scenario would be observed (Quiggin, 2007). For example, there was a severe drought event in 2002 in Australia which resulted in the least productivity of crops and the country suffered a huge economic loss. 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 significant socioeconomic and environmental impacts (van Dijk et al., 2013). To be

specific, Murray–Darling Basin had its capacity fallen to 17% during this drought and it was not recovered to its optimum condition until the end of 2010 (Khan and Gilani, 2021).

1.1.2 Soil acidification

Soil acidification is a natural process. In natural ecosystems, soil gradually becomes more acidic with time which means older and more weathered soils were usually more acidic than younger soils. Nitrate leaching is also considered as a major cause of soil acidification in all ecosystems (Huang et al., 2014). Overused urea can also reduce soil pH and cause acidification. It is reported that application of mineral N fertilisers has globally reduced the pH of agricultural lands by 0.26 values (Tian and Niu, 2015). In addition, crop yield will be decreased by soil acidification as high aluminium concentrations in the acidified surface soil which therefore an inability of the plant to access to water and nutrients when soil dries out (McLay et al., 1994).

Australia land severely suffers from soil acidification. Within the agricultural areas of Australia, it is estimated that between 11 and 21 million hectares of agricultural lands had strongly acidic topsoil (pH 4.3 – 4.8), and from 1 to 3 million hectares are extremely acidic (pH less than 4.3). A much larger area of land (25 to 37 million hectares) estimated to have moderately acidic topsoil (pH 4.8 – 5.5). The largest area of strongly acidic soils (pH 4.3 – 4.8) existed in New South Wales (5 to 7 million hectares), Victoria (4 to 5 million hectares) and Western Australia (1 to 7 million hectares). The largest area of moderately acidic soils (pH 4.8 – 5.5) located in Western Australia (7 to 19 million hectares) and New South Wales (11 to 13 million hectares) and to a lesser extent Victoria (2 to 3 million hectares) (National Land and Water Resources Audit, 2001).

1.1.3 Soil salinization

Soil salinization is a process of accumulation of water-soluble salt in soil profile to level that impacts agriculture production, environmental health and economical welfare (Rengasamy, 2016). There are three main types of salinity which classified by the sources of salts including groundwater related/unrelated salinization and irrigation related salinization (Rengasamy, 2006). Government agencies and the communities including farmer groups in Australia are concerned about the impact of salinity on the value of land and water resources as excess concentration of soluble salt in soil profile would damage soil structure, agricultural productivity, and environmental health. The major attention for salinity in Australia is on irrigation-induced salinity in the Murray Darling Basin and dry-land salinity associated with shallow groundwater, particularly in Western Australia (Rengasamy et al., 2003), as the National Land and Water Resources Audit (2001) estimated that approximately more than a half million square kilometres of Australia's agricultural and pastoral zone have a high potential for developing salinity through shallow water tables.

1.1.4 Soil compaction

The soil compaction can be defined as the process by which the soil grains are rearranged to decrease air-filled space and increase soil bulk density (Committee and America, 2008). Soil compaction involves the changes in physical properties of the soil and these altered physical parameters further influence soil chemical properties, soil microbial properties and plant growth (Nawaz et al., 2013). Soil compaction is a problem that can be observed globally: 68 million hectares of land are estimated to be compacted due to vehicular traffic alone (Hamza and Anderson, 2005). Due to use of heavy machineries, the soil gets compacted, and negative effects may occur depending on the local situation like climate, soil texture, and vegetation type. It is reported that

soil compaction has noticeable negative effects on root growth and crop production (Bakker and Barker, 1998). In Australia, the decreased agricultural production due to soil compaction is approximately AUD 850 million per year (Walsh, 2002). Soil compaction is thus a severe environmental issue and a form of land degradation of which the negative effects may take years to fade away. In addition, subsoil compaction is especially hard to observe from the surface and may accumulate over subsequent growing seasons before critical values are reached, and the effects can be observed.

1.1.5 Soil organic carbon decline

It has been pointed out that soil organic matter (SOM) includes all living (e.g., plant, soil fauna, and microbial communities) and non-living organic material (e.g., fresh residues, simple monomeric compounds, and irregular polymeric compounds) in the soil (Baldock and Skjemstad, 2000). Total soil organic carbon (SOC) stocks in 1990 for the Australian continent were estimated at 19 Pg for the top 30 cm layer (Grace et al., 2006) with a natural flux of ~ 700 Tg CO₂ exchanged between the soil and atmosphere every year. In subtropical regions of New South Wales and Queensland, clay-rich vertisols are the dominant soil type for cropping, representing ~ 2.5 M ha. These soils had moderately high native SOC stocks (~ 70 -100 Mg C ha⁻¹) but tended to lose that C rapidly upon cultivation. Summarizing much of the long-term field trial data in this region, a study has concluded that changes in soil C inputs are the main driving factor for either depletion or sequestration of soil C (Grace et al., 1998). By soil organic matter deficiency, Australian soil is suffering from nutrient depletion, increased bulk density, decreased soil aggregation (silt and clay) stability, decreased microbial diversity and increased greenhouse gas (GHG) emission (Dalal and Chan, 2001).

1.2 Soil as a living system: concept, basic properties, and main functionalities

Soil has been defined differently according to the different contexts. From Dokuchaev (1879) to USDA (United States Department of Agriculture) soil classification, the concept of soil evolved over time and was defined as a natural body constituted by solids, liquid, and gases which developed from parent material to support and maintain its ecological functionalities. Under natural processes, all three phases (solid, liquid, and gas) are intimately mixed with all organic and inorganic matters. The pores which are aggregated by transforming and mixing during natural processes are not only accommodated air and water but also provide great habitat to soil microbial communities (Voroney and Heck, 2015) and establish 'hot spots' for soil microorganisms involved in nutrients cycling (Kuzyakov and Blagodatskaya, 2015). Thus, soil always includes five major components — mineral matter, organic matter, water, air and living beings. Soils have the capacity to support various ecosystems on the earth by their various functionalities (Nannipieri et al., 2017). Soils have many crucial ecological services such as providing food and biomass; storing, filtering, and transforming water, carbon, and other nutrients; providing habitats and gene pools; regulating climate; and providing a physical and cultural environment to the mankind (Tzivilivakis et al., 2005). This thesis is going to focus on soil functions as a medium for plant growth, a regulator of water supply, and a recycler of nutrients.

1.2.1 Soil is a medium for plant growth.

As a medium for plant growth, soil can support plant growth through its physical, chemical and biological properties (Passioura, 2002). Physical properties of soil include colour, texture, structure, porosity, density, consistency, temperature, and aeration

(Letey, 1958). Colour of soils indicate important properties such as organic matter content, moisture, and redox conditions, although this indicator is varied widely based on different soil backgrounds (Schwertmann, 1993). Soil texture, structure, porosity, density, and consistency are related to soil particles ratio and their arrangement (Dexter, 2004). In addition, soil is also widely accepted as a chemical entity (Clark et al., 1998). Moreover, many organisms live in soil and perform various functions for their growth and reproduction. Due to these functions of soil organisms, soils behave like a living system. Soil microorganisms play a critical role in maintaining soil nutrients cycling, which can further improve crop yield.

Soil structure is considered a key factor in supporting soil ecological services as soil structures is important for plants to establish root system and buffer environmental disturbance (Bronick and Lal, 2005). Suitable soil structure and high aggregate stability play a vital role in improving soil fertility, increasing crop productivity, increasing soil porosity and decreasing soil erodibility (Bronick and Lal 2005). In addition, soil structure is highly related to pore size, the number of pores, and distribution of pores and total porosity of the soil (Rabot et al., 2018) or 'the combination of different types of pores' (Pagliai and Vignozzi, 2002). Thus, soil structure governs soil water retention and movement, including infiltration, percolation, permeability, leaching, and drainage (Rawls et al., 1991). For example, rhizosphere soils are drier than surrounding soils under natural conditions due to uptake of water by plant roots and transpiration by plant leaves (Leung et al., 2015). A better soil structure would improve water movement in the root zone, which consequently improves plant water uptake. Similarly, nutrient availability in soil and nutrient absorption capacity of roots is influenced largely by soil structure.

Soil structure influences the habitation, movement, and activity of soil organisms (Schimel and Schaeffer, 2012). Soil organisms were usually the most active in the surface soil zone of 0–15 cm because this zone had accumulated organic residues and available nutrients. Soil structural conditions also influence microbial processes like organic matter decomposition, mineralization, nitrification, and nitrogen fixation. Soil microbes can photosynthesize (Nagarajan and Pakrasi, 2001), respire, and reproduce, and by these processes, soil microbes played a key role in maintaining soil nutrients cycling and further improving crop yield. Although soil biota constitutes a very small portion of the total soil volume, it significantly impacts soil properties and related soil ecological services (Jones et al., 2014).

1.2.2 Soils is a regulator of water supplies.

Earth hydrologic cycle used in describing water continuously moves among the earth's surface including atmosphere, lithosphere, hydrosphere, and biosphere (Ramanathan et al., 2001). Water entered the soil by infiltration, and the infiltrated water move down to the water table by percolation (Carsel and Parrish, 1988). The infiltrated water may store in the soil as soil water content or may be leached to deeper layers and enter the groundwater at the end (Rawls et al., 1982). A part of the water that infiltrates get evaporated, some is absorbed and transpired by plants, some is retained in soil pores and on soil particles, and the remaining water percolates. Although a certain amount of water stored in soil, but still a large amount of water filtered through soil profile. During the period of water remaining in soil, DOM (dissolved organic matter) is decomposed, the pollutants are removed by reaction with soil aggregates or exchanged at the surface of soil particles, and the purified water would enter the groundwater (Schulte et al., 2018). Therefore, soil is a natural filter. Soil water availability is not only governed by soil properties, abut also relies on local climates which determined annual rainfall and

local humidity. In addition, vegetation factors like root depth and leaf area also indirectly affect soil water availability. Such soil water availability has been proved to be intensively related to soil nutrients cycling (Dougill et al., 1998; Kaiser and Kalbitz, 2012).

1.2.3 Soil is a recycler of nutrients.

In general, soils are considered as a sink of carbon (C), nitrogen (N) and phosphorus (P). The C and N are added to soils from the atmosphere through physical and biological fixation mechanisms. The C and N are circulated via the atmosphere, pedosphere, and biosphere systems in the terrestrial ecosystem. A large quantity of C and N are fixed from the atmosphere, and substantial amounts of C, N, P, and S are added to soils as organic residues (Liu et al., 2018). They are chemically and biologically transformed in soil, a large proportion is adsorbed by plants and again added to soils after their death and decomposed by soil microorganisms. A large proportion of these elements are removed by crop harvest and incorporated back to soil as fertilisers. In this way, these elements established ecological cycles. In the end, a large amount of them is transferred into other ecosystems, such as lakes, streams, and oceans, where they contribute to the greater global cycling (Sylvia et al., 2005).

Primary producers including plants and autotrophic microorganisms fix atmospheric CO₂ by the process of photosynthesis. A large quantity of organic C materials is added to soils by the decomposition of dead body, excreta, and exudates of organisms. These residues are deposited on the surface by litterfall, as crop residues, and at variable depths by root death and exudation. The organic matter like litter falls or dead organisms are colonized by soil heterotrophic microorganisms, which derive energy for growth from the decomposition of complex organic compounds. During decomposition,

essential elements are converted from organic compounds into simple inorganic forms which called mineralization. For example, organically combined N, P, and S are decomposed into NH_4^+ , H_2PO_4^- , and SO_4^{2-} ions, and a large amount of C is emitted to the atmosphere as CO_2 . The C in soil substrates used by the microorganisms is incorporated into microbial cell organs or built-up microbial biomass, together with a variable proportion of other elements such as N, P, and S. This intake makes these elements unavailable for plant growth which is called immobilization (Schmidt et al., 1999). During the decomposition process of organic residues, recalcitrant substances accumulate, and soil microbes synthesize some new complex organic compounds. Complexation of these substances with soil mineral matter produces humus. Thus, the important processes in the soil nutrients cycle are immobilization, mineralization, and decomposition. In addition, humification is a concomitant process of decomposition and re-synthesis by which stabilized humic materials are produced in the soil. Therefore, soil plays as a recycler in nutrients cycling.

1.3 Brief review of soil ecology

Ecology has been considered a study of the natural environment, including the relations of organisms to one another and to their surroundings, and this concept was first given by Ernst Haeckel, a biologist from German, in 1869 (Odum, 1977). The increase in public attention had a profound effect on academic ecology. Although ecology developed based on the development of biology, it has emerged from biology as an essentially new and multi-subject generated discipline that links physiochemical and biological processes and builds a bridge between the natural sciences and the social sciences (Odum, 1977). With the booming population, modern society started to concern environmental problems like environmental pollution, food security and biological diversity. After ecology became a fierce topic globally, the study of how

individual organisms and species interface and use resources intensified while researchers expanded the scope of ecology.

1.3.1 Soil health, an ecological term

Soil was considered the primary source to support crop growth by providing ‘plant food’. With the discovery of soil nutrients and soil functionalities such as water holding capacities, soil's main purposes have been considered ‘to provide a better yield of the crop’. Also, with the industrial revolution and the development of chemical fertilizers, soil fertility and productivity became vital components to decide if the soil is healthy. To a large extent, soil fertility and productivity were considered equal to soil health at that time. However, it has been pointed out in later decades that soil fertility or its productivity is just one of the critical functionalities of soil.

Following the overuse of chemical fertilizers and adoption of inappropriate land management practices, land degradation became a serious environmental issue worldwide. Societies and researchers started to pay more attention to soil resources and sustainable development. Meanwhile, with the boost of technologies, soil had been studied from different aspects, from the physical to the biological level and from the macroscopical level to the gene level. Currently, the concept of soil health is more focused on sustainable development without compromising crop yields. Also, it should be noted that the term of soil quality and soil health has recently been used interchangeably by soil scientists (Anderson and Ingram, 1994).

Several definitions of soil health have been proposed during the past decades. The term ‘soil quality’ described soil’s agricultural productivity or fertility. It was pointed out that soil quality was limited to productivity and indicated soil interactions with the surrounding environment, including the implications for human and animal health in

the 1990s (Acton and Gregorich, 1995; Doran and Parkin, 1994; Doran and Safley, 1997; Pankhurst and Doube, 1997; Singer and Sojka, 2002).

Soil was regarded non-renewable resource as soil regeneration via weathering processes requires a relatively long geological time (Huber et al., 2001). Decline of soil health which affects human, animal and plant health (Singer and Sojka, 2002), had become a rising global concern. The soil itself serves as an environmental filter for removing solid and gaseous pollutants from air and water due to its capacity in buffering contaminants (Parr et al., 1992).

Soil is dominated by a solid phase consisted of particles of different sizes surrounded by water and gases, the amount and composition of which fluctuate markedly according to local environments. Water is ordinarily discontinuous, except when the soil is water-saturated and for those pore space without water would be filled by air (Stotzky, 1997). There is an interchange of chemical compounds among solid, liquid, and gaseous phases governed by physicochemical and biological activities (Doran and Parkin, 1994). Maintaining this balance is of great importance to soil health which further proved modern soil scientists to devote more effort to investigate soil health from an ecological perspective.

Most soil biological activity is observed in the topsoil. Despite their small volume in soil, microorganisms are essential in nutrient (nitrogen, sulfur, and phosphorus) turnover and the decomposition of organic residues (Pankhurst, Doube, et al. 1997). In addition, organic residues are, converted to microbial biomass or mineralized to CO₂, H₂O, mineral N, P, and other nutrients (Bloem and Breure, 2003). Mineral nutrients immobilized in microbial biomass are subsequently released when microbes are taken by microbivores such as protozoa and nematodes (Bloem and Breure, 2003).

Additionally, soil microbes are further related to the transformation and degradation of waste and organic compounds (Torstensson et al., 1998). In addition, soil microbe had impacts on nutrients cycling and affects the physical properties of soil such as water holding capacity, infiltration rate (Elliott et al., 1996).

Based on the advantages explained above, the status and dynamics of soil microbes could provide comprehensive knowledge about the overall soil health and could potentially serve as a soil health indicator. Soil microbe responds rapidly to environmental changes; therefore, they can quickly adapt to new environmental conditions. This fast response and adaptation potentially allow analyses of soil microbial communities to be discriminating in soil health assessment, and changes in microbial community structure and activities may therefore function as a great indicator of soil health shifting (Pankhurst et al., 1995) as the turnover rate of microbial biomass is much faster than the turnover of total soil organic matter (Carter et al., 1997).

1.3.2 Soil health and new soil health indicators

The soil health concept attempts to qualify how the soil functions, especially in terms of the resistance, resilience, and recovery toward environmental stress by quantifying soil characteristics. The definition of soil health has been given as “the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation” by the Soil Science Society of America (Doran and Parkin, 1994).

Soil health had two vital components: inherent soil properties and dynamic soil properties (Gregorich et al., 1994; Karlen et al., 2003). Inherent soil properties are defined by both soil formation processes and underlying bedrock. Meanwhile, dynamic

soil properties refer to how the soil properties respond to environmental stresses and field management. As inherent soil nature cannot be changed frequently, thus soil health generally refers to the dynamic of soil properties. Although inherent soil nature cannot be altered easily, it played an essential role in the basic functionality of soil. To be specific, all dynamic soil health indicators must be compared only to soils based on similar inherent soil characteristics. Soil health and soil quality can be considered interchangeably (Laishram et al., 2012) when both two terms only refer to dynamic soil properties, including soil resistance, resilience, and recovery toward environmental stress.

Soil quality is an important concept to farmers, and its importance can be dated back to the first appearance of agriculture in the history of human beings. The soil has been descriptively called “dark”, “light” and “prosperous” by land users or landowners, and centuries-long debates of soil quality and its concept as the measurable objective via scientific approaches has increased dramatically in past few decades (Karlen et al., 2003; Warkentin, 1995). With the development of modern technology, the Soil Science Society of America, the American Society of Agronomy, and the North Central Region Committee on Soil Organic Matter, tried to define soil quality and connect the different soil aspects to the soil quality in 1994 (Doran and Parkin 1994). As soil quality can also be applied to various contexts or different ecosystems, the soil quality concept has been welcomed by a variety of disciplines related to soil science and its applications have been boosted in the past few decades (Doran and Zeiss, 2000).

Improving soil sustainability via improving soil health is a generally accepted idea for all land users and landowners (Delgado et al., 2003). The boundaries include vegetation type (or land use), soil type and local climates are necessary to determine primal soil

functionality before getting into a conclusion of soil health conditions. It had been suggested that soil qualities are normally evaluated by external factors such as land management, local climate, and environmental interactions (Doran and Parkin, 2015). Some previous researchers also suggested that soil health indicators have to be specific for soil land use and location (Andrews et al., 2004). Soil functions ranged from providing ecosystem services to supporting crop yield (Warkentin, 1995), and those functions may be overlapped under a specific context. There is not much research on soil health indicators that considered the crop yield, production sustainability, and environment system dynamics simultaneously.

The final achievement of a soil health assessment should include fundamental soil property, and soil responses to regional main soil constrains under certain boundaries (such as local climate, land uses and soil texture) and functionalities. Therefore, a useful soil health evaluation should include a preliminary report and a guide of sustainable field management (Doran and Zeiss 2000). The soil under healthy conditions would have increased yield and decreased external nutrients requirements to retain yield at the same time. Meanwhile, because of improved soil structure and other soil basic properties such as greater water holding capacity (WHC) and higher cation exchange capacity (CEC), healthier soil always had a stronger resistance toward environmental impacts, a better resilience toward environmental stress, and a faster recovery rate when environmental fluctuates occur. It has been reported that healthier soil had more efficient nutrients cycling via soil microbial community, resulting in a decreased demand for external fertilizer (Gregorich et al. 1994). Nevertheless, different land users or landowners may have varied needs and demands, thus soil health should only be compared within the same boundary and evaluated under the same soil functionalities.

Soil health indicators based on soil properties were selected to reveal the current soil quality and fertility condition and project potential crop yield connected to crop production with sustainable field managements (Doran and Parkin, 2015). The indicators of soil health evaluation were suggested to be interpreted and afforded easily by growers (Sarrantonio et al., 2015). Also, it has been reported that the best soil health indicators should be sensitive to soil functionality fluctuation (Andrews et al., 2004). It is challenging to find that soil health indicators can respond rapidly before yield has been affected as soil buffer capacity can delay the side-effects of soil management practices between two crop rotations. Meanwhile, there is always a ‘profit dilemma’ to stop landowner to accept soil health evaluation. If profitability is not considered during soil health evaluation, then the evaluation equals based on the assumption that farmers would like to compromise the cost of soil profits to soil sustainability, which is not realistic (Wander and Drinkwater, 2000). However, if profitability and productivity are the only outcomes of soil health evaluation, then soil health evaluation will simply become soil productivity evaluation, which distorted the principle of soil health evaluation (Wander and Drinkwater, 2000). Therefore, it is necessary and urgent to establish a new robust and cost-effective soil health indicator.

Current widely used mainstream soil health indicators can generally be divided into three parts: physical, chemical, and biological properties. Among all these soil health indicators, a minimum data set needs to be used in soil health assessment which means soil parameters involved in soil health evaluation need to be representative, comparable, and affordable. However, current soil health indicators have the following limitations:

- 1) Interpretation barrier; there were many soil chemical, physical and biological parameters suggested as soil health indicators, but the interpretation of all these parameters could be difficult, particularly for the farmers and the end-users who may

not have a soil science background; 2) Not robust; soil type, vegetation cover, and climate conditions varied significantly across different agricultural regions, and soil health indicators developed in one region (system or soil type, vegetation, or climate) may not be suitable for another region. Unfortunately, there is no soil health indicator that can perform perfectly to conclude soil health conditions, because of the diversity of soil types, vegetation types, and local climates. Therefore, a new robust soil health indicator is urgently needed to be developed to fix this problem; 3) Data redundancy. Data redundancy generally caused by overlapped results produced from similar but different levels of measurement. To be specific, advanced technologies (i.e., sequencing technique) were adopted in soil science to replace primary soil measurement (i.e., microbial biomass C: N ratio) in indicating microbial community structure. Indeed, soil DNA and 16sRNA data have been reported as excellent indicators to represent the microbial community constitution (Arias et al., 2005). Also, NMR (Nuclear magnetic resonance) fingerprint technology can trace C and N cycling in soil. However, these results may cause data redundancy to end users as simpler approaches would do the same thing when higher data resolution is not the most urgent requirement. Also, data redundancy came after a tremendous cost. These extra costs finally became a massive burden to farmers and decrease the willingness of farmers to monitor their land health condition.

1.3.3 New robust soil health indicator

A robust soil health indicator should be developed to provide more information than a simple quantitative or descriptive report, which includes some parameters representing current soil status. However, it should represent the functional status of the whole soil living system, as there is only limited knowledge in developing soil health indicators available based on microbial responses towards environmental stresses. To be specific,

better understanding of the responses of soil to field managements and environmental perturbations is essential to develop a new robust soil health indicator.

The ecological terms resistance and resilience are originally used to describe the stability of an ecosystem toward an environmental disturbance. It has been reported that resistance and resilience were applied to microbial communities (Allison and Martiny, 2008). However, it is difficult to measure soil resilience and resistance directly, and the authors in this thesis measured the response of soil CO₂ respiration to environment stresses to represent soil resistance and resilience. Allison and Martiny (2008) concluded that soil resilience is the rate at which soil CO₂ respiration returns to its original soil respiration level after being disturbed, while soil resistance as the degree of soil CO₂ respiration remains unchanged when the environmental disturbance occurred. Although metabolic quotient ($q\text{CO}_2$) cannot distinguish between effects of disturbance (pulse impact) and environmental stress (press impact), it still a valuable index in measuring how efficiently the soil microbial biomass is utilizing C resources, and the degree of substrate limitation for the microbial community in soil (Wardle and Ghani, 1995). In this thesis, these parameters were calculated by the fluctuation of soil microbial respiration under the impact of environmental disturbances. Overall, the healthier soil should have a higher resistance and resilience to certain soil constrains.

1.3.4 Resistance of microbial community

Resistance to compositional change under disturbances was enhanced if a microbial community contains many versatile physiologies or physiological plasticity (Evans and Hofmann, 2012). Bacteria have been proved to adopt environmental change rapidly by expressing a range of metabolic capabilities and therefore the existing community can adjust their structure to adopt new conditions through gene expression by individual

cells (Meyer et al., 2004). Adaptive gene expression referred to the natural selection process on gene expression (Whitehead and Crawford, 2006), and this phenomenon has been observed and confirmed in an experimental model on yeast evolution (Ferea et al., 1999). In addition, the ability to use many different carbon and energy sources might be a typical pattern in natural microbial communities (Eiler, 2006), which would further provide microbial stability to adopt environmental changes. As the microbial community is a crucial driver of soil health in the proposed soil structure, microbial community resistance is assumed to equal the resistance of the soil.

1.3.5 Resilience of microbial community

Although microbial structure has been proved sensitive to environmental disturbances, the microbial community might still be resilient and respond rapidly return to its composition before disturbance. There are several features of soil microbe suggested that resilience could be common. Firstly, microorganisms generally have fast growth rates; thus, they had the potential to recover rapidly when they were impaired by environmental disturbances. Secondly, microbes tended to have higher physiological flexibility. For example, purple non-sulfur bacteria were phototrophs under anaerobic conditions and became heterotrophs under aerobic conditions (Allison and Martiny, 2008). Finally, if self-adaptation is not an option, then the rapid evolution through mutations or horizontal gene exchange could allow microbial taxa to adapt to new environmental conditions and recover from disturbance. All these facts anticipated that abundance is reduced by a disturbance, but some microbial taxa may benefit from the new conditions and increase in abundance. This indicates that resilience would help soil microbial community to bounce back to its pre-disturbed situation. In the present study, soil respiration and metabolic quotient ($q\text{CO}_2$) has been measured and calculated to

generate soil resistance and resilience index instead of measuring microbial community with high-cost approaches.

1.4 Research gap, question, and hypothesis

Soil health decline is a severe consequence of climate change, overused agricultural machinery, and intensive application of chemical fertilizers, world widely. However, currently used soil health indicators in the soil health monitoring framework were relatively latency, insensitive, and expensive. Therefore, it is necessary to monitor soil health conditions with a cheap, easy-to-understand, and robust index. Traditionally, soil physical and chemical properties are widely used in soil monitoring processes, however, soil microbial properties should be adopted into the soil health assessment as soil microbial community are sensitive to environmental stresses and respond rapidly to environmental disturbance in comparison to soil physicochemical properties. Although the impacts of environmental disturbance, such as compaction or drought, on soil health and crop yield is widely studied, how soil microbial communities respond to environmental disturbance remains largely unknown. Therefore, it is essential to generate new knowledge to fill this research gap by investigating on how soil microbial community respond to environmental disturbances and develop a new index to represent soil health conditions.

The main aim of this thesis was to investigate the microbial responses to soil constraints as a measure of soil health in sugarcane and grain systems. The specific objectives of this thesis were to:

a) determine the mechanisms responsible for soils' microbial functional response and thresholds to environmental disturbance (Chapter 3);

b) expand the mechanism of the responses of soil microbial community following soil compaction (or drought) stress into both conventional and improved soil management practices contexts (Chapter 4 and 5);

c) generate soil resistance and resilience index based on the responses of soil microbes to compaction and/or drought (Chapter 4 and 5);

d) enrich the knowledge of soil microbial activity and nutrient pools responses to different practices of removing compaction stress, such as deep trenching mill-mud application and shallow furrow application, in fields context (Chapter 6).

Three incubation experiments and a field trial were designed to test the following hypothesis:

1) Soil biological functions such as respiration and microbial biomass and activity are reliable indices for the assessment of soil health and resilience to compaction and water stresses.

2) Soil with improved management practice history has higher resistance against compaction stress and higher resilience against following removal of compaction stress.

3) The response pattern of soil microbial communities to drought stress is highly related to the applied stress levels rather than the history of field management practices.

4) Mill-mud application and removal of soil compaction stress would increase the size of the microbial community and microbial activity (indicating soil resistance and resilience), which further improves soil health conditions and increases sugarcane yield.

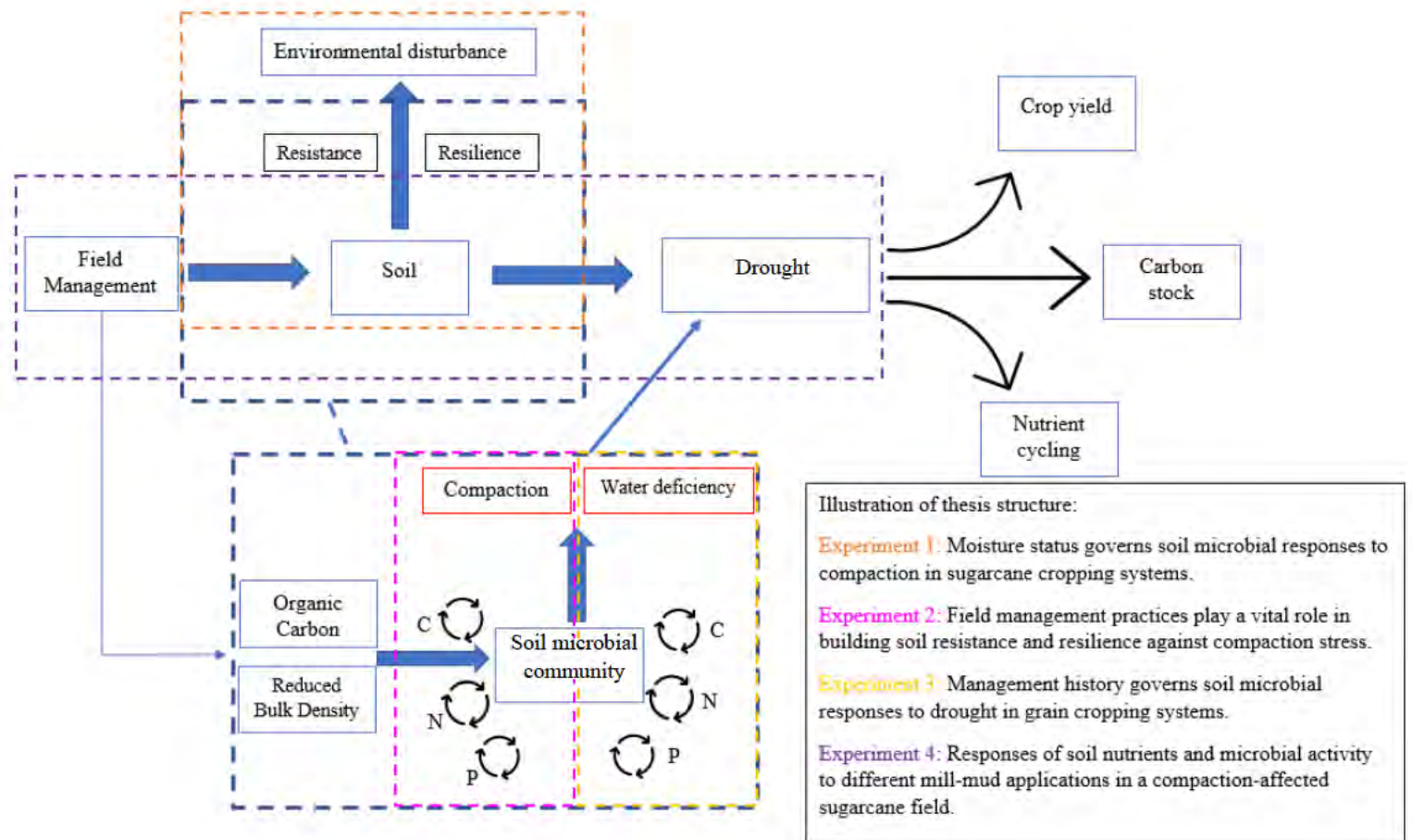


Fig. 1.1 Thesis structure diagram

Chapter 2 Materials and Methods

2.1 Introduction

All result chapters (3, 4, 5 and 6) of this thesis have been prepared as journal papers with specific “material and methods” section. This chapter covers the general materials and methods used in this thesis.

2.2 Experimental sites

In the first experiment (chapter 3), fresh soils with an initial water content of 15% (w/w) were collected from 0-10 cm depth of two sugarcane farms at Rocky Point (sandy soil; 27° 43' S, 153° 17' E) and Ingham (clayey soil; 18° 39' S, 146° 07' E), Queensland, Australia (Fig. 2.1). The mean annual temperature of the region was 29.3 °C for Ingham and 29.4 °C for Rocky Point and mean annual precipitation was 2137.6 mm for Rocky Point and 2084.8 mm for Ingham (Australia Bureau of Meteorology, 2021). Sandy soil was classified as Planosols and clayey soil was classified as Nitisols based on the FAO world reference base (WRB, 2014) and classified as Arenosols and Dermosols based on Australia soil classification (Isbell, 2016). At each site, the soil samples were randomly collected from five locations of the farm within sugarcane growing field and bulked as a composite sample. The sandy soil contained 22% coarse sand, 48% fine sand, 15% silt and 15% clay with an initial pH (1:5 water) of 6.2. The total C (TC) and N (TN) contents of sandy soil were 10.9 g kg⁻¹ and 0.8 g kg⁻¹, respectively. The clayey soil contained 5% coarse sand, 24% fine sand, 26% silt and 45% clay with an initial pH (1:5 water) of 5.7. The total C and N contents of clayey soil were 12.2 g kg⁻¹ and 0.9 g kg⁻¹, respectively.

In the second experiment, the experimental site located in a sugarcane producing area in Foresthome (18°36' S, 146°12' E), North Queensland, Australia (Fig. 2.2). The soil was classified as red Dermosol (Isbell, 2016), which was also classified as Nitisol base on 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 compared to conventional field management. The conventional experimental field block has been cropped with sugarcane (*Saccharum officinarum*, Q240) in the past 30 years and improved management experimental field block has been cropped with sugarcane (*Saccharum officinarum*, Q240) in the past 14 years. The improved management block was managed with minimum tillage, mound planting and legume plantation in comparison to conventional furrow management. The distance between two is less than 1 km.

In the third experiment, 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' S, 117° 38' E), Western Australia in 2019 (Fig. 2.3). At each site, the soil samples were randomly collected from five locations of the farm and bulked as a composite sample. The soil classified as Arenosols in 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 and N 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) used in the improved practice management, while monoculture of wheat (*T. aestivum*) used in the conventional management in the last 5 years before soil sampling.

In the fourth experiment, the trial site was located in a sugarcane producing area, Burdekin (19°30' S, 147°20' E), Queensland, Australia (Fig. 2.4). The soil was a Mesonatric Brown Sodosol (Isbell, 2016), which is also classified as Solonchaks based on FAO world reference base (WRB, 2014). The mean annual temperature was 29.2°C and mean annual precipitation was 741.0 mm (Australia Bureau of Meteorology, 2021). The experimental field has been cropped with sugarcane (*Saccharum officinarum*) for the past 15 years. As a common practice, fine agricultural gypsum was applied to a depth of ~10 cm using the broadcasting method. Gypsum was applied to improve soil structure and calcium (Ca) concentration (5 tonnes hectare⁻¹). Soil at this field site has been affected by compaction due to routine use of heavy machinery (e.g., harvester) for management and harvesting.

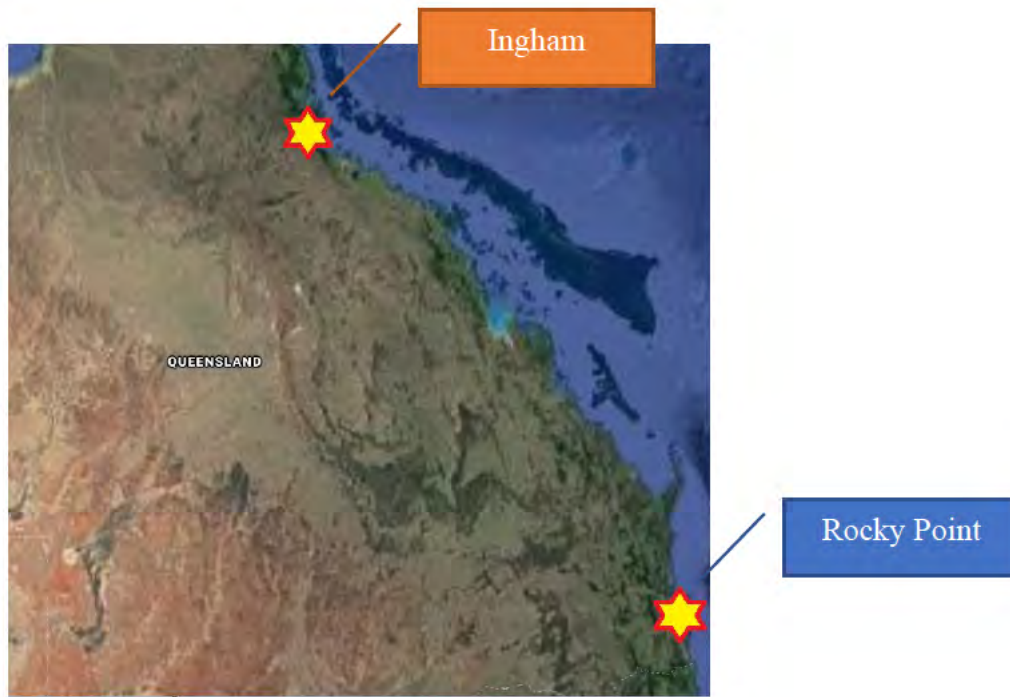


Fig. 2.1 Location of two sugarcane farms in Rocky Point and Ingham, Queensland, Australia.

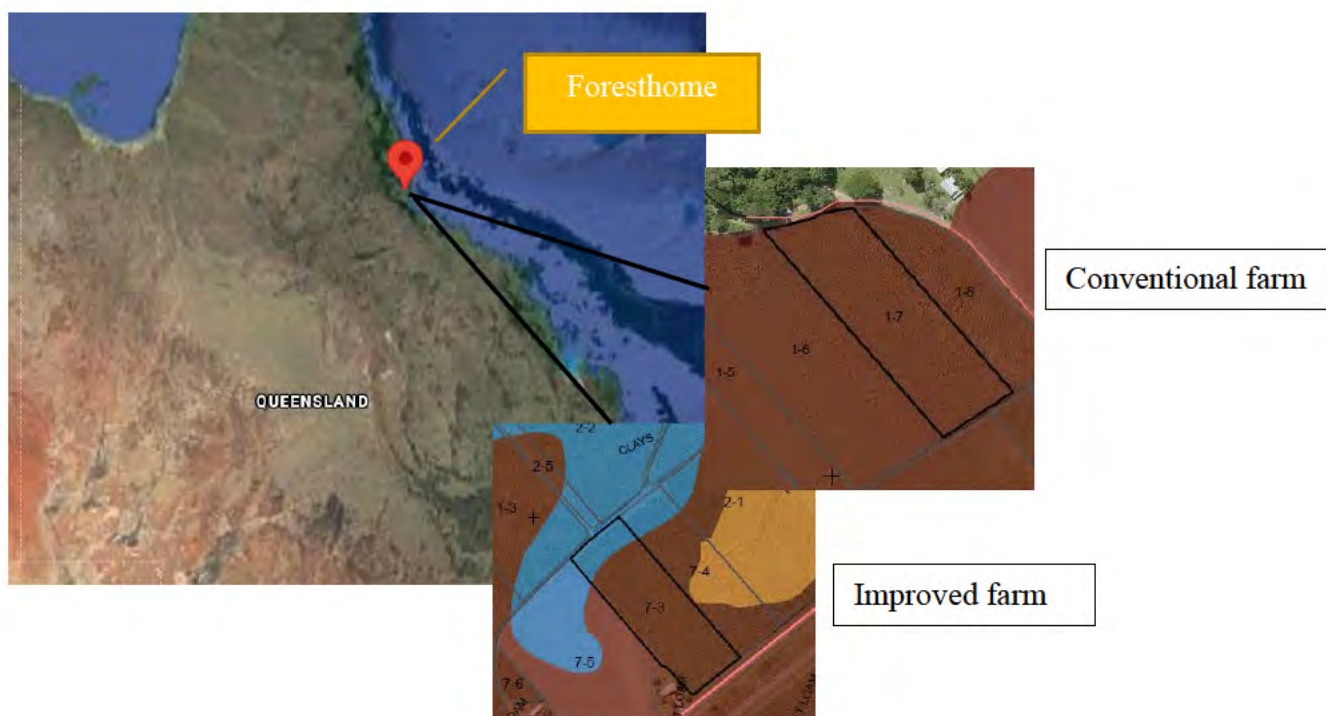


Fig. 2.2 Location of two sugarcane farms in Foresthome, Queensland, Australia.

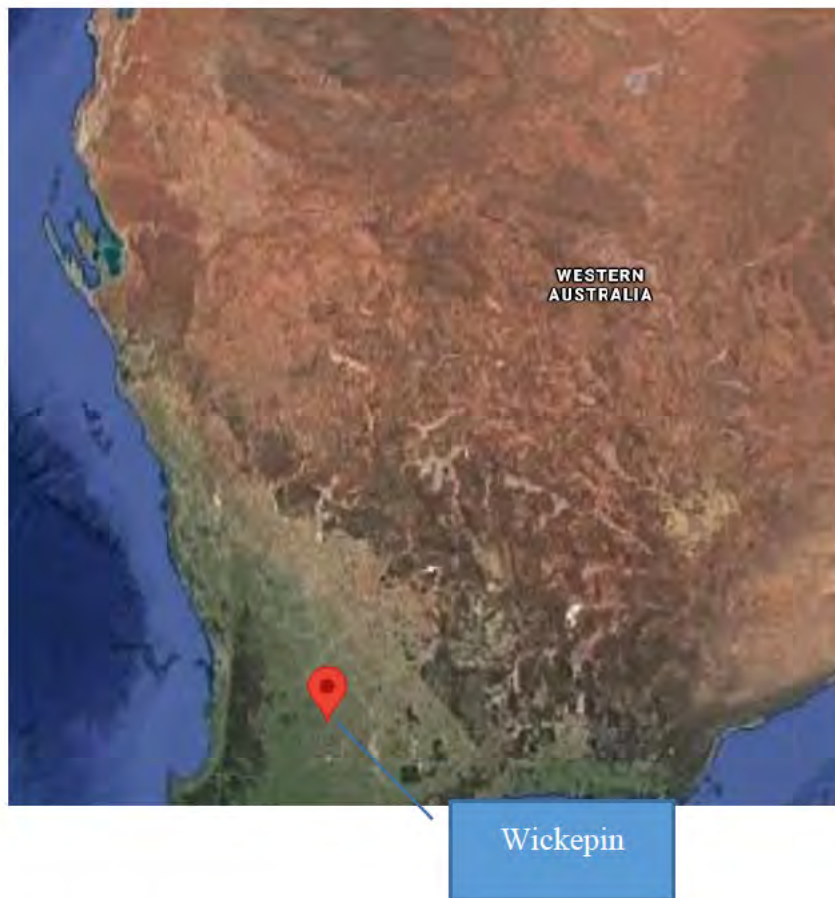


Fig. 2.3 Location of two wheat farms in Wickepin, Western Australia.

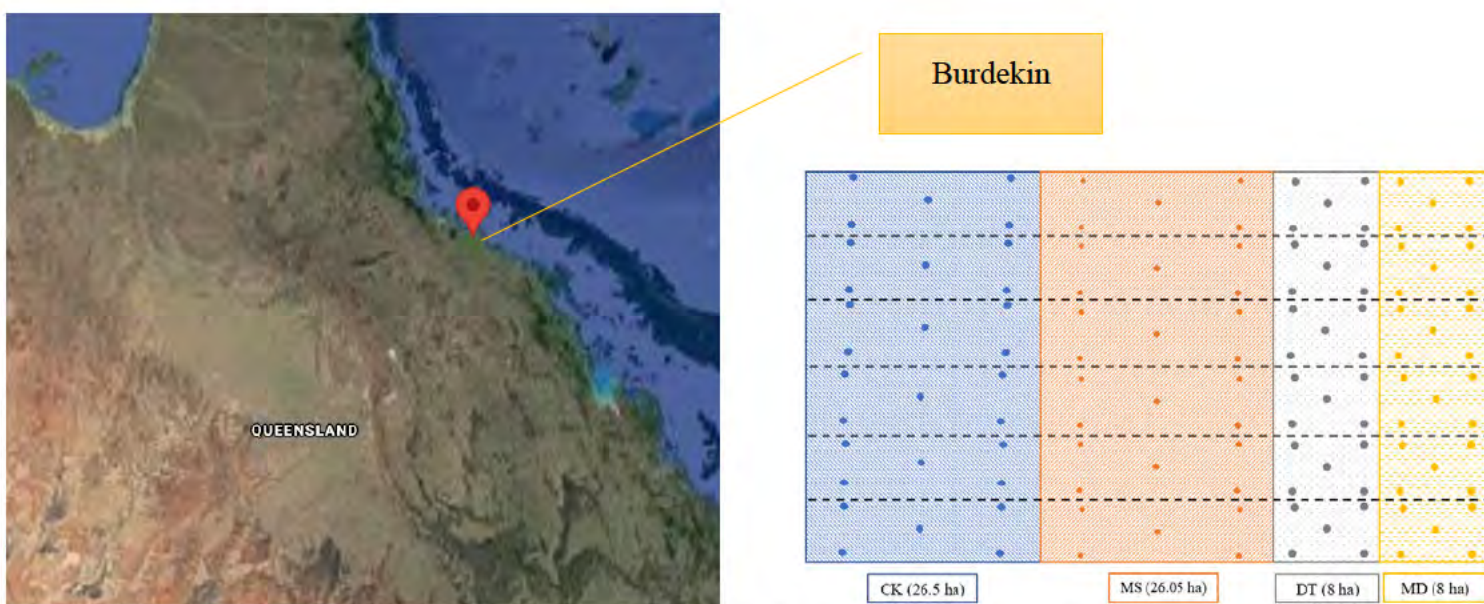


Fig. 2.4 Location of sugarcane farms in Burdekin, Queensland, Australia

2.3 Soil samples processing and analysis

All soil samples collected from experimental sites were well mixed, sieved (<2 mm) and large roots collected before analyses. Samples were divided into air-dried and fresh subsamples. The air-dried samples were finely ground (< 150 µm) before the pH, and soil total C and total N measurements. Fresh samples were kept at 4° C during transportation and storage before the extraction for chemical and biochemical measurements which conducted within one week after sampling to minimize the influence caused by temperature and transportation. Detailed soil sampling plan of each experiment was described in chapters 3, 4, 5, and 6, respectively.

2.3.1 Measurement of soil texture, pH, EC, total C and N

Particle size distribution was conducted using soil hydrometer method developed by Day (1965) and particle size analysis instrument (Maxingsizer 3000, Malvern Panalytical UK). Soil pH (1:5 soil to water ratio) and EC value was measured with a glass electrode method described by (Rayment and Lyons, 2011). Soil total C and N contents were measured by LECO CN analyzer (TruMac NO. 830-300-400; Fig. 2.5).



Fig.2.5 The LECO CN analyzer (TruMac NO. 830-300-400).

2.3.2 Soil mineral N content

The fresh soil samples were extracted with 2 M KCl at a soil to solution ration of 1:5 using an end-to-end shaker for 1 h and filtered with Whatman paper No. 42 (Rayment and Lyons, 2011). The concentrations of NO_3^- -N and NH_4^+ -N in the extracts were measured by a SEAL AA3 Continuous Segmented Flow Analyzer (SEAL Analytical Limited, USA; Fig. 2.6). Total extractable mineral N of the samples was calculated as the sum concentrations of NO_3^- -N and NH_4^+ -N. The results were expressed on an oven-dry basis.



Fig.2.6 SEAL AA3 Continuous Segmented Flow Analyzer (SEAL Analytical Limited, USA).

2.3.3 Microbial biomass C and N and hot water extractable organic C and N

The microbial biomass C and N were determined using the chloroform fumigation-extraction method (Vance et al. 1987) on fresh soil samples. Briefly, fumigated and un-fumigated soils (10 g dry weight equivalent) were extracted by 40ml of 0.5 M K_2SO_4 . Samples were shaken by end-to-end shaker for 30 min and then filtered by Whatman paper No. 42. The microbial biomass C and N were calculated as the differences in soluble organic C and N between chloroform-fumigated and un-fumigated soil samples, after analysis by a SHIMADZU TOC-L (shimazu, Kyoto, Japan) TOCN analyzer (Fig. 2.7).

Hot water extractable organic C (HWEOC) and total N (HWEN) were measured using the method described by Chen et al. (2000). Briefly, 4.0 g (oven-dry equivalent) soil sample was incubated with 20 mL distilled water in a capped test-tube at 70 °C for 18 h. After incubation the test-tubes were shaken on an end-over-end shaker for 5 min and filtered through a Whatman 42 paper (Whatman Ltd., Maidstone, UK), followed by a 0.45- μ m filter membrane. Concentrations of organic C and total extractable N in the filtrate were determined using a SHIMADZU TOC-L (Shimadzu, Kyoto, Japan) TOCN analyzer. The results were expressed on an oven-dry basis.

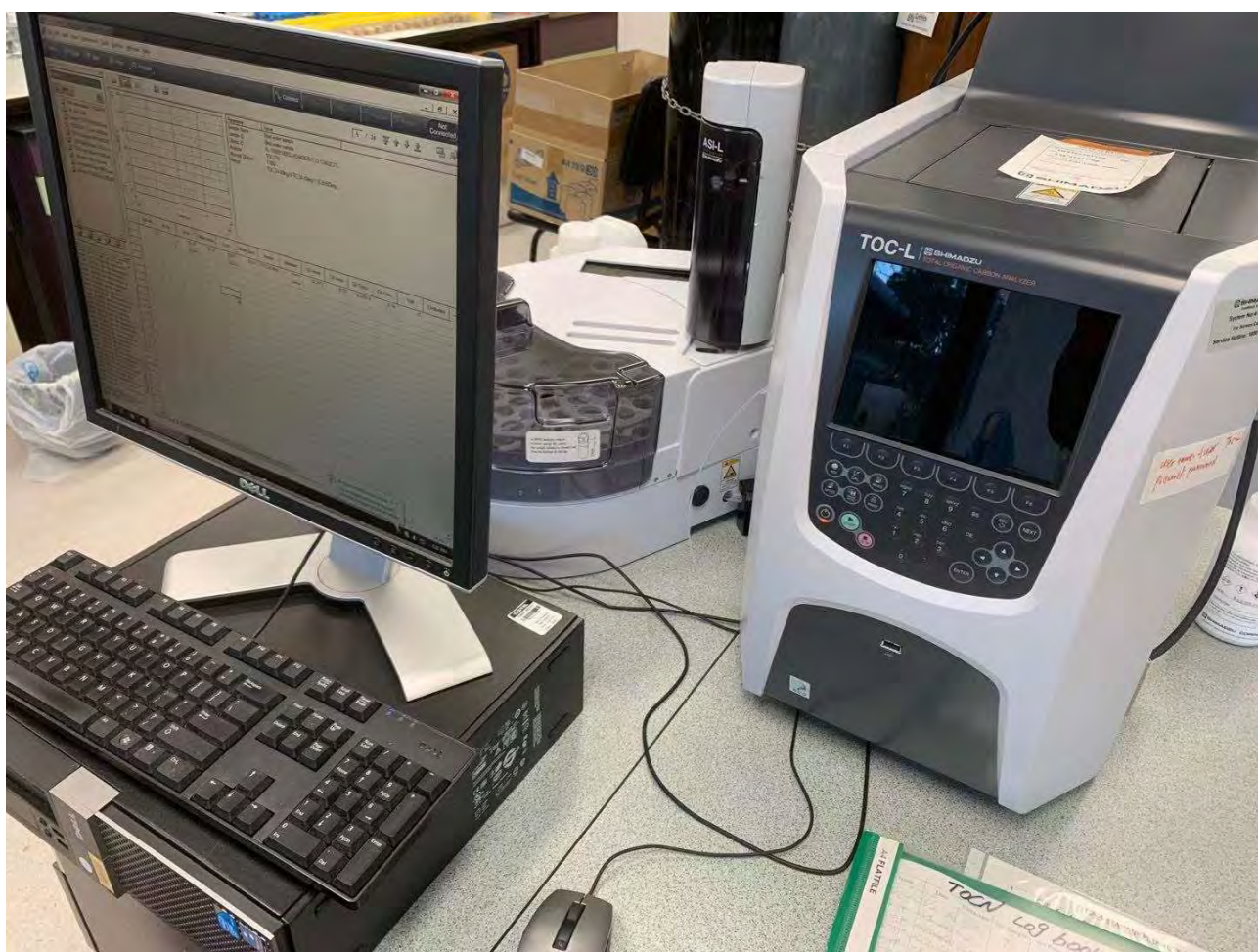


Fig.2.7 The SHIMADZU TOC-L TOCN analyzer (shimadzu, Kyoto, Japan).

2.3.4 CO₂ respiration and enzyme activities

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 1 minute. Gas samples were collected from the headspace of the glass jars 8 hours after closure using a 25 mL gas-tight syringe and immediately transferred to pre-vacuumed 12mL glass vials (Labco, UK). Gas samples were analysed for CO₂ concentration using a gas chromatograph (Shimadzu GC-2010 Plus; Fig. 2.8) method. Linearity tests on CO₂ concentration increases were conducted on all treatments over a subset of sampling times during the incubation. The activity of β -glucosidase (hydrolyzing cellulose to glucose), chitinase (hydrolyzing chitin and other glucosamine polymers) and phosphatase (hydrolyzing phosphomonoesters), in soil were measured at optimum pH for acidic soils (Tabatabai 1994).

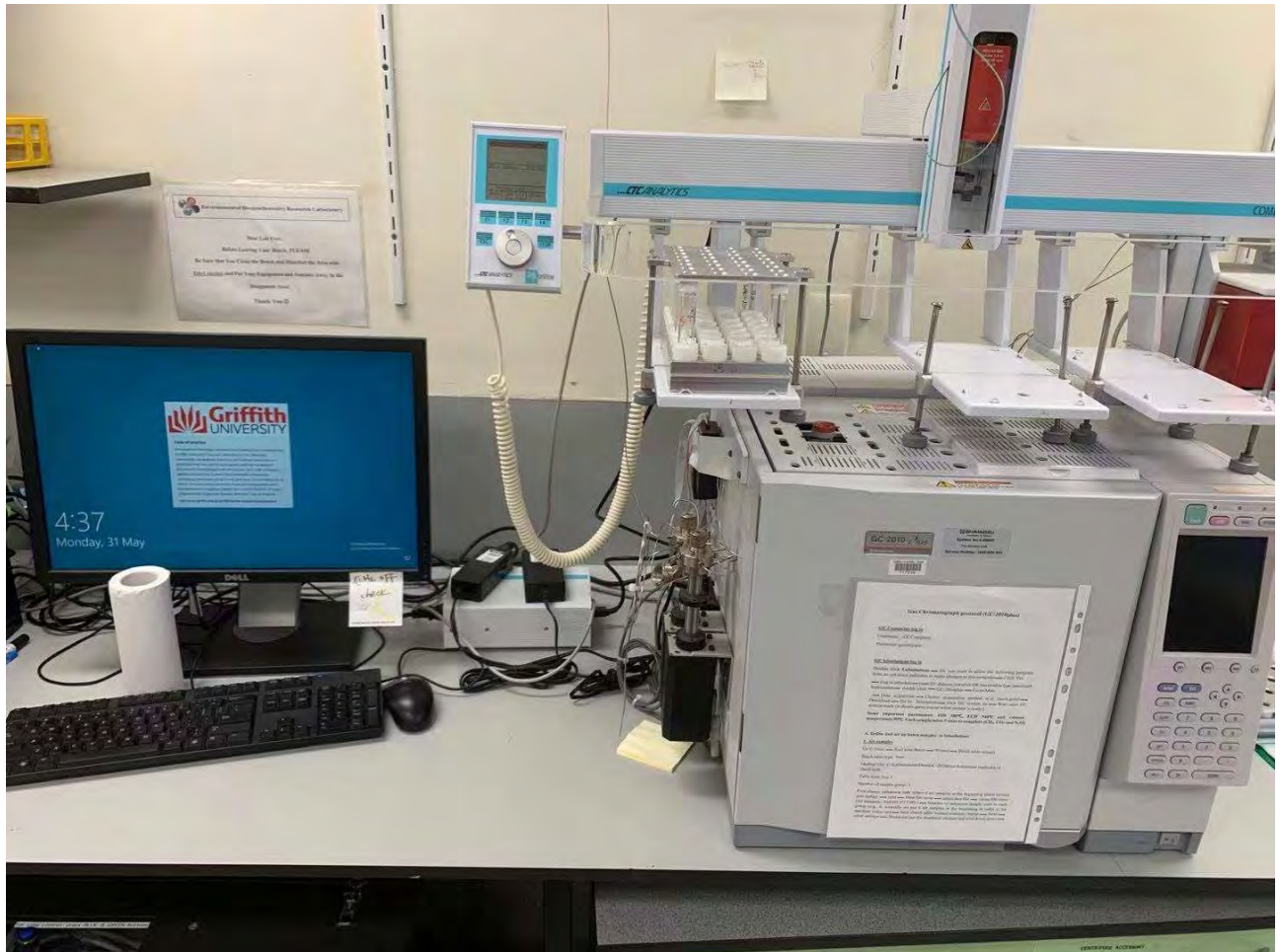


Fig.2.8 Gas Chromatograph (Shimadzu GC-2010 Plus, Kyoto, Japan).

Chapter 3 Soil microbial responses as an indicator of compaction resilience is govern by soil moisture status in sugarcane cropping systems

Abstract

Soil compaction combined with local extreme weather events, such as intensive rainfall or prolonged drought, may cause a significant decrease in soil microbial activity and threaten sustainable crop productivity. Soil biology provides a wide range of functions in soil, contributing to fertility, aggregate stability, carbon (C) turnover and pollutant degradation, amongst others. However, there is a paucity of information on soil microbial responses to compaction and drought stresses. To address this, two contrasting sugarcane soils (Planosols-sandy and Nitisols-clayey) were exposed to a combination of compaction and drought stress, and microbial responses were investigated going into stress (resistance) as well as their response coming out of stress (resilience). We artificially applied a gradient of bulk densities ($0.9 - 1.5 \text{ g cm}^{-3}$) and water fill pore space (WFPS; 21% to 100%). Under high water content (e.g., WFPS of 47% to 100%), low compaction levels (1.1 and 1.2 g cm^{-3}) increased soil cumulative microbial respiration in Planosols treatments by 18% in comparison to nil compaction treatment (0.9 g cm^{-3}), while high compaction levels ($1.3-1.5 \text{ g cm}^{-3}$) decreased soil cumulative microbial respiration by 25% with increased compaction. In contrast, in Nitisols with high water content, highest compaction treatment (1.5 g cm^{-3}) significantly increased soil cumulative microbial respiration by 12% in comparison to nil treatment (0.9 g cm^{-3}). Our data revealed that the Nitisols had a higher resistance

index of microbial C use efficiency, while the Planosols had higher resilience index of microbial C use efficiency to compaction and moisture stress. This could be attributed to greater aggregation potential (associated with more finely textured particles) and higher diversity of the microbial community in the Nitisols, which provide a higher functional stability to resist environmental disturbance, while fast drainage and higher adaptation of microbial communities in the Planosols would provide a faster recovery from applied stresses. This also indicates that soil microbial responses to compaction stress is governed by soil texture and moisture status.

3.1 Introduction

Climate change and human activities are altering the environment by increasing the atmospheric CO₂, 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 that 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 indicated that soil compaction would negatively affect 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) would significantly affect soil physicochemical and biological properties such as soil water-filled pore space (WFPS);(Logsdon and Karlen, 2004), soil C and nutrient cycles and 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 capacity 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). Organic C is utilized as energy source via respiration and microbial substrates assimilated into microbial biomass during microbial decomposition. This utilization is often considered as substrate use efficiency or C use efficiency which is an important microbial parameter in investigating the fate of C in soils (Bölscher et al., 2016). Soil respiration and its relation to changes in soil microbial biomass and enzyme activity are considered as 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). Although the metabolic quotient fails to distinguish between effects of disturbance (pulse impact) and environmental stress (press impact), it is still a valuable index in measuring how efficiently the soil microbial biomass is utilizing C resources, and the degree of substrate limitation for the microbial community in soil (Wardle and Ghani, 1995).

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 was to assess the mechanisms responsible for sugarcane soils' microbial functional response to compaction and water stresses. The underlying hypotheses were: 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) Nitisols tends to have a greater resistance to compaction while Planosols soil tend to have higher resilience to compaction

3.2 Materials and Methods

3.2.1 Sample preparation and experimental design

Soils were collected from 0-10 cm depth of two sugarcane farms at Rocky Point (Planosols (sandy soil); 27° 43' 51" S, 153° 17' 20" E) and Ingham (Nitisols (clayey

soil); 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 has been 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 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 total C and N contents of Nitisols 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 represent drought stress) or 35% GWC (high water content group that represent 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 (RS phase). At the end of 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 then further incubated in the dark at 22 ± °C till the end of experiment at day 74 (RL phase). The experiment had 24 treatments in total (2 soil types, 2 water content levels, 6 compaction levels) with 3 replicates for each treatment (in total 18 replicates for 6 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} , and 0.9 represent 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} , and 0.9 represent 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 treatment (nil compaction and no water stress) in both Planosols and Nitisols was 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 1 - 4 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) was measured using a glass electrode (Rayment and Lyons, 2011). Soil mineral N was extracted using 2M KCl at a 1:4 ratio of soil to extractant using an end-over-end shaker for 1h, filtered by a Whatman 42 filter paper and concentrations of NH_4^+ -N and NO_3^- -N were measured using a SEAL AA3 Continuous Segmented Flow Analyzer (SEAL Analytical Limited, USA). The total C (TC) and total N (TN) contents of soil samples were measured by dry-combustion using a LECO CNS-2000 analyzer (LECO Corporation, MI, USA). The hot water extractable organic C (HWEOC) and total N (HWETN) contents of the samples were measured using the method described by (Chen et al., 2000). Briefly, 4.0g (oven-dry equivalent) of fresh soil was incubated with 20mL 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 5 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 analyzer. The results were expressed on an oven-dry basis. Water fill pore space (WFPS) was calculated by following equation:

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

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.

3.2.3 Analysis of soil biological properties

Soil microbial biomass C (MBC) and 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 1 minute before closure. Gas samples were collected from the headspace of the glass jars 8 hours after closure using a 25 mL gas-tight syringe and immediately transferred to pre-evacuated 12mL glass vials (Labco, UK). Gas samples were analysed for CO₂ concentration using a gas chromatograph (Shimadzu GC-2010 Plus). Linearity tests on CO₂ 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 ($q\text{CO}_2$), defined as the C respired per unit of MBC per day, and calculated from the ratio of CO₂-C emission rate ($\text{mg CO}_2\text{-C kg}^{-1} \text{ soil day}^{-1}$) to MBC ($\text{mg C kg}^{-1} \text{ soil}$) 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).

3.2.4 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):

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

Where, 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:

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

Where, 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} becomes higher than the absolute value of D_{35} , then the RL index will have a negative value. Mathematically, the RL index of control treatment will be infinitely close to +1, while it has been considered as +1 in the RL equation.

3.2.5 Statistical analysis

Differences at $P < 0.05$ between treatments were considered significant and all variables were tested for normality of distribution using the Kolmogorov–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 CO₂ emissions and soil properties.

3.3 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 Nitisols soil treatments than Planosols soil treatments, while no significant differences were observed between the initial and final concentrations of TC and TN across treatments.

3.3.1 Soil bioavailable carbon and nitrogen contents

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 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) till the end of RS phase at day 35, and before the removal of compaction stress by ploughing (Fig. 3.1a). During the RL phase which started at day 35, the concentration of HWEOC significantly ($P < 0.05$) increased in all treatments (day 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 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 experiment (Fig. 3.1b). However, there was no significant difference among all treatments at the end of 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 experiment. Regardless of soil type, all treatments with high water content showed lower concentration of HWEOC compared with other treatments, at the end of RL phase (Figs. 3.1a and 3.1b). This observation would indicate the dependency of C mineralization rate (both Planosols and Nitisols) to soil moisture status, during recovery from an environmental stress.

Planosols generally showed a decrease in HWETN content during the first 2 weeks of incubation, while the values increased toward the end of RS phase at day 35 (Fig. 3.1c). By the end of 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 till 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 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. 3.1d). However, 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 RL phase, no significant differences were observed between 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 ($\text{NH}_4^+ + \text{NO}_3^-$) contents did not show any significant difference among all Planosols treatments in the first week of RS phase (Table 3.1), while the observed values were significantly lower than the Nitisols treatments (except H1.4 treatment). Although different Planosols treatments showed different pattern of N mineralisation during the RS phase, but their mineral N concentration 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 rate compared with other treatments, while control treatment (H0.9) had the highest ($P < 0.05$) N mineralisation rate at the end of 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 RS phase compared with 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 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 7 days of RL phase. However, the Nitisols treatments with low water content (L0.9 to L1.5) generally had lower mineral N concentrations compared with control and high water content (H1.1 to H1.5) treatments by the end of experiment.

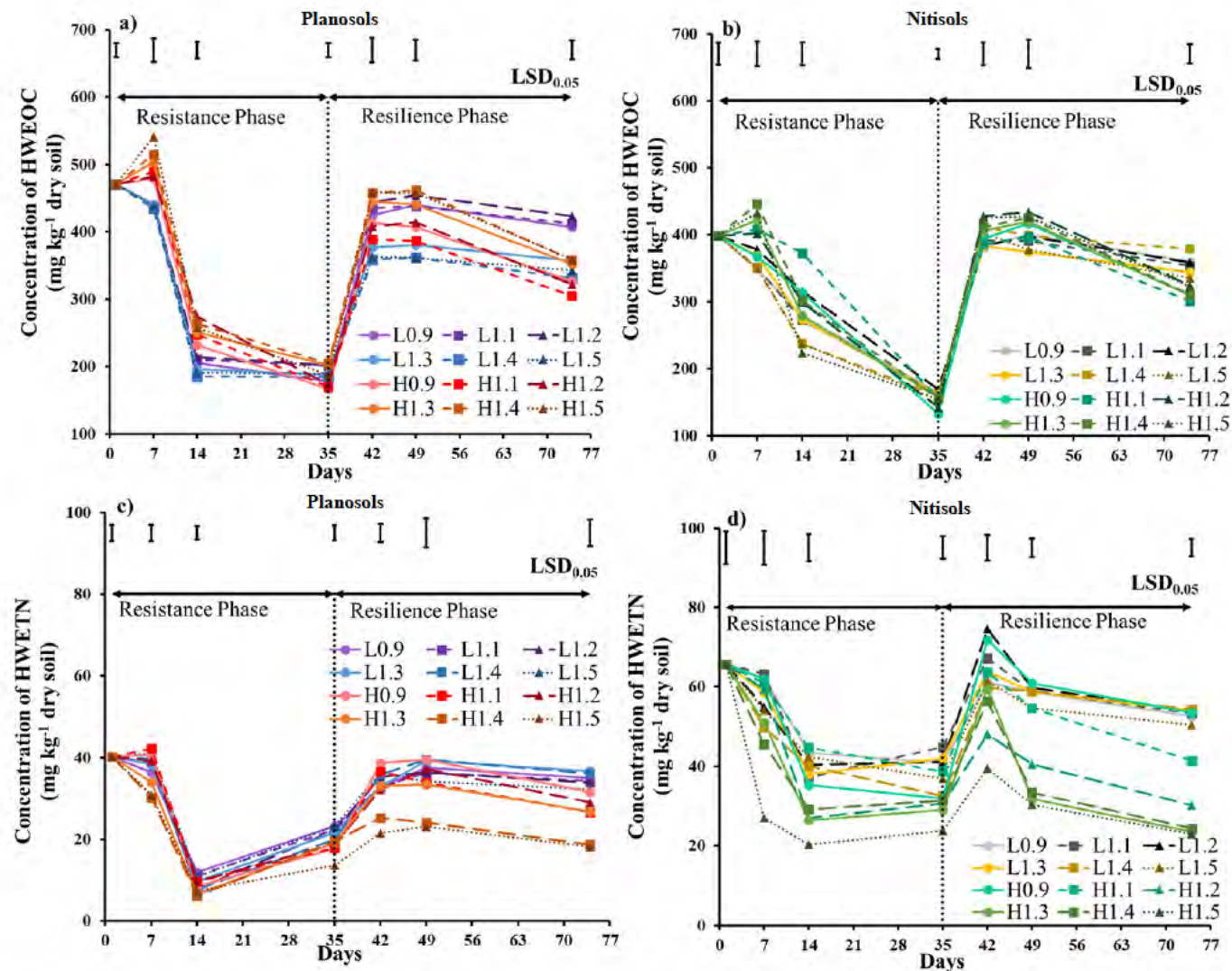


Fig. 3.1 Effect of combined compaction and moisture stresses on concentration of hot water extractable organic C (HWEOC) and hot water extractable total N (HWETN) in sandy (a and c) and clayey (b and d) sugarcane soils. L, low moisture level (15% GWC); H, high moisture level (35% GWC). Numbers follow 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 ranged 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 ranged 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. 3.1a means sandy soil with moisture content of 15% GWC and bulk density of 0.9 g cm⁻³ and H1.5 in fig. 3.1b 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 3.1 Mineral nitrogen 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
Planosols (Sandy soil)	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	
Nitisols (Clayey soil)	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 treatments at $P < 0.0$

3.3.2 Soil enzyme activities and microbial biomass carbon and nitrogen contents

The change 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 RL phase (day 74; Table 3.2). 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 RS phase. However, no specific pattern of recovery was observed in β -glucosidase enzyme activity of Nitisols treatments during RL phase. The chitinase enzyme activity in Planosols treatments with low water content (L0.9 to L1.5) were generally higher than control (H0.9) and high water content (H1.1 to H1.5) treatments at the end of RS phase (Table 3.2). However, this difference increased during RL phase and resulted in significantly ($P < 0.05$) higher chitinase enzyme activity in low water content than control and high water content treatments (except H1.5) by the end of experiments (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 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 control treatment (H0.9), were significantly ($P < 0.05$) higher than other treatments in the first week of RS phase (Fig. 3.2a). However, by the end of RS

phase (day 35) Planosols treatments with high water content (H1.1 to H1.5) showed significantly ($P < 0.05$) higher MBC contents than 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 7 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. 3.2b). By the end of 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 Planosols treatments showed a similar pattern in both RS and RL phases (Fig. 3.2c), 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 RS phase). In Nitisols treatments, the H1.3 treatment showed significantly ($P < 0.05$) higher MBN content in the first week of RS phase, while no significant differences were observed between other treatments (Fig. 3.2d). At the end of 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 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 experiment (day 74).

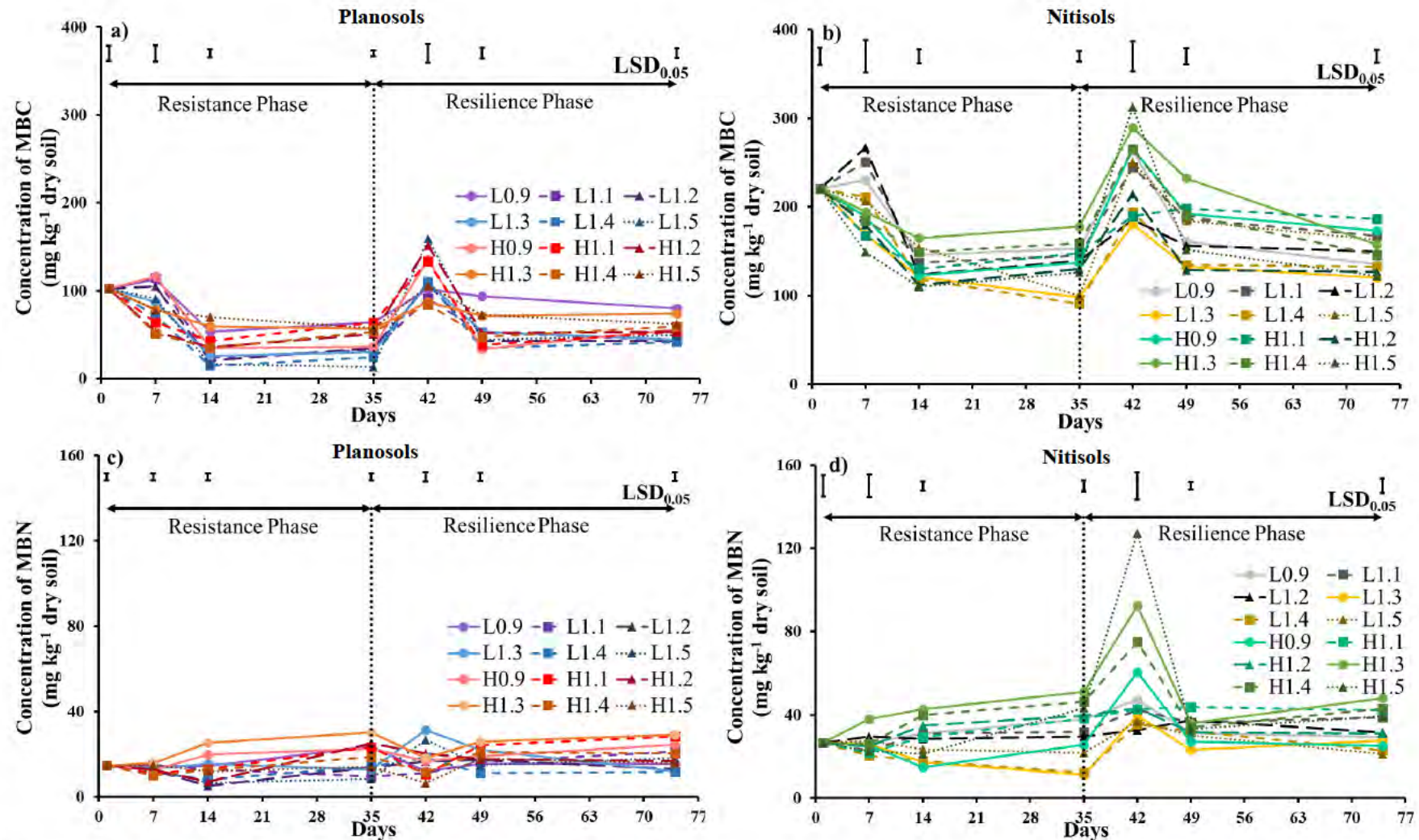


Fig. 3.2 Effect of combined compaction and moisture stresses on soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) contents in sandy (a and c) and clayey (b and d) sugarcane soils. L, low moisture level (15% GWC); H, high moisture level (35% GWC). Numbers follow by letter L or H indicates bulk density (e.g., 0.9 means 0.9 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 3.2 Selected enzyme activities of sugarcane soils under different moisture and compaction stresses.

Treatments	Soil types	β -Glucosidase activities ($\mu\text{g } p\text{-nitrophenol h}^{-1} \text{g}^{-1} \text{dry soil}$)						Chitinase activities ($\mu\text{g } p\text{-nitrophenol h}^{-1} \text{g}^{-1} \text{dry soil}$)					
		day7	day14	day35	day42	day49	day74	day7	day14	day35	day42	day49	day74
		Planosols (Sandy soil)	L0.9	215.9c	261.4c	82.2ef	126.6f	228.0c	60.2g	269.3b	223.1a	111.8c	98.9d
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	
Nitisols (Clayey soil)	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 $P < 0.05$

3.3.3 Soil microbial respiration and metabolic quotient

Figs. 3.3a and 3.3c shown the values of cumulative soil microbial respiration (presented as cumulative CO₂ emission) in Planosols (sandy soil) under different compaction level in low and high water contents. In low moisture condition, the highest and lowest values were observed in L1.5 and L0.9 treatments, respectively, while in high moisture condition, the high compacted treatments (H1.3, H1.4 and H1.5) had lower cumulative respiration than other treatments at day 35. In contrast, by the end of RL phase at day 74, the Planosols treatments with low water content (L0.9 to L1.5) generally showed lower cumulative respiration than control (H0.9) and high water content (H1.1 to H1.5) treatments. In low moisture condition, the L0.9 treatment showed the lowest cumulative respiration and the values generally increased with increasing the compaction level (Fig. 3.3a). However, in high moisture condition, the control treatment (H0.9) showed lower cumulative respiration than other treatments (except H1.5 treatment) by the end of experiment (Fig. 3.3c).

The values of cumulative soil microbial respiration in Nitisols (clayey soil) treatments showed different trends for high ($BD \geq 1.3 \text{ g cm}^{-3}$) and low ($BD < 1.3 \text{ g cm}^{-3}$) compacted treatments at the RS phase. The cumulative respiration values in treatments with $BD \geq 1.3 \text{ g cm}^{-3}$ were lower with high water content (Fig. 3.3d) and higher in low water content conditions (Fig. 3.3b). In low moisture condition, the highest and lowest values were observed in L1.5 and L0.9 treatments, respectively, while in high moisture condition, the control treatment (H0.9) showed the highest cumulative respiration and the values generally decreased with increasing the compaction level. At the end of 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 control (H0.9) and highwater content (H1.1 to H1.5) treatments. In low moisture condition, the cumulative respiration values were generally increased with increasing the compaction level (Fig. 3.3b). However, in high moisture condition, the control treatment (H0.9) showed the highest cumulative respiration and the observed values generally decreased with increasing the compaction level (day 74; Fig. 3.3d).

The changes in metabolic quotient (qCO_2) values did not show a specific pattern among Planosols treatments in the first two weeks of RS phase (Table 3.3), while the observed values were generally higher than the Nitisols treatments. At the end of RS phase, all Planosols treatments with high water content (H1.1 to H1.5) showed significantly ($P < 0.05$) lower qCO_2 values than 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 RL phase (except H1.3 treatment). However, by the end of 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 RS phase. However, by the end of experiment at day 74, There were no significant differences between Nitisols treatments with high water content (H1.1 to H1.5) and 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.

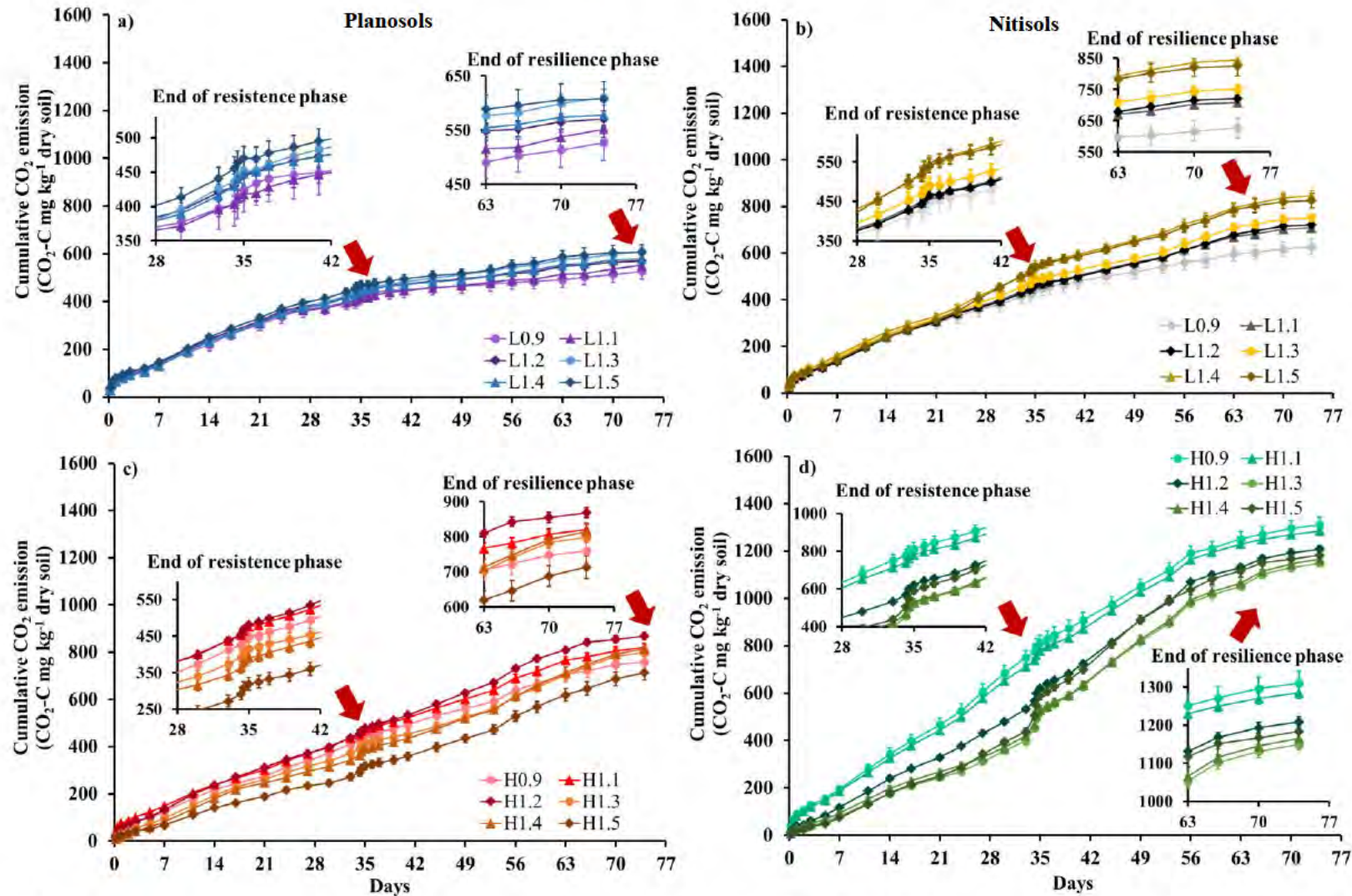


Fig. 3.3 Effect of combined compaction and moisture stresses on cumulative CO₂ emissions in sandy (a and c) and clayey (b and d) sugarcane soils. L, low moisture level (15% GWC); H, high moisture level (35% GWC). Numbers follow by letter L or H indicates bulk density (e.g., 0.9 means 0.9 g cm⁻³). Vertical bars are standard error of three replicates.

Table 3.3 Metabolic quotient ($q\text{CO}_2$) of sugarcane soils under different moisture and compaction stresses.

Soil types	Treatments	Metabolic quotient ($\mu\text{g CO}_2\text{-C h}^{-1} \text{mg}^{-1}$ microbial biomass C)						
		day7	day14	day35	day42	day49	day74	
Planosols (Sandy soil)	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	
	Nitisols (Clayey soil)	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 3.4 Stepwise multiple linear regression equations between cumulative CO₂ emissions and soil properties at the end of resistance and resilience phases.

Incubation phase	Equation	R ²	P value
Resistance phase (day 35)	$Y (\text{CO}_2) = -27.04 (\text{Labile C: N Ratio}) - 3.32 (\text{Chitinase}) + 484.1$	0.57	0.05
Resilience phase (day 74)	$Y (\text{CO}_2) = 27.21(\text{Labile C: N Ratio}) + 36.71(\text{MBC: MBN Ratio}) + 1.19 (\text{Chitinase}) + 345.65$	0.81	0.05

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

The principal component analysis (PCA) between soil (Planosols and Nitisols) physicochemical and biological properties at the end of RS (day 35; Fig. 3.4a) and RL (day 74; Fig. 3.4b) phases indicated that principal component 1 (PC1) and 2 (PC2) were able to 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 were HWEOC, HWETN, MBC, MBN, β -glucosidase activity and cumulative respiration, while the WFPS and BD of soil samples showed the highest correlation coefficient (>0.8) for PC2. The PCA analysis at the end of RS phase (Fig. 3.4a) was able to clearly separate 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 showed 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 RL phase (Fig. 3.4b) indicated a different pattern of recovery in soil properties under low and high moisture stresses, while this pattern was 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 CO₂ emission) and other soil (Planosols and Nitisols) physicochemical and biological properties (Table 3.4). The results indicated 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 ($R^2 = 0.57$, $P < 0.05$, $n = 72$). 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 ($R^2 = 0.81$, $P < 0.05$, $n = 72$).

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. 3.5a). 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. 3.5b). In contrast to RS index, the RL index of qCO_2 in Planosols treatments were higher than Nitisols treatments. The RL of qCO_2 in Planosols treatments increased with increasing the compaction level, with higher recovery form stress observed in low water content than high water content condition (Fig. 3.5c). This behaviour would indicate 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 form drought stress than waterlogging stress (Fig. 3.5d).

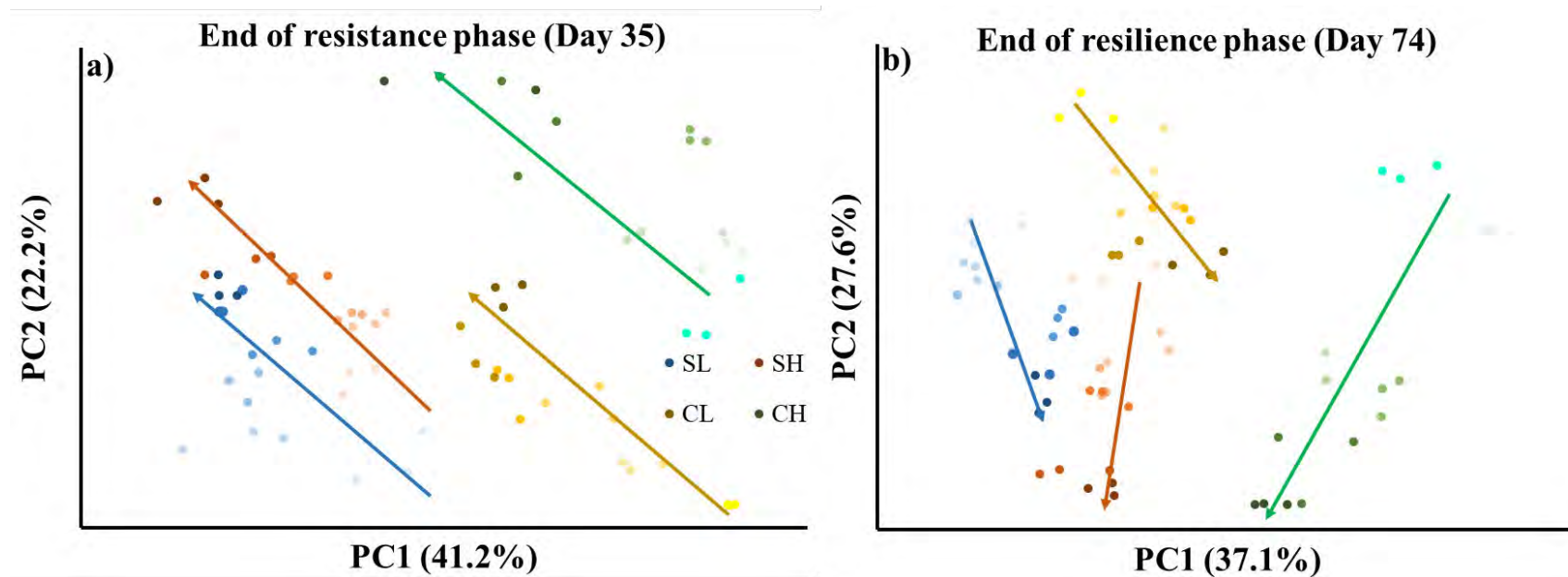


Fig. 3.4 Scores plot of principal component analysis (PCA) showing the separation of different soil samples under compaction and moisture stresses at resistance (a) and resilience (b) phases and loading values of the individual soil parameters (PC1 and PC2) for soil samples. SL, sandy soil with low moisture (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.

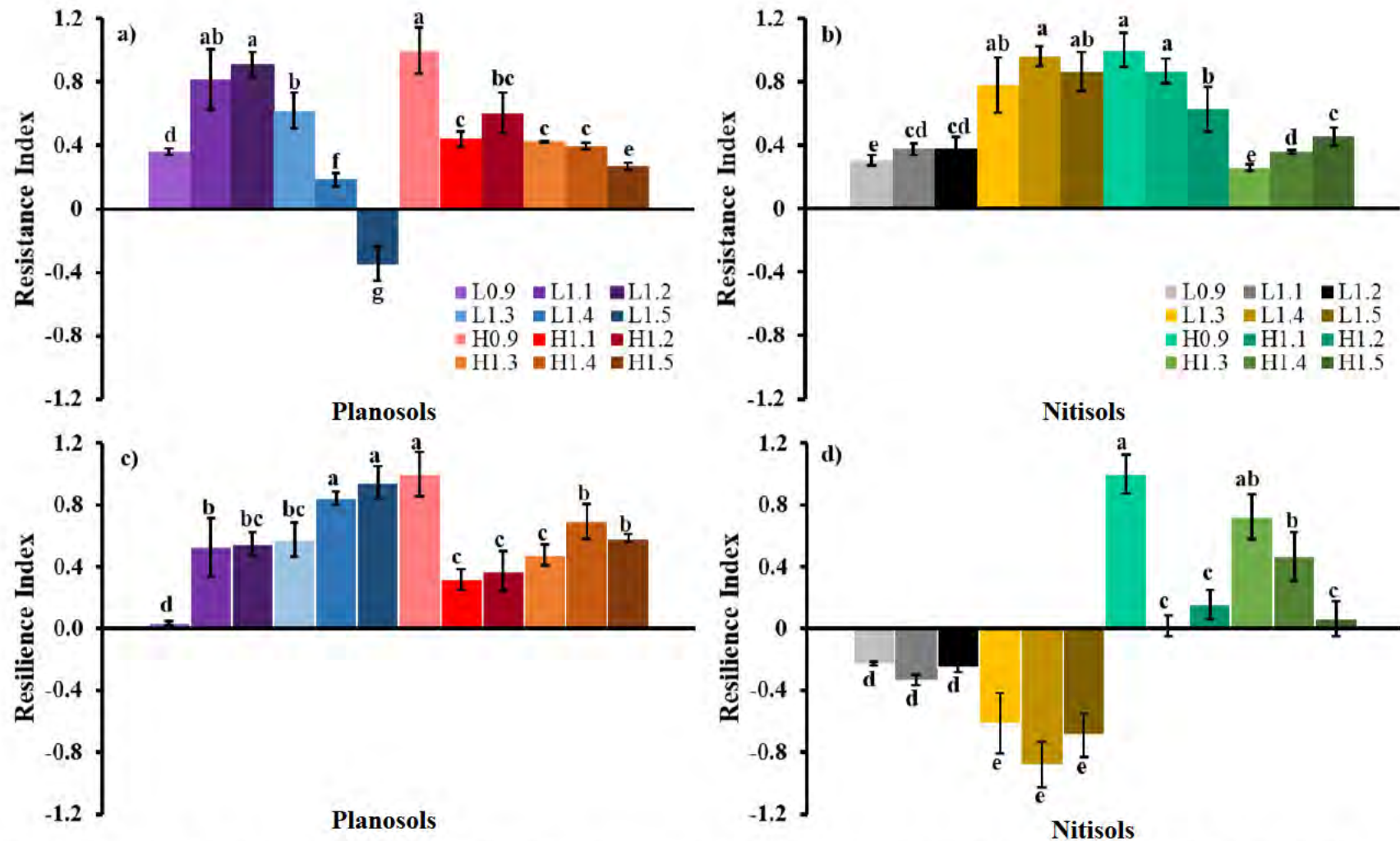


Fig. 3.5 The resistance (day 35) and resilience (day 74) indices of soil microbial carbon use efficiency (metabolic quotient) in sandy (a and c) and clayey (b and d) soils. L, low moisture level (15% GWC); H, high moisture level (35% GWC). Numbers follow by letter L or H indicate bulk density (e.g., 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.

3.4 Discussion

3.4.1 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 indicated 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. 3.1a 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 were 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 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 of dry soils or ploughing of 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 indicated 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 indicated the importance of soil moisture status in resilience of soil organic matter to mineralisation process (Hawkes et al., 2017; Hu et al., 2021).

3.4.2 Microbial responses to soil compaction and moisture stresses

Soil biological properties play a critical role in the proper functioning of soil ecosystems, while 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 for assessment of 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 would change 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 would reduce 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 would increase soil microbial biomass and activities by increasing soil bioavailable C pools due to the breakdown of soil aggregates, while waterlogging and drought stresses would reduce soil microbial biomass and activities by creating anaerobic condition in 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 this experiment (Figs. 3.2 and 3.3). This behaviour 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 WFPF 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 indicated that the magnitude of microbial respirations in the soils were 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 3.4). Chuckran et al. (2020) and Deng et al. (2018) also indicated 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 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 $q\text{CO}_2$ values observed in Nitisols treatments at the end of RS and RL phases (Table 3.3). 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.

3.4.3 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 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 indicated 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 illustrated 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).

Our results also showed 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 reported 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.

Our results indicated that the Nitisols was neither RS nor RL to the drought stress under soil bulk densities $\leq 1.2 \text{ g cm}^{-3}$, 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 indicated 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 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 RS phase.

3.5 Conclusions

We demonstrated that soil microbial activities are directly affected by soil moisture status, whereas compaction may affect soil physio-chemical 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 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 Nitisols treatments with high water content (H0.9 - H1.5), microbial respiration linearly decreased with the increasing of compaction stress. This observation indicated 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 adaptation of microbial community to frequent disturbance would provide a faster

recovery from environmental stresses. Our 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 structure 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.

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Chapter 4 Field management practices play a vital role in building soil resistance and resilience against compaction stress

Abstract

Soil microbes are sensitive to compaction stress and consequently affect soil C and N cycling, further governs plant growth and yield. To mitigate the compaction stress on soil microbial community, it is essential to understand the mechanism of soil microbe responses against compaction. Nitisols with conventional and improved management history from the same sugarcane farm at Foresthome, North Queensland, Australia, were exposed to compaction stress (1.4 g cm^{-3}), and microbial functions were investigated during compaction and ploughing cycle. The improved management block was managed with minimum tillage, mound planting, and legume plantation in comparison to conventional furrow management. Compaction was applied at the commencement of the experiment (resistance phase) and removed at day 28 (resilience phase) via plant residue application combined with ploughing. Overall, the concentration of labile C was 42% higher in treatments had improved management history. Within treatments had 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. Interestingly, we found that legume residue incorporation along with improved management minimized fluctuation of net cumulative CO_2 emissions between compacted and non-compacted treatments by the end of RL phase; however,

we did not find the same pattern in treatments had conventional management. Our results revealed 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 caused by compaction. Improved field management can increase soil RS and RL to compaction stress, which is shown as a faster stabilization of microbial properties after compaction stress and its removal due to their higher organic matter content and complexity of microbial community. These data confirmed that 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.

4.1 Introduction

Soil compaction is a vital factor contributing to global land degradation and rising more interests 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 the 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) indicated that soil compaction had negative effect on crop growth and commonly causes yield loss. Radford et al., (2001) also reported that mean production of wheat reduced by 23% in compacted soil in comparison with uncompacted soil. Research by Cherubin et al. (2016) reported that soil compaction in Brazil resulted in 50% reduction in sugarcane yield potential in the fourth and fifth ratoons, while (Bell et al., 2007) concluded 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 would provide crucial knowledge to better understanding of soil microbial responses and 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 the mainstream field management practices to meet the increased demand for sustainable agriculture. Conservation agricultural management include reducing tillage and using plant residues and/or trash blanket for permanent ground cover (Chabert and Sarthou, 2020). In addition, incorporation of organic amendments such as compost showed 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) reported that conservation field management practices would reduce 15% of operation cost in comparison with conventional field management. The no-tillage field management practice would also increase soil organic C by about 5% compared to conventional management practice (Deen and Kataki 2003). However, the reduction in crop yield associated with conservation management practice can be compensated by benefits of sustainable agriculture in a long-term. Arvidsson et al. (2014) reported that no-tillage practice would reduce crop yield by 10% compared to conventional practice. Vyn and Raimbult (1993) also reported a significant reduction of corn yield in no-tillage field 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 reported that composted sugarcane residue can help in maintaining high crop yield and preventing soil degradation. Hernández et al., (2014)

also reported an increase in crop yield and soil fertility via field application of organic compost. Although there are many studies conducted to investigate the impacts of organic residue amendment on soil properties, detailed knowledge related to the responses of soil microbial communities to different field management practices remained 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 and dependency to 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 would increase soil microbial performance and further improve soil health condition (Flavel and Murphy, 2006).

Soil RS and RL concept, which derived from the term ‘microbiome RS and RL’, is widely used in describing soil microbiome 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 recover to their pre-stress state after removal of compaction. This study aims to investigate the responses of soil microbial community following soil compaction stress and estimate soil RS and RL against compaction in conventional and improved soil management practices. The underlying hypotheses were: 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 would not improve soil RL against compaction stress due to the rapid loss of labile C through soil microbial respiration process; c) incorporation of plant residues would improve soil microbial activities and their recovery from compaction stress.

4.2 Material and method

4.2.1 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), North Queensland, Australia. The soil was classified as Red Dermosol in 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 has been cropped with sugarcane in the past 30 years and improved management field has been cropped with sugarcane in the past 14 years. The legume plantation in improved management field, during the fallow period (every 5 years), was a mix 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 conducted in April 2020 in six replicates. The plots under conventional (1.63m row space) and improved (1.83m 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 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 RS phase, half of the treatments (CP, CCP, IP, ICP, CPO, CCPO, IPO and ICPO) 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 condition at 22 ± °C till the end of 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). The letter 'C' denotes conventional management, followed by no-compaction in 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 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 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 (NH₄⁺-N and NO₃⁻-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 CO₂ emissions) during the incubation experiment, using three randomly selected replicates of each treatment.

4.2.2 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 a glass electrode method described by (Rayment and Lyons, 2011). Soil mineral N (NH₄⁺-N and NO₃⁻-N) was extracted by 2M KCl at a 1:5 ratio of soil to extractant using an end-over-end shaker for 1 h, filtered by a Whatman 42 filter paper (Rayment and Lyons, 2011) and concentrations of NH₄⁺-N and NO₃⁻-N were determined by a SEAL AA3 Continuous Segmented Flow Analyzer (SEAL Analytical Limited, USA). Soil total C (TC) and N (TN) contents were measured by the combustion method using a LECO CNS-2000 analyzer (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 h. After incubation the tubes were shaken on an end-over-end shaker for 5 min 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 analyzer. The results were expressed on an oven-dry basis.

4.2.3 Soil biological analysis

Soil MBC and 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). Concentrations of soluble C and N pools of the fumigated and non-

fumigated soil samples were determined using a SHIMADZU TOC-VCSH/ CSN TOCN analyzer (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 1 minute before closure. Gas samples were collected from the headspace of the glass jars 8 hours after closure using a 25 mL gas-tight syringe and immediately transferred to pre-evacuated 12mL glass vials (Labco, UK). Gas samples were analysed for CO₂ concentration using a gas chromatograph (Shimadzu GC-2010 Plus). Linearity tests on CO₂ 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 ($q\text{CO}_2$), defined as the C respired per unit of MBC per day, and calculated from the ratio of CO₂-C emission ($\mu\text{g CO}_2\text{-C kg}^{-1}\text{ soil day}^{-1}$) to concentration of MBC ($\text{mg C kg}^{-1}\text{ soil}$) 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 (hydrolyzing cellulose to glucose) was measured at pH 6.0 (Tabatabai, 1994).

4.2.4 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):

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

Where, 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:

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

Where, 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} becomes higher than the absolute value of D_{28} , then the RL index will have a negative value.

4.2.5 Statistical analysis

Differences at $P < 0.05$ between treatments were considered statistically significant and all variables were tested for normality of distribution using Kolmogorov-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 CO₂ 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).

4.3 Result

At the end of incubation, soil samples were well-mixed and analyzed 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).

4.3.1 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 RS phase (Table 4.1). Regardless of soil management history, compaction stress generally increased labile C concentration compared with non-compacted treatments at day 7. After the first week of incubation, concentration of HWEOC decreased over time, in treatments without legume residue amendment, till

the end of 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 RL phase (Day 35). The incorporation of legume residue significantly ($P < 0.05$) increased the concentration of HWEOC in treatments with conventional management history but decreased concentration of HWEOC in treatments with improved management history in comparison with surface application of legume residue at day 35, respectively. This observation indicates that combined ploughing and organic residue amendment practices increased the mineralization and depolymerization 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 RL phase, we also found that concentration of HWEOC 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 RS phase (Table 4.1). Also, like HWEOC, the compaction stress increased concentration of HWETN compared with non-compacted treatments by the end of RS phase (day 28). At day 28, we observed a slight decrease in concentration of HWETN in treatments with improved management history, while this trend was not clear in conventionally managed treatments. The results also indicated 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 significantly ($P < 0.05$) increased

soil HWETN content in all treatments. Additionally, at day 35, treatments that received surface application of legume residue had significantly ($P < 0.05$) higher concentration of HWETN than treatments that received legume residue incorporation, regardless of soil management history. By the end of 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.

4.3.2 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 4.1). The mineral N content generally increased in treatments with both management history at the end of RS phase and the first week of RL phase (day 35). The removal of compaction stress significantly 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 4.1). 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 non-compacted 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 RL phase. Although this pattern was similar in the treatments with improved management history, but the changes were less clear compared with conventionally managed treatments. At the end of 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 4.1). 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 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 4.1 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 start with letter C referred 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 start with letter I referred 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.

4.3.3 Soil microbial pools

In general, present study indicated that compaction stress increased concentration of MBC in treatments with conventional management history (CC and CCP), while decreased concentration of MBC in treatments with improved management history (IC and ICP) compared to control treatments (CCK and ICK), at day 7 (Table 4.2). However, concentration of MBC reached to a stable level at day 28 in all treatments, regardless of treatments' management history and compaction status. In the first week of RL phase, we observed that there were no significant changes in concentration of MBC in control treatments (CCK and ICK), while the MBC contents slightly decreased in non-compacted 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 experiment (day 70), we found that concentration of MBC increased in non-compacted and ploughed treatments (CP and IP), while decreased in control treatments (CCK and ICK), regardless of soil management history (Table 4.2). In addition, the increase in MBC content was lower in compacted treatment with improved management history (IC) than conventional management history (CC) by the end of experiment. Comparison of compacted and ploughed treatments at the end of RL phase also indicated that the concentration of MBC was significantly ($P < 0.05$) higher in treatment with improved management history (ICP) than treatment with conventional management history (CCP). This observation indicates that improved management history may provide a higher potential for soil microbial community to recover after removal of compaction stress, without organic residue amendment. After legume residue amendment, concentration of MBC boosted in all treatments regardless 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 RL phase. However, at the end of experiment (day 70), the concentration of MBC decreased in treatments with residue incorporation method (CPO, CCPO, IPO and ICPO), but 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 4.2). After removal of compaction stress at the end of RS phase, the concentration of MBN significantly ($P < 0.05$) increased in treatment with improved management history (ICP), while significantly ($P < 0.05$) decreased in treatment with conventional management history (CCP) at day 35. At the end of 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 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 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 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 4.2). In the first week of 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 legume residue amendment at the end of experiment. With legume residue amendment at the end of RS phase, we observed a significant ($P < 0.05$) increase in β -Glucosidase activity in all treatments regardless 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 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 4.2 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 (μ g 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 4.1.

4.3.4 Cumulative CO₂ respiration and net cumulative CO₂ respiration

Treatments with improved management history had higher cumulative CO₂ emissions than treatments with conventional management history, while there were no significant differences in net cumulative CO₂ emissions between treatments with improved and conventional management history, by the day 28 (Figs. 4.1a and 4.1c). The compacted and ploughed treatments (CCP and ICP) had the highest cumulative CO₂ respiration followed by compacted-only treatments (CC and IC), while control treatments (CCK and ICK) had the lowest cumulative CO₂ respiration among treatments with the same management history (Figs. 4.1a). 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 legume residue resulted in higher cumulative CO₂ emissions in treatments with conventional management history in comparison with improved management treatments (CO vs IO, and CCO vs ICO), while no significant differences observed between legume residue incorporated treatments (CPO, CCPO, IPO and ICPO), regardless of their management history (Fig. 4.1b). In addition, surface application of legume residue showed higher net cumulative CO₂ emission in treatment with conventional management history (CCO) in comparison to treatment with improved management history (ICO), while incorporation method of legume residue amendment resulted in lower net cumulative CO₂ emissions in conventional management treatments compare to improved management treatments (Fig. 4.1d). This observation indicates that surface application of legume residue to treatments with conventional management would result in higher loss of applied organic C via CO₂ emissions. In addition, legume residue incorporation to soil with improved management history minimised the fluctuation of net cumulative

CO₂ emissions between compacted and non-compacted treatments by the day 70 , while this was not the case in treatments with conventional management history (Fig. 4.1d).

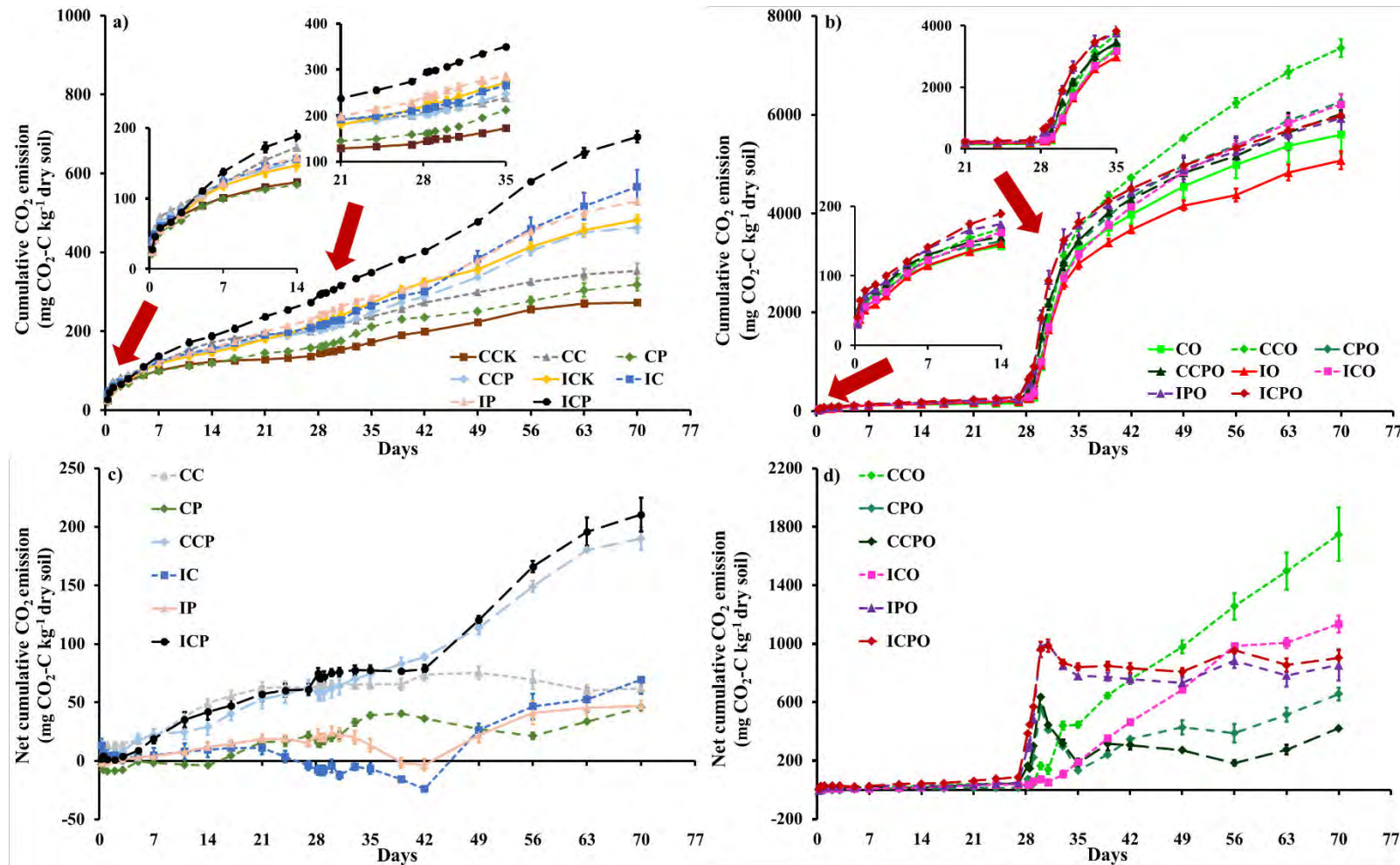


Fig. 4.1 Cumulative (a and b) and net cumulative (c and d) CO₂ emissions from different treatments during the incubation period. The values of net cumulative emission for each treatment were calculated based on their differences from their control treatment at each sampling time (i.e. CCK and ICK for treatments without organic residue amendment, and CO and IO for treatments with organic residue amendment). Description of treatments are the same as Table 4.1.

4.3.5 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 RS phase (day 28), while there was no significant difference among treatments in the first 7 days of incubation (Fig. 4.2a). The microbial activity significantly ($P < 0.05$) increased in all treatments at day 28, regardless of soil management history and compaction status. At day 35, 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. 4.2a). However, at the end of 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 at day 35 in all treatments regardless field management history (Fig. 4.2b). However, at the end of RL phase the observed values significantly ($P < 0.05$) increased in non-ploughed treatments regardless management history, while generally decreased in ploughed treatments with improved management history (IPO and ICPO).

The compaction stress significantly ($P < 0.05$) increased qCO_2 values in treatments with conventional management history, compared with non-compacted treatments, while significantly ($P < 0.05$) decreased qCO_2 values in treatments with improved management history, at day 7 (Table 4.3). However, at the end of RS phase (day 28) the compacted treatments had generally higher qCO_2 values than non-compacted 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 $q\text{CO}_2$ 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 RL phase (Day 70), the compacted and ploughed treatment (CCP) showed the highest $q\text{CO}_2$ value in treatments with conventional management history, while similar treatment with improved management history (ICP) showed the lowest $q\text{CO}_2$ value. The legume residue amendment significantly ($P < 0.05$) increased $q\text{CO}_2$ value in all treatments regardless of their management history. However, the soil with improved management history generally had lower $q\text{CO}_2$ values following legume residue amendment. In addition, the compacted and ploughed treatments (CCPO and ICPO) showed the highest $q\text{CO}_2$ values within treatments with the same management history.

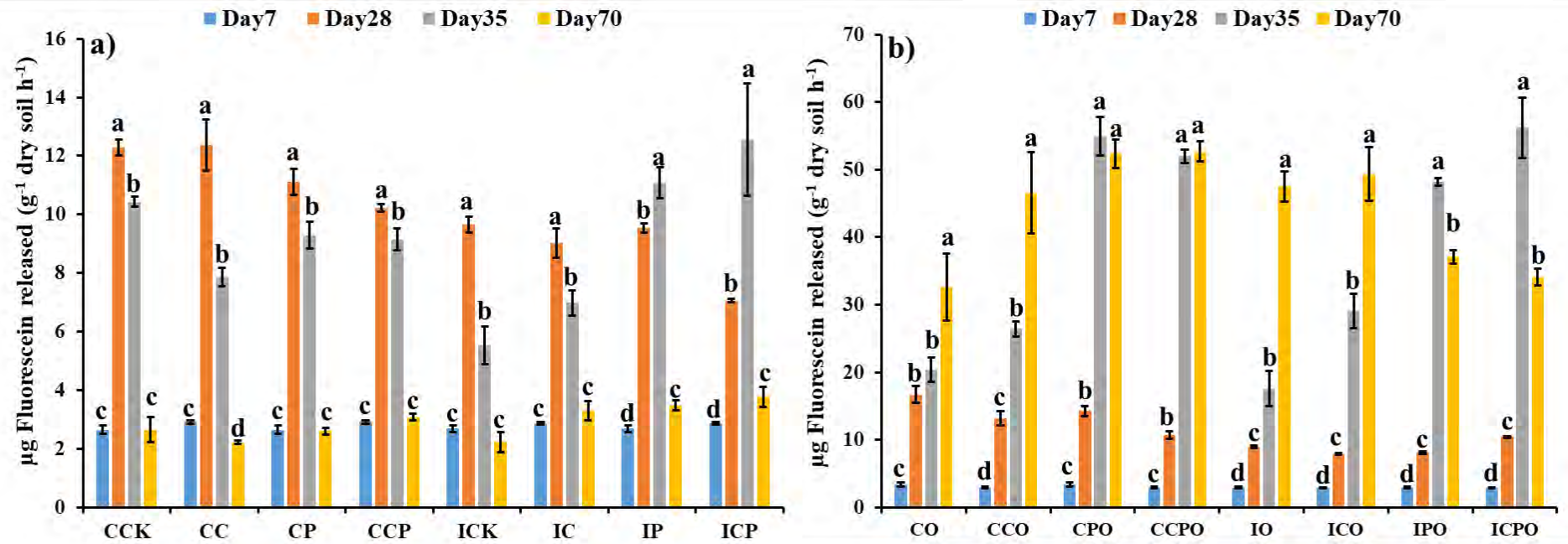


Fig. 4.2 Dynamics of soil microbial activity during the incubation period, in treatments under different management practices, without (a) and with (b) organic residue amendment. Description of treatments are the same as Table 4.1. Vertical bars are standard error of three replicates. Means of the values for different sampling events, within a treatment, with the same letter are not different at the 5% level of significance.

Table 4.3 Dynamics of soil metabolic quotient during the incubation period, in treatments under different management practices, with and without organic residue amendment.

Treatment	$q\text{CO}_2$ ($\mu\text{g CO}_2\text{-C h}^{-1} \text{mg}^{-1} \text{MBC}$)				
	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 4.1.

4.3.6 Statistical analysis

The PCA results showed that principal components (PC) PC1 and PC2 explained 27.2% - 46.1% and 22.6% - 29.5% of the data variance, respectively (Fig. 4.3). The PCA analysis was also able to clearly separate 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 parameter with the highest correlation coefficient (> 0.85) for PC2 were microbial activity, β -glucosidase, and CO₂ emissions. The treatments with conventional and improved management history were clearly separated based on PC1 and PC2 at days 7 and 28 of the experiment (RS phase; Figs. 4.3a and 4.3b). 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. 4.3c and 4.3d). Interestingly, in the first week following legume residue amendment (day 35), treatments with conventional and improved management history were clearly separated from each other based on PC1 and PC2 components (Fig. 4.3e). 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 RL phase in day 70 (Fig. 4.3f).

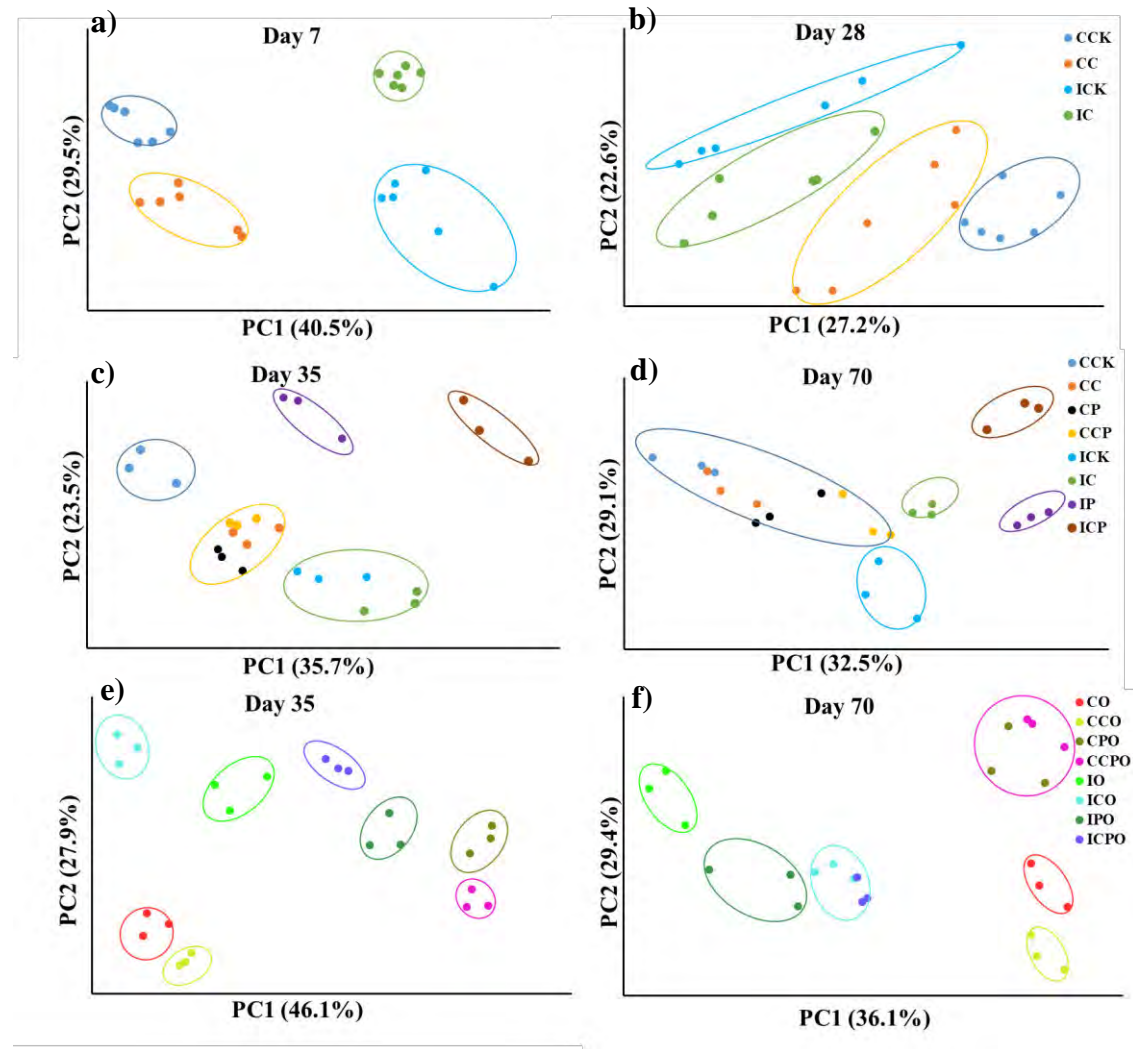


Fig. 4.3 Scores plot of principal component analysis (PCA) showing the separation of treatments under different management practices, with and without organic residue amendment, and loading values of the individual soil parameters (PC1 and PC2) for each treatment. Description of treatments are the same as Table 4.1.

The compaction stress resulted in a significantly ($P < 0.05$) lower RS index of C use efficiency ($q\text{CO}_2$) in both soils in comparison to their control treatments (CCK and ICK; Fig. 4.4a). 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 $q\text{CO}_2$ 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. 4.4b). In addition, the ploughing-only treatment with conventional management (CP) showed a significantly ($P < 0.05$) higher recovery rate of $q\text{CO}_2$ 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 legume residue amendment, all of treatments showed very low recovery rate of $q\text{CO}_2$ in both surface application and incorporation methods. Generally, surface application of legume residue resulted in higher RL index of $q\text{CO}_2$ compared with the incorporation method. The results also indicated no significant difference between RL index of $q\text{CO}_2$ 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 $q\text{CO}_2$ compared with CPO treatment (Fig. 4.4c).

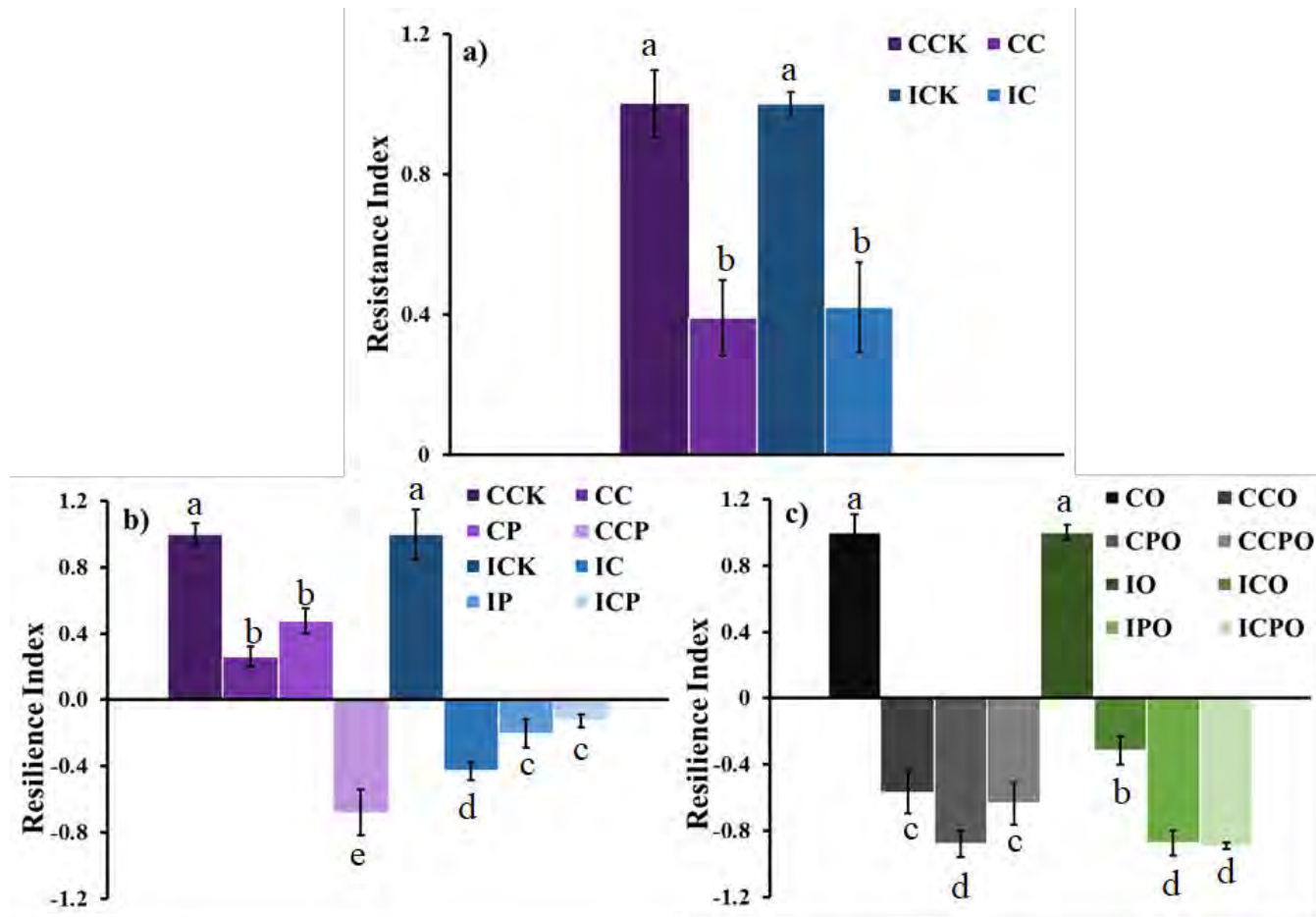


Fig. 4.4 The resistance (a; day 28) and resilience (b and c; day 70) indices of soil microbial carbon use efficiency (metabolic quotient) in treatments under different management practices, with (b) and without (c) organic residue amendment. Description of treatments are the same as Table 4.1. Vertical bars are standard error of three replicates. Means of the treatments by the same letter are not different at the 5% level of significance.

4.4 Discussion

In order to tackle issues related to the effect 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, which are able to alleviate soil compaction in the field and improve soil health and fertility (Yang et al., 2019). It has been suggested that 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 mineralization (Rogers and Burns, 1994) and soil microbial activity (Schneider et al., 2017). It has also 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.

4.4.1 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 considered as one of the best indicators

for assessment of the impacts of field management practices on soil health status (Chen et al., 2004).

This study showed that labile C was the main C source for microbial consumption, which supported by (Teutscherova et al., 2017) who indicated 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 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 indicated 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 present study, which could be confirmed by previous studies which also reported that concentration of labile C would be increased by 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 showed that concentration of labile C increased in compacted treatments, while this pattern was not clear after ploughing practice at the end of 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 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 surface application of legume residue had higher labile C content compared with legume residue incorporated treatments, within the same management history. Additionally, there was 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 experiment. This indicated that 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 showed any significant impact on concentration of HWETN among treatments. After legume residue amendment, labile N significantly increased in all treatments, which could be due to the acceleration of N mineralisation process by soil microbial community under stress relieved condition. In addition, concentration of HWETN was lower in ploughed treatments at the end of 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 concentration of HWETN was significantly lower in treatments with improved management in comparison to treatments with conventional management, at the end of RL phase and without 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 high concentration of labile C would trigger 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 showed a cumulation in concentration of soil mineral N at the end of RS phase. Also, we found that ploughing practice would increase 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 in consistent with a study which indicated that ploughing practice would result in accumulation of mineral N in soil profile (Myrbeck et al., 2012). As expected, concentration of soil mineral N was 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.

4.4.2 Microbial responses to soil compaction and its removal

Soil moisture content is considered as an important factor in regulating microbial activity and diversity (Pérez Castro et al., 2019). In the present study, we found 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 would decrease soil microbial pools (Beylich et al., 2010; Dick et al., 1988; Tan et al., 2005), however in some cases, compaction resulted 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 would indicate 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 Tadro, 2002). At the end of RL phase, we found that content of microbial biomass C increased back to its initial level in soil with improved management history in comparison to soil with conventional management history, which indicates that improved field management would help to build a higher RL against compaction stress. With legume residue amendment, we found a sharp increase in soil microbial pools in all treatments, while 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 utilization 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 concentration of MBN in comparison to conventionally managed soil, but compaction stress showed no significant impact on MBN content in soil with conventional management. 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 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 legume residue amendment, we observed 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, the 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 non-compacted treatments, regardless of their management history. Siczek and Fraç, 2012 also reported that severe compaction stress would decrease soil enzyme activity mainly by decreasing soil porosity which restricted 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 soil microbial community were more sensitive in treatments with

improved management history compared to treatments with conventional management history. Legume residue incorporation method increased soil microbial activity at the start of 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 CO₂ 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 stabilized 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 recognized 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 CO₂ 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 CO₂ emission in comparison with treatments with conventional management, but there was no significant difference in net cumulative CO₂ emissions between treatments with improved and conventional management history, by the end of RL phase. This observation indicates that the difference between treatments under the same compaction stress only resulted 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 CO₂ emission, followed by compaction-only and ploughing-only treatments. As expected, a significant increase in

CO₂ emissions was observed in all residues 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 CO₂ 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 CO₂ emission in treatments with conventional management in comparison to treatments with improved management, while the incorporation method resulted in lower net cumulative CO₂ emission 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, we found that legume residue incorporation along with improved management, minimized fluctuation of net cumulative CO₂ emissions between compacted and non-compacted treatments by the end of RL phase, while this was not the case in treatments with conventional management history. This may indicate that soil with conventional management history had dramatic responses to compaction stress following legume residue amendment. A possible explanation could be the ecological concept that the simple microbial community structure which build by conventional management practice had relative lower stability to compaction stress which further confirmed that improved management would increase complexity of soil microbial structure and its stability to compaction stress.

Our results indicated a similar response of the investigated soils to compaction stress regardless of their management history. The results also showed a higher RL in soil

with improved management history to compaction-ploughing process, as soil with conventional management was more unstable to compaction-ploughing process 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 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 RL index also indicates that the absolute value of difference between the stressed treatments and control treatment was higher at the end of incubation experiment than the end of RS phase.

4.5 Conclusion

The response of soil microbial community to compaction is governed by water content and water filled pore space, which would be stimulated by different field management practices as soil microbial community are sensitive to soil disturbance. Improved field management is able to increase soil RS and RL to compaction-ploughing process, which shown as a faster stabilization 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 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 CO₂ emissions and soil microbial activity. This confirmed that 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 the field adaptation of the proposed soil health index in this study and determination of soil microbial community composition and key functional genes involved in C and N cycling via modern molecular technologies. This would help better understanding of the mechanisms regulating soil microbial responses and soil nutrient turnovers in the compaction affected agricultural lands.

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Chapter 5 Improved field management history builds soil microbial tolerance to drought in Australian grain cropping systems

Abstract

Inappropriate anthropogenic activities in the past decades resulting in climate changes and consequently severe drought stress which in turn have led to soil degradation and crop yield decline. Soil microbes are more sensitive to drought stress and consequently affect soil C and N cycling, further reducing plant growth and yield. To mitigate the effects of drought stress on soil microbial community, it is essential to understand the mechanism of soil microbe responses against drought stress. To address this, two soils (Planosols) with conventional and improved management history were exposed to drought, and microbial responses were investigated going into stress as well as their response to removal of stress by rewetting and organic amendment application. We artificially applied three levels of water content to simulated severe drought (15% WHC), moderate drought (30% WHC) and no drought (55% WHC) condition. The present study found that drought stress decreased soil microbial C pool size by 38% to 44% and cumulative CO₂ respiration by 24% to 33% regardless of soil management history. With organic amendment application, the cumulative CO₂ respiration of control and moderate drought applied treatment had similar values in soil with conventional management history, while moderate and severe drought affected treatments in soil with improved management had similar cumulative CO₂ respiration by the end of the experiment. The present study showed that different field management practices might

not change soil microbial response patterns to drought as improved field management will not affect soil water content directly. Instead, improved field management could help soil build resilience to drought, which is shown as the tolerance to moderate drought and resistance to severe drought due to increased organic matter content and complexified microbial community structure over improved field management. Also, the organic residue application also increased the microbial biomass content and enzyme activity regardless of soil management history. This confirmed that organic residue application improved soil chemical conditions, increased the organic C and N supply for the soil microbial community, enhanced nutrient cycling processes which further improved soil health conditions.

5.1 Introduction

The World Economic Forum that estimated drought cost \$ 6–8 billion per year globally due to agricultural and related business 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 significant 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 was a deficit of water to support soil ecological functions (Sheffield et al., 2012). Drought resulted in severe soil health decline as soil nutrients in the drying soil profile became less bioavailable at the root surface when soil water

deficit restricted nutrients transport via mass flow and diffusion processes, particularly in the topsoil (Ma et al., 2015). Drought also decreases nutrient uptake of plants by root shrinkage 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 would decrease 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 would react to prolonged periods of drought, and how they recover from stress when it rains again. It is also critical to study the sustainability of soil ecosystem under drought stress and provide practical and site-specific soil management practices that farmers can apply to optimise the performance of their soils and improve crop yield when drought is removed. It is expected from a sustainable ecosystem to minimise the impact of a disturbance and to adopt and recover from the impact rapidly (Ingrisch and Bahn, 2018). Soil biological properties were 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 were 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 enzymes activities are considered as one of the

most widely used and robust biological indicators of soil health and its resistance (RS; the ability of a soil to maintain its functional stability after a disturbance) and resilience (RL; the 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 indicated 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). However, there is only limited knowledge regarding adaptation of soil microbial properties in predicting soil health condition, particularly in drought-affected grain field.

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 tillage control and lengthening and diversifying crop rotations, often by including legume crops and using crop residues and/or cover cropping for permanent ground cover (Chabert and Sarthou, 2020). Organic field management also proved to be more profitable and environmentally friendly and deliver equal or more yield that contains less (or zero) pesticide residues than conventional farming (Reganold and Wachter, 2016). Deen and Kataki, 2003 indicated that conservation field management would have 15% less cost in comparison with conventional field management. This study also reported that no-tillage field management would increase about 5% of soil organic carbon in comparison to conventional management. Vyn and Raimbult (1993) also reported 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 improved

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 nutrients 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) indicated that Bedminster compost (compost of garbage and sewage sludge) increased 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 investigated the impacts of organic residue amendments on soil properties, scientific knowledge regarding soil microbial responses to environmental disturbance like drought under different field management history remained largely uncertain. 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 re-wetting of dry soils. Also, limited information is available regarding governing factors in soil microbial community responses and changes of soil nutrient pools following organic residue amendments and during the recovery from drought stress. This information would be critical for better predicting of soil health conditions in the context of boosting crop yield and developing sustainable agriculture.

Despite widespread interest in assessing the detrimental impacts of drought and re-wetting cycles in agricultural systems, the response of the belowground nutrient turnover processes to drought stress and how the microbial communities mediate such responses were 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 the fate of soil nutrient

pools under different drought stress scenarios. Therefore, the main objective of this study was to assess the mechanisms responsible for grain farming soils' microbial functional responses to drought stress under different field management histories. The underlying hypotheses were: a) soil biological functions such as microbial respiration and microbial biomass and activity are reliable indices for assessment of soil health and resilience to drought stress; b) the response pattern of 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 practise are more resistant and resilient to drought stress than soils under monoculture practice, due to their higher nutrients bioavailability.

5.2 Materials and methods

5.2.1 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 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 of the farm and bulked as a composite sample. The soil is classified as Arenosols in Australia 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 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 (2018), Lupin (2017), Barley (2016), and Wheat (2015) were used in the improved

practice management, while monoculture of wheat used in the conventional management in the last 5 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 uniform compaction throughout the soil profile), and incubated in dark and aerobic condition at $22 \pm 0.5 \text{ }^\circ\text{C}$ for the first 28 days of the experiment (RS phase). At the end of 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 $60 \text{ }^\circ\text{C}$ 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 $22 \pm \text{ }^\circ\text{C}$ till the end of the experiment at day 70 (RL phase). The experiment was conducted in 12 treatments with total 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 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 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 1 - 4 days depending on the expected levels of CO_2 emissions) during the incubation experiment, using three randomly selected replicates of each treatment.

5.2.2 Analysis of soil chemical properties

The value of soil pH (1:5 soil to water ratio) was measured with a glass electrode method described by (Rayment and Lyons, 2011). The TC and TN contents were measured by combustion method via a LECO CNS-2000 analyzer (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 25mL of water in a capped falcon tube at 70°C for 18 hours. Tubes were shaken on an end-over-end shaker for 5 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 (Shimadzu, Kyoto, Japan) TOCN analyzer. 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 1h 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.

5.2.3 Analysis of soil biological properties

Soil microbial biomass C (MBC) and N (MBN) contents were measured by the 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). Three replicates of each treatment were placed at each gas sampling event into 2 L airtight glass jars that were then continually flushed with ambient compressed air for 1 minute. Gas samples were collected from the headspace of the glass jars 8 hours after closure using a 25 mL gas-tight syringe and immediately transferred to pre-vacuumed 12mL glass vials (Labco, UK). Gas samples were measured for CO₂ concentration using a gas chromatograph (Shimadzu GC-2010 Plus) method. Linearity tests on CO₂ concentration increases were conducted on all treatments over a subset of sampling times during the incubation. These samples were taken initially after the glass jars closed 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 ($q\text{CO}_2$), defined as the C respired per unit of MBC per day, and calculated from the ratio of CO₂-C emission ($\mu\text{g CO}_2\text{-C kg}^{-1}\text{ soil day}^{-1}$) to concentration of MBC ($\text{mg C kg}^{-1}\text{ soil}$) 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 various environmental samples, including soils at pH 7.6 (Green et al., 2006) and was used to measure soil microbial activity in this study.

5.2.4 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):

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

Where, 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:

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

Where, 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.

5.2.5. Statistical analysis

Significant differences were considered at $P < 0.05$ between treatments and all variables were tested for normality of distribution using the Kolmogorov–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 CO₂ 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 RS phase).

5.3 Result

The investigated soils' basic properties (pH, TC, and TN) 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 significantly ($P < 0.05$) higher in treatments with improved management than treatments with conventional management history.

5.3.1 Labile organic C and N pools

Table 5.1 showed 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 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 5.1). This may indicate the higher sensitivity of the soil with improved management history to drought stress. At day 35, the IS treatment had the highest concentration of HWEOC, while conventional management treatments showed significantly ($P < 0.05$) lower concentrations among the treatments without wheat residue amendment. However, no significant differences had been 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 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 (hot water extractable total N) 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 5.1). After removal of drought stress at day 28, no significant difference was observed among all treatments without wheat residue amendment till the end of experiment. However, in treatments with wheat residue amendment, concentration of HWETN was significantly ($P < 0.05$) higher in severe drought treatment (ISO) than 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 5.1). At the end of RL phase (day 70), improved management treatment with severe drought stress (ISO) showed the highest concentration of HWETN in comparison with other treatments.

Table 5.1 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 start with letter C referred 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 start with letter I referred 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 $P < 0.05$.

5.3.2 Mineral N pool

Concentration of $\text{NH}_4^+\text{-N}$ was generally higher in treatments with improved management history. However, concentration of $\text{NH}_4^+\text{-N}$ 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, present study showed that removal of severe drought stress (CS and IS treatments) without wheat residue amendment, resulted in the highest concentration of $\text{NH}_4^+\text{-N}$ but there was not significant difference between control and moderate drought stress treatments regardless of the management history. Interestingly, significant ($P < 0.05$) differences observed between severe (CS) and moderate (CM) drought stress treatments in conventional management history, while in treatments with improved management history severe drought stress (IS) and other two (IC and IM) treatments showed a significant ($P < 0.05$) difference in $\text{NH}_4^+\text{-N}$ concentration. At the end of experiment, concentration of $\text{NH}_4^+\text{-N}$ was higher in IS treatment that 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 showed that the IMO treatment had the highest concentration of $\text{NH}_4^+\text{-N}$, while the CSO treatment had the lowest concentration at day 35. At the end of experiment, the removal of severe drought stress (CSO and ISO) resulted in the highest concentration of $\text{NH}_4^+\text{-N}$ within treatments with the same soil management history.

In general, concentration of was higher in treatments with improved management history throughout the entire incubation experiment. However, there was a sharp decrease in $\text{NO}_3^-\text{-N}$ concentration after wheat residue application to both of 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) tend to have the lowest concentration of NO_3^- -N within the same management history at the end of 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, till the end of 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 observed among treatments with the same management history, although improved management treatments tend to have higher concentration of NO_3^- -N in comparison with conventional management treatments.

5.3.3 Soil microbial properties

In general, concentration of microbial biomass C (MBC) was higher in control treatments (CC, IC, CCO and ICO) throughout experimental period, regardless of management history (Table 5.2). 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 day 28. 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. Concentration of MBC kept increasing in treatments with conventional management, while it became stabilized 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, concentration of MBC decreased with increasing of drought stress level in both soils regardless of their management history, although concentration of MBC was higher in treatments with improved management practice. At the end of 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, concentration of MBC in treatments with improved management history slightly decreased by the end of the experiment (day 70).

In general, the present study showed that drought stress significantly ($P < 0.05$) decreased concentration of microbial biomass N (MBN) in treatments received conventional management, while there was no significant difference in soil received improved management till the end of RS phase (day 28). After removal of drought stress, there was no significant changes in soil with conventional management by day 35, but a sharp increase occurred in stressed treatments (CM and CS) at day 70 (Table 5.2). Interestingly, improved management treatments showed a different pattern after removal of drought stress. We found that drought stress did not show a significant impact on concentration of MBN in soil with improved management at day 35. However, the stressed treatments (IM and IS) showed significantly ($P < 0.05$) higher concentration of MBN than control (IC) by the end of experiment (day 70). Table 5.2 also showed that severe drought stress (IS) had the highest concentration of MBN, while the control treatment (IC) had the lowest concentration. There was a significant

($P < 0.05$) increase in concentration of MBN after wheat residue application, regardless of management history at day 35, while the concentration of MBN decreased with increasing of drought stress level. Interestingly, at day 70, 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.

5.3.4 Soil β -glucosidase activity

Overall, activity of β -glucosidase increased over time till the end of incubation (Table 5.2). 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 level resulted in reduction of the activity of β -glucosidase at the day 28, regardless of soil management history. After removal of drought stress, no significant differences observed among treatments with conventional management history, while significantly ($P < 0.05$) increased activity of β -glucosidase observed in severe drought treatment (IS) with improved management history at day 35. At the end of experiment, recovery from drought stress increased activity of β -glucosidase regardless of stress levels and soil management history. With wheat residue application, there was no significant difference among treatments at day 35. This may indicate that addition of organic matter would boost β -glucosidase activity and mask the impacts caused by drought stress. At day 70, no significant difference found among treatments with improved management history, while β -glucosidase activity was higher in severe drought stressed treatment (CSO) than the control treatment (CCO) in conventionally managed treatments.

Fig. 5.1 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 experiment, while this pattern was not clear in severe drought stressed treatment (CS) with conventional management (Fig. 5.1a). Additionally, total microbial activity increased in a short period after removing the stress in improved management treatments, but decreased at the end of experiment, although this pattern was not clear in severe drought stresses treatment (IS). With 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 experiment (Fig. 5.1b). This pattern observed in all treatments regardless of soil management history and drought stress levels.

5.3.5 CO₂ and net CO₂ respirations

In general, treatments with improved management had significantly ($P < 0.05$) higher cumulative CO₂ 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 CO₂ respiration regardless of soil management history (Fig. 5.2a). Also, there was a significant ($P < 0.05$) increase of cumulative CO₂ respiration in severe drought stressed treatments at the end of RS phase (day 28), while this pattern was not clear in control (CC and IC) and moderate stressed treatments (CM and IM), regardless of soil management history. Additionally, the differences in cumulative CO₂ respiration were smaller between drought stressed treatments and control in soil with conventional

management than soil with improved management history (Fig. 5.2c). This may indicate that soil with improved management was more sensitive to changes in soil moisture levels, which confirm the observed higher microbial property levels, such as higher MBC and enzyme activities, when there were no external organic matter inputs. According to Fig. 5.2b, wheat residue amendment significantly ($P < 0.05$) increased cumulative CO₂ respiration, regardless of soil management history and drought stress levels during the RL phase of the experiment. The cumulative CO₂ respiration of 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 CO₂ respiration by the end of experiment. This may indicate that severe drought may decrease C use efficiency in soil with conventional management history, while soil with improved management history could tolerate moderate and severe drought which further indicate a higher drought resilience in comparison to soil with conventional management. Interestingly, we found that the differences of cumulative CO₂ decreased at day 56 but increased at the end of experiment (day 70) in both drought stressed treatments (CMO and CSO) with conventional management. However, the net cumulative CO₂ emissions of soil with improved management history decreased throughout the experiment after wheat residue amendment (Fig. 5.2d). This may indicate that soil with conventional management history tends to have lower resistance and higher recovery toward drought following wheat residue application.

Table 5.2 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 (μ g p-nitrophenol h ⁻¹ g ⁻¹ dry soil)				q CO ₂ (μ g 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
	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 5.1.

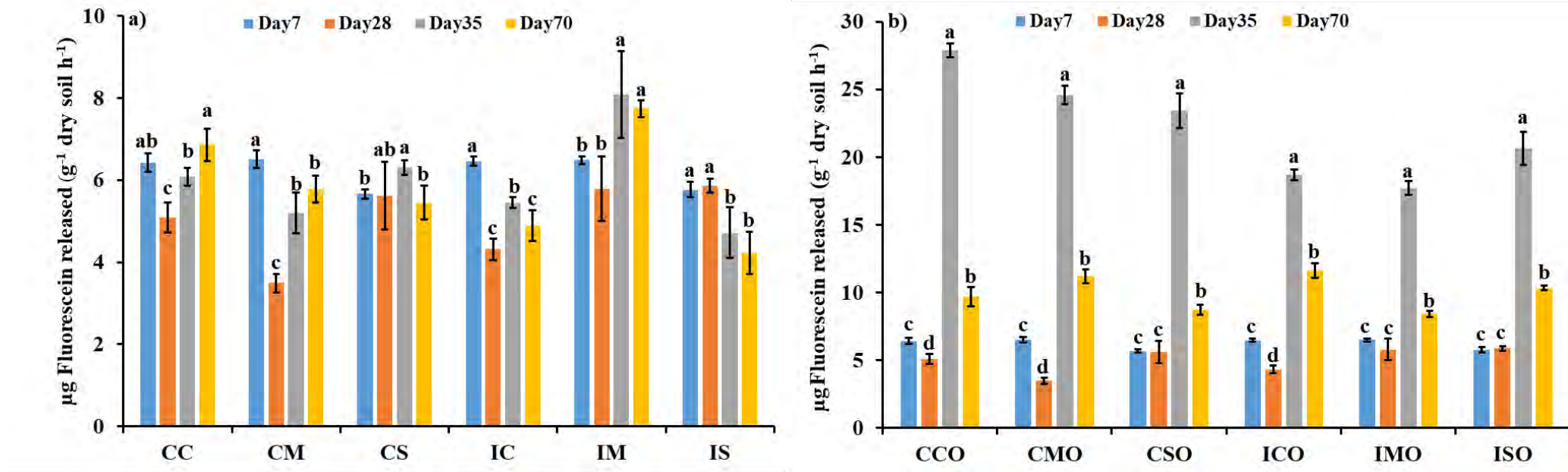


Fig. 5.1 Dynamics of soil microbial activity during the incubation period, in treatments under different management practices, without (a) and with (b) organic residue amendment. Description of treatments are the same as Table 5.1. Vertical bars are standard error of three replicates. Means of the values for different sampling events, within a treatment, with the same letter are not different at the 5% level of significance.

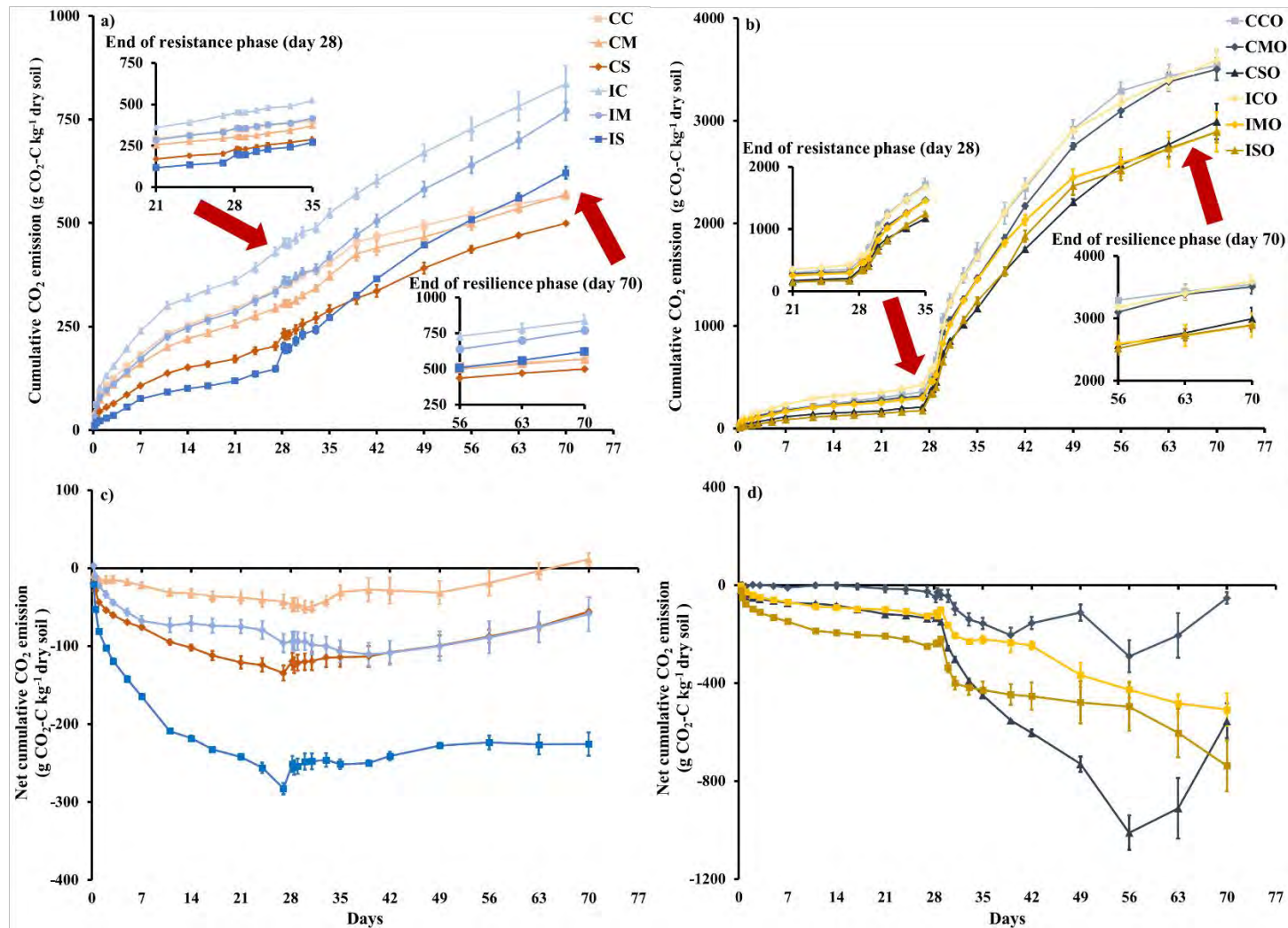


Fig. 5.2 Cumulative (a and b) and net cumulative (c and d) CO₂ emissions from different treatments during the incubation period. The values of net cumulative emission for each treatment were calculated based on their differences from their control treatment at each sampling time (i.e. CC and IC for treatments without organic residue amendment, and CCO and ICO for treatments with organic residue amendment). Description of treatments are the same as Table 5.1. Vertical bars are standard error of three replicates.

5.3.6 $q\text{CO}_2$ values

Table 5.2 shown clearly that $q\text{CO}_2$ value increased in moderate drought level regardless management history at day 7. There was an increase of $q\text{CO}_2$ happened at day 28 regardless soil drought level and management history. Without wheat residue application, soil had improved management history has significant lower $q\text{CO}_2$ level in comparison to soil has conventional management history at day 35 but higher $q\text{CO}_2$ level at the end of experiment (day 70). It is important to mention that $q\text{CO}_2$ level tend to be higher in moderate drought affected treatments regardless soil management history. This may indicate that soil responses to drought would not be affected by soil management history. With wheat residue application, there was an increase of $q\text{CO}_2$ found in all treatments after drought removal while there was no significant difference found among treatments within same soil management history, although $q\text{CO}_2$ level in conventional managed soil significantly ($P < 0.05$) higher than in improved management managed soil. Similar to no wheat residue applied treatments, soil had improved management history has significant higher $q\text{CO}_2$ level in comparison to soil has conventional management history at the end of experiment (day 70). This may indicate that application of wheat residue would increase $q\text{CO}_2$ values but had no impact on soil response process to drought stress.

5.3.7 Relationships between soil physicochemical and microbial properties and the PCA analysis

The correlation coefficients (r) between soil physicochemical and biological properties ($n=144$) are shown in table 5.3. The results indicated 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 5.3 confirmed that soil total microbial activity

(FDA), β -glucosidase activity and cumulative CO₂ respiration were highly related to soil labile pools and mineral N contents ($P < 0.01$).

The PCA results showed that principal components (PC) PC1 and PC2 explained 34.6% - 57.6% and 12.8% - 28.7% of the data variance, respectively (Fig. 5.3). 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 CO₂ respiration. The treatments with conventional management clearly separated based on PC1, while treatments with improved management clearly separated based on PC2 at day 7 (Fig. 5.3a). At day 28, soils under both management history were clearly separated based on PC1 (Fig. 5.3b). After removal of drought stress, soils with both management history were clearly separated based on PC2 at day 35 and day 70 (Fig. 5.3c and 5.3d). Interestingly, with wheat residue application, treatments with conventional management history clearly separated based on PC1, while treatments with improved management history 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 (Fig. 5.3e and 5.3f). According to Fig 5.4a, it showed that RS index of C use efficiency would be decreased by increasing drought stress regardless soil management history. This may indicate that response of soil microbe to drought stress is govern by water content rather than soil management history. This also proved in Fig. 5.4b, without wheat residue input, soil with conventional management history and soil with improved management history had similar pattern in response to drought stress level. With the presence of wheat residue, soil with improved management history had significant higher RS index

of C use efficiency in comparison to soil with conventional management history (Fig. 5.4c).

Table 5.3 Correlation coefficients (r) between treatments' physicochemical and biological properties during the incubation period (n=144)

	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)

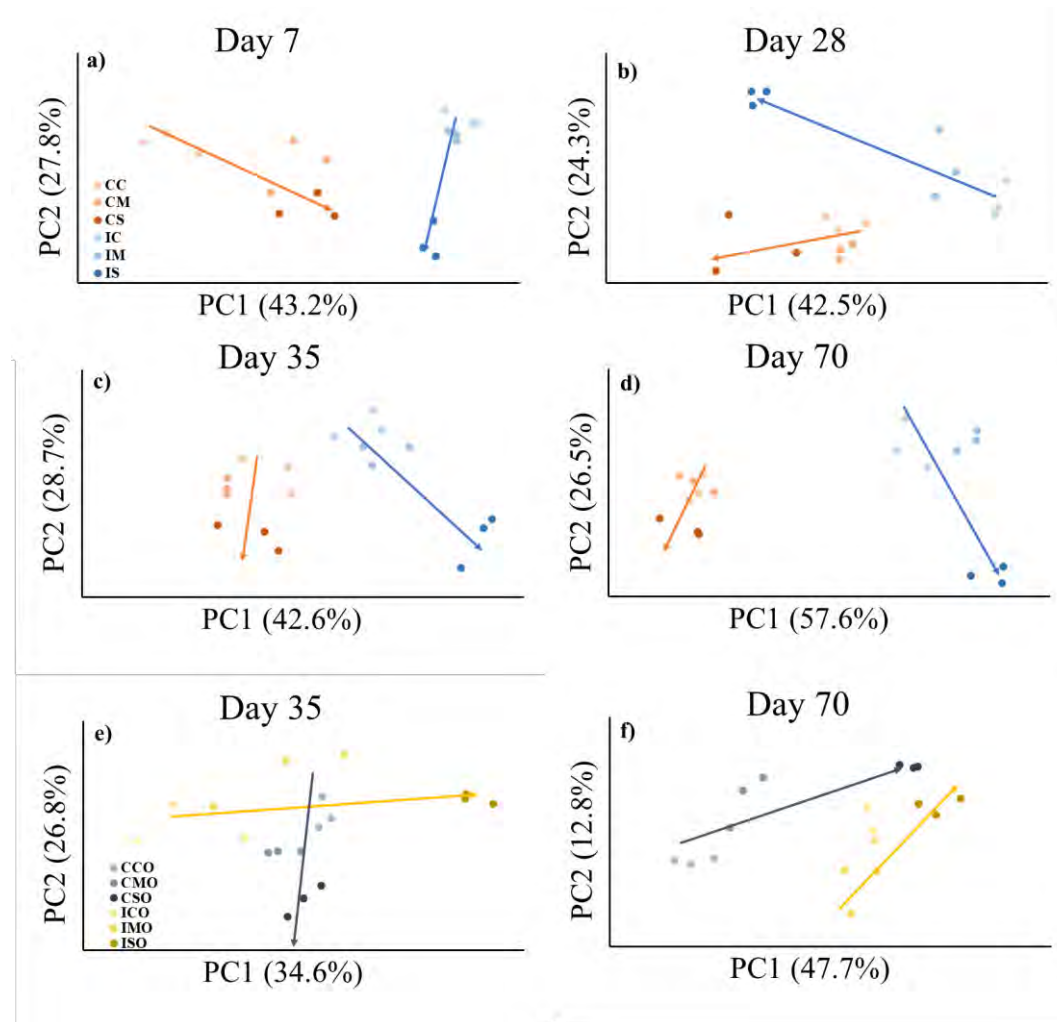


Fig. 5.3 Scores plot of principal component analysis (PCA) showing the separation of treatments under different management practices, with and without organic residue amendment, and loading values of the individual soil parameters (PC1 and PC2) for each treatment. Description of treatments are the same as Table 5.1. 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.

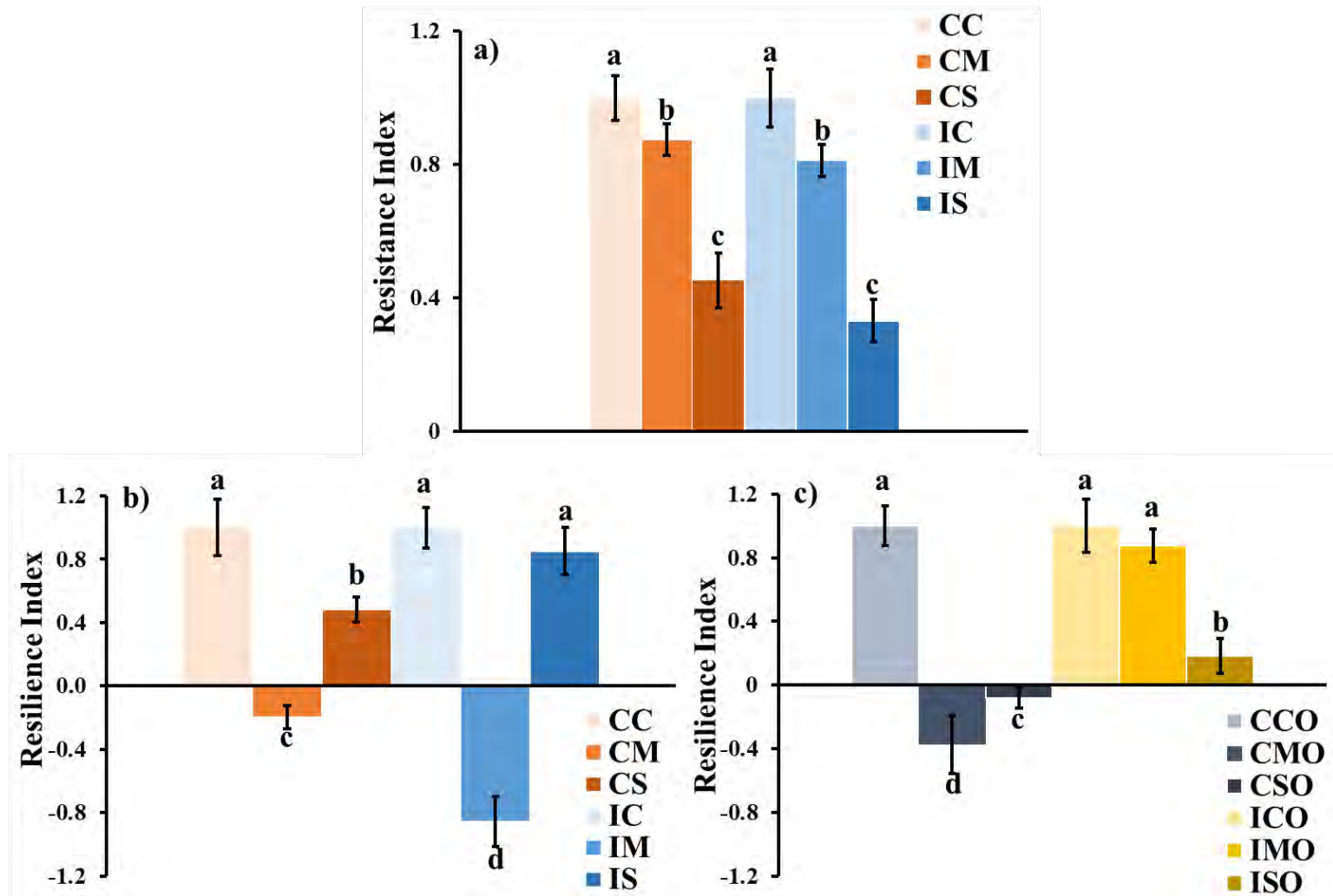


Fig. 5.4 The resistance (a; day 28) and resilience (b and c; day 70) indices of soil microbial carbon use efficiency (metabolic quotient) in treatments under different management practices, with (b) and without (c) organic residue amendment. Description of treatments are the same as Table 5.1. Vertical bars are standard error of three replicates. Means of the treatments by the same letter are not different at the 5% level of significance.

5.4 Discussion

To address issues related to the effects of drought stress on the 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 organic residue amendment can ameliorate drought stress and improve soil health through increasing water retention, reducing soil acidity, increasing soil microbial activity and increasing bioavailability of soil organic matter (Jeffery et al., 2011). In addition, agricultural field management practices such as ploughing may increase crop yield by improving 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 increase 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.

5.4.1 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 assessing soil health. 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

recognized as soil labile C and N pools and are considered as the best indicators for the effects of field management practices on soil health condition (Chen et al., 2004). The present 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 due to the soil microbial community consumption. A study by Liu et al. (2018) also suggested that labile C was consumed by 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 soil labile pool in improved management practice that could further provide a better environment for soil microbial community. Application of 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 showed that labile C accumulated in severe drought stressed treatments after wheat residue input. 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) reported higher organic matter content in severe drought stressed soil and indicated that organic amendments can improve the size and activity of soil microbial community under stress conditions. In addition, moderate drought stress showed no significant impact on concentration of labile C in soil received improved management practice compared to soil received conventional management, which

indicates that proper management practices and the application of organic residues could help improve soil resilience against moderate drought stress.

In terms of HWETN, an overall downward trend was observed in all treatments before removal of drought stress at the end of RS phase (day 28). After removing drought stress, no significant difference was observed among all treatments without wheat residue amendment until the end of the experiment. However, after wheat residue application, labile N content was significantly ($P < 0.05$) higher in severe drought (ISO) than control (ICO) in treatments with improved management history, while there was no significant difference among conventionally managed treatments regardless of drought stress level. The labile N content was relatively higher in soil that received improved management in comparison with soil that received conventional management. This also confirmed that proper management practices could help to improve soil resilience against drought stress in intensive grain cropping systems.

This study showed an increase in concentration of soil mineral N during the RS phase. In addition, concentration of NH_4^+ -N showed a slight increase after wheat residue application at the start of RL phase. It has been reported that soil mineral N concentration would increase following organic amendment to soil (Eghball et al., 2004). Interestingly, concentration of NO_3^- -N slightly increased in treatments without wheat residue amendment while 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 emission contributed by mineralization of soil organic matter, which is consistent with previous observations reported in a field study conducted in Hunan of China (Wu et al., 2017).

5.4.2 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, we found that drought stress decreased soil microbial C pool size regardless of its management history. This observation indicates that proper field management practices would increase nutrient pools for soil microorganisms while not altering the soil microbial responses to drought stress. In addition, following the removal of drought stress at the start of 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 CO₂ respiration data, we found that conventionally managed soil had higher microbial pools and lower cumulative CO₂ respiration than improved practice managed soil. This indicates that C use efficiency was higher in soil with conventional management than soil with improved management practice. With wheat residue application, we observed a sharp increase in soil microbial pools in all treatments, while MBC content decreased with increasing drought stress level. This observation indicated that plant residue input would increase nutrient pools for soil microbial community as soluble organic C and N in soil would provide an available energy source for utilization by soil microorganisms. This may further increase 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 practice while significantly decreased MBN content in treatments with conventional practice.

After removing drought stress, MBN content immediately increased in treatments had improved management history when there was no external wheat residue input. With wheat residue application, we observed a sharp increase of microbial pools in all treatments, while MBN contents decreased with increasing of drought stress level. Interestingly, by the end of RL phase, we observed that severe drought stress increased MBN content in conventional management treatment (CSO) but decreased its content in improved management treatment (ISO).

Field application of soil organic amendments has significantly increased soil enzyme activity (Albiach et al., 2000). In the present study, the β -glucosidase activity tended to be higher in the treatments with improved management history than the treatments with conventional management at the beginning of the RS phase. Also, wheat residue application significantly increased activity of β -glucosidase regardless of soil management history and drought stress level. 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) showed that soil organic C content significantly correlated 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 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 RL phase, soil microbial activity increased till the end of experiment, but this pattern was not clear in severe drought stressed treatment (CS) under conventional management without wheat residue application (Fig. 5.1a). 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 wheat residue application, microbial activity significantly boosted at day 35 and then decreased by the end of experiment (Fig. 5.1b). 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 suggest a partial alleviation of constraint during the RL phase of the experiment.

Soil microbial respiration has been increasingly recognized as an indicator of soil fertility and health to field management practices (Bastida et al., 2008). By the end of the RL phase, significant differences in cumulative CO₂ respiration were observed among all treatments in the present study. Interestingly, cumulative CO₂ respiration decreased with increasing drought stress levels regardless of soil management history, which can be attributed to the decrease in soil MBC pool and microbial activity by increasing drought stress level. In addition, the present study showed that treatments with conventional management history had similar net CO₂ respiration pattern 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 CO₂ 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 CO₂ 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 found that cumulative CO₂ respiration of control and moderate drought stress treatments had similar values in soil with conventional management history, while moderate and severe drought stress treatments in soil with improved management had similar cumulative CO₂ respiration (Fig. 5.2b). This may indicate that severe drought stress decreased C use efficiency in soil with conventional management history, while soil with improved management history responded similarly to both moderate and severe drought stress which further indicates a higher drought resilience in comparison to soil with conventional managements. Interestingly, we found that difference of cumulative CO₂ decreased at the beginning but increased at the end of the experiment in both drought-stressed treatments with conventional management. However, net cumulative respiration of soil with improved management history was different from soil with conventional field management as it was decreasing throughout the RL phase after wheat residue application. This may indicate that soil with conventional management history had slightly lower resistance but significantly higher recovery from moderate drought stress following wheat residue application. A possible explanation could be an ecological concept that simple ecosystems have lower resistance but higher recovery from moderate but not severe environmental stresses, which further confirm that proper field management practices would increase complexity of soil microbial structure and resilience to severe drought stress.

5.6 Conclusion

The present study has clearly shown that water content regulates the response of soil microbial communities to drought, and the field management would amplify these

responses. Improved field management could help soil build resilience to drought, which is shown as the tolerance to moderate and severe drought due to their high organic matter content and complexity of microbial community. However, the soil under conventional field management would have a lower resistance but higher recovery to moderate drought stress. Also, this study 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. Furthermore, the HWEOC pool was highly related to the cumulative CO₂ respiration and microbial activity. This confirmed that organic residue application improved soil chemical conditions, increased the organic C and N supply for the soil microbial community, enhanced nutrient cycling processes, and improved soil health conditions. Future research should focus on determining microbial community composition shifting to better understand mechanisms for microbial responses and nutrient dynamics in the drought-affected grain cropping system. Furthermore, field study is necessary to bring this lab-induced index into practical level.

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Chapter 6 Responses of soil nutrients and microbial activity to different mill-mud applications in a compaction-affected sugarcane field

Abstract

Remove of compaction and organic amendment are commonly used to mitigate the compaction-induced declines in crop yield, soil carbon and soil health. However, the response of microbial activities and nutrient pools to the combination of mill-mud amendments and decompaction in soil profile are largely unknown. A field trial was conducted at Burdekin, Australia, to investigate the effects of different decompaction managements on soil nutrient cycling, associated biological activities and sugarcane yield. This experiment includes four treatments comprising: control (CK, without mill-mud), mill-mud shallow furrow (MS), deep trench without mill-mud (DT) and deep trench mill-mud application (MD). Surface mill-mud application increased concentration of soil Colwell P six-fold in comparison to the control. Deep trench application of mill-mud increased concentrations of hot water extractable organic C (HWEOC) by 30-70% and hot water extractable total N (HWETN) by 30-90% at the application depth. Soil microbial biomass C and N were also higher in mill-mud applied layers. As expected, in comparison to the control, mill-mud applied treatments increased plant cane yield by 7% (MS treatment) and 14% (MD treatment). The deep trench without mill-mud (DT) practice also increased the plant cane yield by 11% compared to the control, which may be due to release of native organic C. Deep trench combined with mill-mud application (MD) increased the supply of organic C and N and

nutrients to the microbial community within the entire soil profile, enhanced nutrient cycling processes, improved soil environmental conditions and soil health for sugarcane growth and thus increased sugarcane productivity.

6.1 Introduction

Compaction, a type of soil disturbance which commonly occurs in heavy machinery over-used fields, leads to severe crop yield loss and soil health decline worldwide (Shah et al., 2017). Compaction may substantially impact soil functions, such as restricting root growth and limiting soil water and nutrient availability. For example, it has been reported that 20% of sugarcane yield losses were due to compaction in Bundaberg, Australia (Bell et al., 2002), 12-23% wheat grain yield decline due to compaction has been reported in a field study conducted in Morocco (Oussible et al., 1992) and an average of 16% sugarbeet yield decline has been reported in Hungary (Lipiec et al., 2003). The effect of compaction on crop productivity, soil carbon (C) and nutrient pools and dynamics has been assessed in a range of field studies (Colombi and Keller, 2019; George et al., 2011; Shukla et al., 2020). It has been reported that compaction, excessive tillage and the depletion of soil organic matter are the main contributors to sugarcane (*Saccharum officinarum*) (Garside et al., 2005), maize (*Zea mays*) (Chen and Weil, 2011) and spring barley (*Hordeum vulgare*) (Czyż, 2004) yield decline. However, the response of soil microbial activity to compaction removal practices has been less explored. Such studies could provide some insight into the underlying mechanisms and improve the knowledge of field managers to enhance their decision making in applying appropriate field management practices to minimize the effects of compaction.

Adequate soil bulk density, organic C and nutrient pools support soil microbial performance and further determine soil health conditions (Flavel and Murphy, 2006;

Magdoff and Weil, 2004). High soil bulk density (higher than 1.47 g cm^{-3} for clay soil; higher than 1.8 g cm^{-3} for sandy soil) affects soil health condition by reducing soil porosity which decreases soil microbial activity and restricts root growth (Li et al., 2002). Labile C, which is largely produced by organic matter decomposition processes, is considered the main food source of soil microbes (Valenzuela-Solano and Crohn, 2006). Labile C limitation or organic matter depletion may result in soil health decline (Liu et al., 2018). While the importance of mineral nutrients for crop growth has been recognized, organic nutrients are also highly related to crop yield and soil health condition (e.g., Liu et al., 2018). Brackin et al. (2015) highlighted that the organic form of N was more suitable for plant root uptake, while inorganic N concentration in soil was, to some extent, overestimated for plant nutrition due to its extraction method. Organic P may not be directly utilized by plants, however, enhancing microbial activities would increase P mineralization, thus enhancing P availability (Gilbert et al., 2008). In addition to other physical (e.g., soil bulk density) and chemical indicators (e.g., soil nutrient pools and availability), soil microbial biomass C and N, microbial activities and the bacterial-to-fungal ratio are also considered important and sensitive soil health indicators because these parameters can change rapidly in response to shifts in environmental conditions (Bhandari et al., 2018; Bünemann et al., 2018). As a key driver of nutrient cycling, the soil microbial community plays a vital role in soil C mineralization (Nicolardot et al., 1994). A measure of overall microbial activity was suggested to be an important indicator of the impacts of field management practices on soil health (Arias et al., 2005; Schloter et al., 2003).

In recent decades, sugarcane yield decline has occurred in many sugarcane growing regions in Australia due to compaction and inappropriate field practices. Depletion of soil organic matter and overuse of heavy machinery in the field are reportedly

responsible for declines in soil structure and sugarcane yield, particularly in monoculture sugarcane-growing areas (Garside et al., 2005; Pankhurst et al., 2003). To prevent further yield loss and soil health decline, the application of organic amendments such as mill-mud (a primary by-product from sugar production) or compost has been suggested to increase soil organic matter, reduce soil bulk density and improve soil structure (Ishak and Brown, 2018; Pattison et al., 2011; Qureshi et al., 2007). Mill-mud application was an efficient way to increase sugarcane yield by improving soil physical, chemical and biological properties (Gilbert et al., 2008; Morris et al., 2007; Naidu and Syers, 1992). McCray et al., 2015 reported that broadcast application of mill-mud significantly increased sugarcane yield via boosting soil nutrients concentrations in a Florida sandy soil. Several studies worldwide have indicated that mill-mud application substantially increased soil organic C and nutrients transformation by providing substrates for microbial utilization (Fauci and Dick, 1994; Lima et al., 2009; Tan, 2009). It has been suggested that the mill-mud can also modify soil microbial pool size and microbial carbon use efficiency in a poorly structured soil (Fang et al., 2020). Hence, mill-mud application is considered one of the most suitable field practices to ameliorate or remove compaction impacts. Based on the above discussion, more attention should be paid to the response of microbial activities and changes in soil nutrient pools to organic amendments used for removing compaction. This will enable better predictions of soil health condition in the context of boosting crop yield and developing sustainable agriculture.

This study aimed to investigate and compare the responses of soil microbial activity and nutrient pools to different compaction removal practices, such as deep trenching mill-mud application and shallow furrow application, in sugarcane fields. The following underlying hypotheses were tested: a) the application of mill-mud would

increase soil nutrient pools including total and labile pools, b) different application methods (shallow furrow application and deep trench application) would lead to different distribution patterns of organic C and nutrients at different depths in the soil profile; c) mill-mud application and soil compaction stress removal would increase the size of the microbial community and microbial activity which further increase soil health condition and sugarcane yield.

6.2 Material and method

6.2.1 Site description and field treatments

The experimental site was located in a sugarcane producing area, Burdekin (19°30'S, 147°20'E), Queensland, Australia. The soil is a Mesonatric Brown Sodosol (Isbell, 2016) which is also classified as Solonchaks based on FAO world reference base (WRB, 2014). The mean annual temperature is 29.2°C and mean annual precipitation is 741.0 mm (Australia Bureau of Meteorology, 2021). The experimental field has been cropped with sugarcane for the past 15 years. As a common practice, fine agricultural gypsum was applied to a depth of ~10 cm using the broadcasting method. Gypsum was applied to improve soil structure and calcium (Ca) concentration (5 tonnes per hectare). Soil at this field site has been affected by compaction due to routine use of heavy machinery (e.g., harvester) for management and harvesting. Organic amendments (e.g., application of mill-mud) have been used to remove compaction constraints by some farmers. In this study, there were four treatments including: a) control treatment (CK, without application of mill-mud); b) mill-mud shallow furrow (ca. 20 cm depth) addition treatment (MS, mill-mud shallow furrow); c) deep trench treatment (DT, deep trench without mill-mud); d) deep trench mill-mud application (ca. 40 cm depth) treatment

(MD, deep trench with mill-mud application). Mill-mud was applied in January 2018 at a rate of 35 tonnes per hectare (dry weight based) and sugarcane harvested in July 2019. Amendments were applied to the targeted subsoil levels (ca. 20 cm depth and 40 cm depth) in each trench two weeks before mung bean (*Vigna radiata* (L.) Wilczek) planting using a large trencher and conveyer spreader to drop mill-mud into trenched slots in the area where the cane row will be planted. Mill-mud materials (a mixture of mill-mud and mill-ash) were slightly acidic (pH = 6.4) and mill-mud nutrients include: C (9.1% of dry matter), N (0.42% of dry matter), P (0.73% of dry matter) and K (0.61% of dry matter).

Soil sampling took place in July 2019 with six replicates from each treatment. Adjacent CK (26.05 ha), MS (26.05 ha), DT (8 ha) and MD (8 ha) treatments were divided into six subplots. The soil cores (5) were randomly sampled with a 5 cm diameter auger and divided into four layers of 0–10 cm, 10–20 cm, 20–40 cm and 40–60 cm depth in each subplot and bulked together to make a composite sample. Fresh soil samples were sieved (<2 mm) and stored at 4 °C prior to chemical and biochemical analyses (within 1 week).

6.2.2 Soil physicochemical analysis

The soil sand, silt and clay contents were measured by Maxing sizer (Malvern Panalytical, UK). Soil bulk density was estimated by soil weight divided by its volume using the method described by (Maynard and Curran, 2007). Soil pH and EC values were measured in a 1:5 volumetric suspension of soil to distilled water (Rayment and Lyons, 2011). Soil mineral N (NH_4^+ -N and NO_3^- -N) was extracted by 2 M KCl at a 1:5 ratio of soil to extractant using an end-over-end shaker for 1 h, filtered by a Whatman 42 filter paper (Rayment and Lyons, 2011) with concentrations of NH_4^+ -N and NO_3^- -

N determined by a SEAL AA3 Continuous Segmented Flow Analyzer (SEAL Analytical Limited, USA). Soil Colwell P was extracted by 0.5 M NaHCO₃ at a 1:5 ratio of soil to extractant using an end-over-end shaker for 16 h, filtered by a Whatman 42 filter paper (Rayment and Lyons, 2011) with concentrations of Colwell P measured by a SEAL AA3 Continuous Segmented Flow Analyzer (SEAL Analytical Limited, USA). Soil total C (TC) and N (TN) contents were measured by the combustion method using a LECO CNS-2000 analyzer (LECO Corporation, MI, USA). Soil total P was measured using an inductively coupled plasma optical emission spectrometer after digestion (ICP-OES; Perkin Elmer; Optima 8300). Hot water extractable organic C (HWEOC) and hot water extractable total N (HWETN) 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 h. After incubation, the tubes were shaken on an end-over-end shaker for 5 min 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 analyzer. All results were expressed on an oven-dry basis.

6.2.3 Soil biological analysis

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). Concentrations of soluble C and N of the fumigated and nonfumigated soil samples were determined using a SHIMADZU TOC-VCSH/ CSN TOCN analyzer. 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 and was used to measure soil microbial activity in

this study (Green et al., 2006). The activities of soil β -glucosidase (hydrolysing cellulose to glucose) at pH 6.0 and soil phosphatase (hydrolysing phosphomonoesters) at pH 6.5 were measured according to methods described by Tabatabai (1994).

6.2.4 Statistical analysis

Univariate analysis of variance (ANOVA) was used for soil properties data using the IBM SPSS Statistics 23 software package (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp., Released 2013). The differences at $P \leq 0.05$ between treatments were considered statistically significant and all variables were tested for normality of distribution using the Kolmogorov-Smirnov test. Pearson linear correlations were used to describe the relationships between soil properties. In addition, data on all soil properties were analysed using principal component analysis (PCA) to distinguish the effects of mill-mud addition and deep trench application on soil.

6.3 Results

6.3.1 Soil physical and chemical properties

Soil contains around 5% clay, 38% silt and 57% sand at topsoil (0-10 cm) and clay content increased with increased soil depth. Soil bulk density in MS was significantly ($P < 0.05$) lower than other treatments at the 0-10 cm (1.07 g cm^{-3}) and 10-20 cm depth (1.38 g cm^{-3}), while there were no significant differences in bulk density observed among CK, DT and MD treatments at these two depths (1.47 to 1.60 g cm^{-3} at the 0-10 cm depth and 1.54 to 1.71 g cm^{-3} at the 10-20 cm depth) (Table 6.1). At the 20-40 cm soil depth, MD bulk density (1.49 g cm^{-3}) was significantly lower than MS (1.78 g cm^{-3}) (Table 6.1). The CK had the highest bulk density (2.32 g cm^{-3}) compared with the other treatments at the 40-60 cm depth, while the DT had the lowest bulk density (1.67

g cm⁻³) (Table 6.1). All soil samples were slightly acidic. Soil pH values in CK (5.72) and MS (5.92) were significantly ($P < 0.05$) higher than MD (5.09) at the 0-10 cm depth (Table 6.1). A similar trend in soil pH was observed at the 10-20 cm soil depth (Table 6.1). At the deeper soil profiles (20-60 cm), soil pH in CK and MS were significantly higher than DT and MD ($P < 0.05$). In addition, the treatments that received mill-mud (MS and MD) inputs had higher pH values than the treatments without mill-mud application (CK and DT) at the deeper soil profiles (20-60 cm). Total C and N content in the mill-mud shallow applied treatment (MS) were generally higher than other treatments at the 0-20 cm soil depth. Additionally, the treatment with deep application of mill-mud (MD) had significantly higher TC and TN concentrations than other treatments at soil depth of 20-40 cm. Interestingly, at the 40-60 cm soil depth, DT had the highest TC content (0.18%) and MD had the highest TN content (0.058%) (Table 6.1).

6.3.2 Labile organic C and N pool

There were no significant differences in concentrations of HWEOC among all treatments at the soil surface (0-10 cm), despite MS having relatively higher HWEOC concentration (Table 6.1). For the 10-20 cm depth, the DT and MD treatments had significantly greater HWEOC than MS and CK, with the lowest HWEOC in CK. There were no significant differences in HWEOC between the two deep trench treatments (DT and MD). At the 20-40 cm soil depth, mill-mud deep trench applicated treatment (MD) had the highest concentration of HWEOC (196.9 mg kg⁻¹, $P < 0.05$), followed by DT and MS treatments, with the lowest value in CK (Table 6.1). A similar trend was found in the 40-60 cm depth (Table 6.1).

There were no significant differences in concentrations of HWETN among all treatments at the soil surface (0-10 cm). At the 10-20 cm depth, HWETN concentrations in MD treatment were significantly greater than other three treatments, while there was no significant difference among CK, MS and DT treatments (Table 6.1). At the 20-40 cm depth, as expected, HWETN concentration in the treatments receiving mill-mud (MS and MD) were at least 70% higher ($P < 0.05$) than the two treatments without mill-mud. At the 40-60 cm soil depth, mill-mud application significantly increased concentrations of HWETN in MS and MD treatments (3.39 and 2.18 mg kg⁻¹), compared to CK and DT (1.65 and 0.66 mg kg⁻¹). Interestingly, surface applied mill-mud did not increase labile carbon store in entire soil profile (0-60 cm) while deep trench applied mill-mud significantly increased labile C store in entire soil profile (Table 6.1).

6.3.3 Inorganic nitrogen pools

Concentrations of soil NH₄⁺-N were in the range of 4.00 to 4.12 mg kg⁻¹ among MS, DT and MD treatments, being significantly ($P < 0.05$) higher than in CK (3.77 mg kg⁻¹) (Table 6.1) at the soil surface (0-10 cm). However, there were no significant differences observed in NH₄⁺-N concentrations between MS, DT and MD. At the 10-20 soil depth, shallow applied mill-mud increased NH₄⁺-N concentrations (4.18 mg kg⁻¹) in MS while CK had the lowest NH₄⁺-N concentrations (3.73 mg kg⁻¹). Concentrations of NH₄⁺-N in MS and MD were significantly higher ($P < 0.05$) than in the other treatments at the 20-40 soil depth. In addition, we found MD had the highest ($P < 0.05$) NH₄⁺-N concentrations (4.10 mg kg⁻¹) at the 40-60 cm soil depth, followed by MS (3.24 mg kg⁻¹). No significant differences in soil NO₃⁻-N concentration were found in the 0-10 cm soil depth among all treatments. MS had significantly higher ($P < 0.05$) NO₃⁻-N concentration (1.10 mg kg⁻¹) than the other treatments at the 10-20 cm

soil depth. The treatments receiving mill-mud (MS and MD) had significantly ($P < 0.05$) higher NO_3^- -N concentration than the other treatments at the 20-40 cm soil depth. At the 40-60 cm depth, MD had the highest NO_3^- -N concentration (0.95 mg kg^{-1} , $P < 0.05$), while there were no significant differences among the CK, MS and DT treatments.

Table 6.1. Selected physicochemical properties of sugarcane field soil under different field mill-mud applications.

Depth (cm)		BD (g cm ⁻³)	Clay%	Silt%	Sand%	pH	C%	N%	HWEOC (mg kg ⁻¹)	HWETN (mg kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)
0-10	CK	1.46a	4.8b	40.7a	54.5c	5.72a	0.75ab	0.051a	192.6a	6.05a	3.77b	1.01a
	MS	1.07b	3.8c	39.8a	56.4bc	5.92a	0.93a	0.071a	217.8a	7.04a	4.12a	1.12a
	DT	1.52a	5.0b	35.9b	59.1a	5.4ab	0.56bc	0.053a	199.9a	6.36a	4.00a	0.99a
	MD	1.47a	6.6a	39.8a	53.6c	5.09b	0.34c	0.067a	197.2a	6.13a	4.11a	1.04a
10-20	CK	1.69a	5.1a	37.3a	57.6a	6.2a	0.58a	0.044b	137.9c	4.98b	3.73c	0.95b
	MS	1.38b	5.8a	38.8a	55.5ab	6.40a	0.68a	0.061a	154.8b	5.34b	4.18a	1.10a
	DT	1.71a	7.8a	38.4a	53.8b	5.15b	0.38b	0.037b	175.4a	5.04b	4.04b	0.90bc
	MD	1.65a	5.6a	37.7a	56.8a	4.75c	0.35b	0.072a	187.1a	6.62a	4.01b	0.82c
20-40	CK	1.82a	6.5b	41.9a	51.6b	6.35a	0.22b	0.033b	114.2c	2.83b	3.69b	0.78c
	MS	1.78a	8.6a	41.3a	50.1b	6.70a	0.13b	0.029b	133.6b	4.78a	4.59a	1.37a
	DT	1.61ab	7.7a	36.7b	55.5a	5.13b	0.22b	0.028b	140.3b	2.29b	2.96c	0.81c
	MD	1.49b	7.8a	40.4a	51.8b	5.43b	0.38a	0.068a	196.9a	5.67a	4.55a	1.01b
40-60	CK	1.88a	8.0b	42.4a	49.6a	6.47a	0.09b	0.022c	75.2c	1.65b	2.57c	0.77b
	MS	1.88a	10.0a	40.1a	50.0a	6.75a	0.08b	0.029b	84.5b	3.39a	3.24b	0.79b
	DT	1.73b	10.2a	38.3a	51.5a	5.35b	0.18a	0.029b	89.3b	0.66c	2.64c	0.81b
	MD	1.91a	8.2ab	38.5a	53.4a	5.64b	0.12b	0.058a	130.7a	2.18b	4.1a	0.95a

BD, bulk density; HWEOC, hot water extractable organic C; HWETN, hot water extractable total N. Control treatment (CK, without mill-mud); Mill-mud shallow furrow (ca. 20 cm) addition treatment (MS, mill-mud shallow furrow); Deep trench treatment (DT, deep trench without mill-mud); Deep mill-mud application (ca. 40 cm) treatment (MD, deep trench with mill-mud application). The reported data are the means of six replicates. Means within a column for each depth followed by the same letter are not different at the $P < 0.05$ level of significance.

6.3.4 Phosphorus pool

Total P concentrations in MS and MD significantly increased ($P < 0.05$) after mill-mud application at the 10-20 cm depth (380.8 mg kg^{-1} in MS) and the at the 20-40 cm depth (294.5 mg kg^{-1} in MD) compared with CK and DT at same soil depth (Fig. 6.1a). As expected, shallow farrowed mill-mud application (MS) increased total P concentration at the targeted soil level (10-20 cm) but also increased total P at the soil surface (0-10 cm) (Fig. 6.1). The deep trench mill-mud application (MD) had the highest soil total P at the deep layers (20-40 cm and 40-60 cm) (Fig. 6.1). On the other hand, total P concentration decreased with soil depth (from 20 to 60 depth) in the conventional field practice treatment (CK) (Fig. 6.1a).

Colwell P concentration decreased with soil depth (from 20 to 60 depth) in the treatments without mill-mud application (CK, DT) (Fig. 6.1b). The mill-mud application treatments (MS and MD) exhibited significantly greater amounts of Colwell P in soil compared with CK and DT (without mill-mud application). Shallow furrow applied mill-mud (MS) increased Colwell P concentration in the surface soil (0-20 cm) but had no impact on the deeper soil profile (20-60 cm) (Fig. 6.1b). On the other hand, deep trench mill-mud application (MD) significantly increased Colwell P levels in deeper soil (20-60 cm) but had no effects on levels in surface soil (0-20 cm) (Fig. 6.1b).

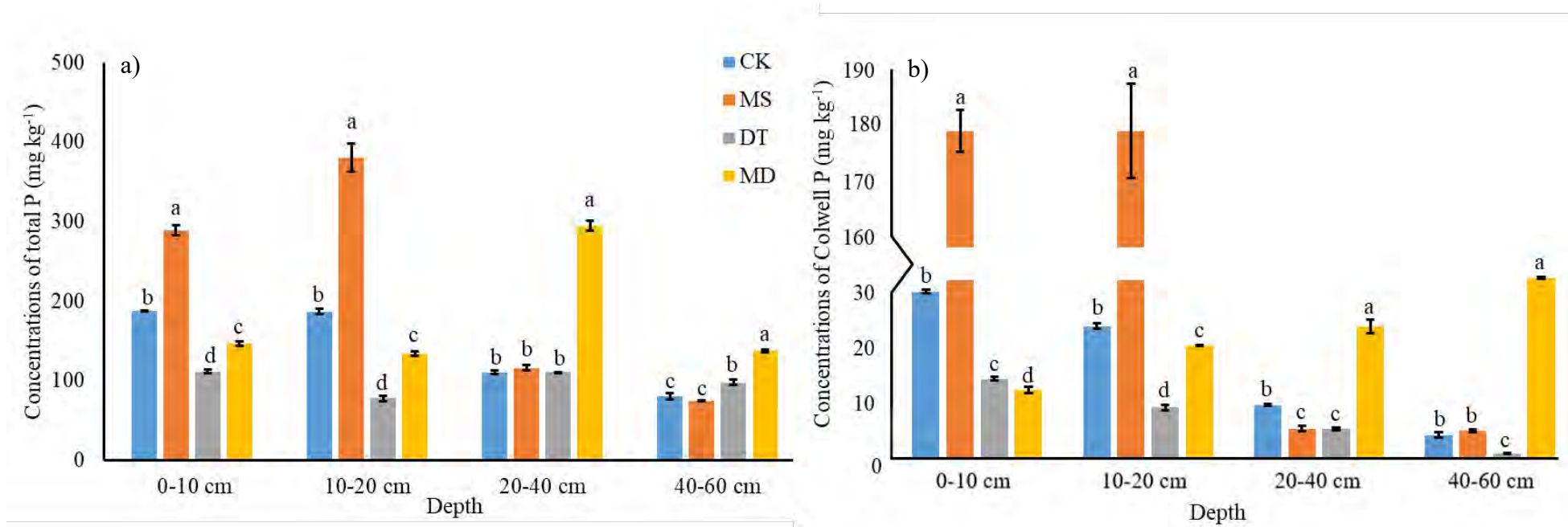


Fig. 6.1 Responses of soil total P (a) and Colwell P (b) to different mill-mud application in a compaction-affected sugarcane field. Control treatment (CK, without mill-mud); mill-mud shallow furrow (ca. 20 cm) addition treatment (MS, mill-mud shallow furrow); deep trench treatment (DT, deep trench without mill-mud); deep mill-mud application (ca. 40 cm) treatment (MD, deep trench with mill-mud application). The reported data are the means of six replicates. Means of the treatments by the same letter are not different at 5% level of significance within each soil depth.

6.3.5 Microbial C and N Pools

Mill-mud application significantly ($P < 0.05$) increased levels of MBC in the MS (0-20 cm) and MD (0-60 cm) treatments, compared with no mill-mud applied treatments (CK and DT) (Fig. 6.2a). Notably, deep trench without mill-mud application (DT) increased the concentration of MBC in the lower soil profile (20-60 cm) in comparison to the control treatment (CK). Increases in MBC mainly took place in the upper layers (0-20 cm) for the MS treatment (Fig. 6.2a). As expected, increases in MBC took place in deeper layers (20-60 cm) for the MD treatment (Fig. 6.2a). In terms of MBN, surface applied mill-mud (MS) increased the concentration of MBN which is similar to the pattern seen in MBC (Fig. 6.2b). However, deep trench mill-mud application (MD) had the lowest MBN level in nearly all layers (Fig. 6.2b). In general, MBC levels decreased with soil depth, but this trend was not clear for MBN. Interestingly, mill-mud application (MS and MD) significantly ($P < 0.05$) increased the microbial C: N ratio in comparison to the control treatment (CK), although this pattern became unclear in the MS treatment in the 40-60 cm soil layer (Fig. 6.2c). Notably, deep trench practice (DT) shows no impact on the microbial C: N ratio in the 0-40 cm soil profile, but significantly increased microbial C: N ratio at the 40-60 cm soil depth (Fig. 6.2c).

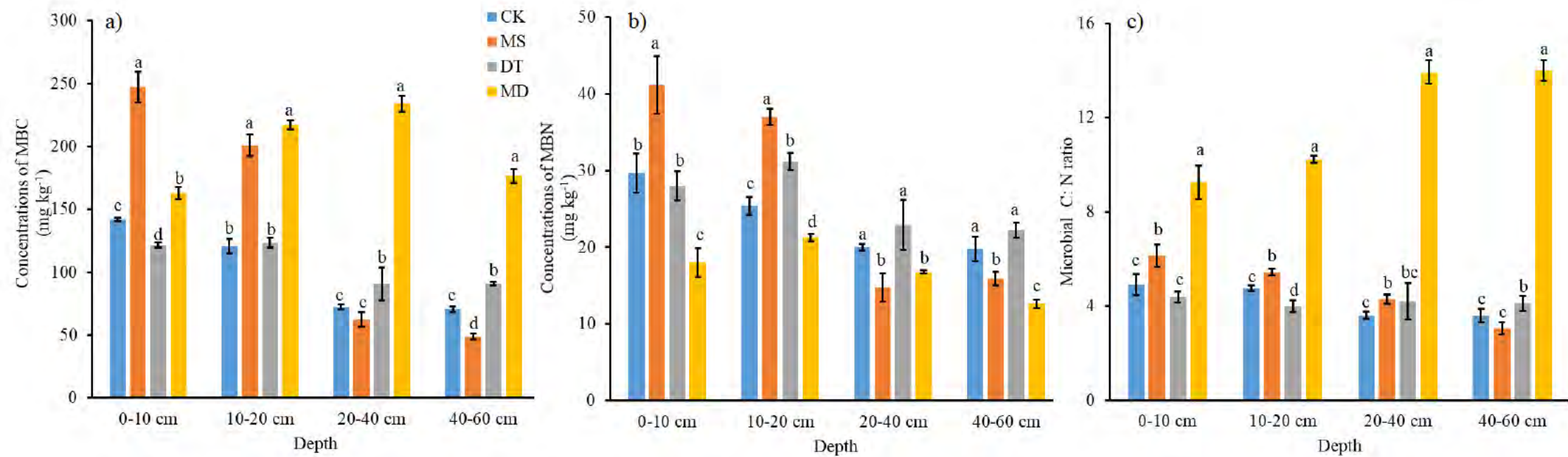


Fig. 6.2 Responses of soil microbial biomass C (MBC) (a) and N (MBN) (b) and microbial C: N ratio (c) to different mill-mud application in a compaction-affected sugarcane field content. Control treatment (CK, without mill-mud); mill-mud shallow furrow (ca. 20 cm) addition treatment (MS, mill-mud shallow furrow); deep trench treatment (DT, deep trench without mill-mud); deep mill-mud application (ca. 40 cm) treatment (MD, deep trench with mill-mud application). The reported data are the means of six replicates. Means of the treatments by the same letter are not different at 5% level of significance.

6.3.6 Microbial and enzyme activities

Overall, microbial activity for CK, MS and DT treatments decreased with soil depth, but MD tends to increase microbial activity up to 40 cm and then declines in the 40-60 cm soil layer. It is notable that the deep trench application (DT) increased microbial activity in the deeper soil profile (20-60 cm) in comparison to the control (CK) which is confirmed with the MBC result. The MS treatment had the highest microbial activity in the surface soil (0-20 cm) while the MD treatment had the highest MBC at the 20-40 cm soil depth (Fig. 6.3a).

Activities of both enzymes (β -glucosidase and phosphatase) were highest at the 10-20 cm depth for the MS treatment, and highest at the 20-40 cm depth for MD, which corresponded well with the target depth of mill-mud application for the MS and MD treatments. In the CK treatment, activities of both enzymes decreased with soil depth, while in the DT treatment, β -glucosidase activity tends to decline with soil depth, but phosphatase increased in the 20-40 cm and 40-60 cm layers (Fig. 6.3b and Fig. 6.3c).

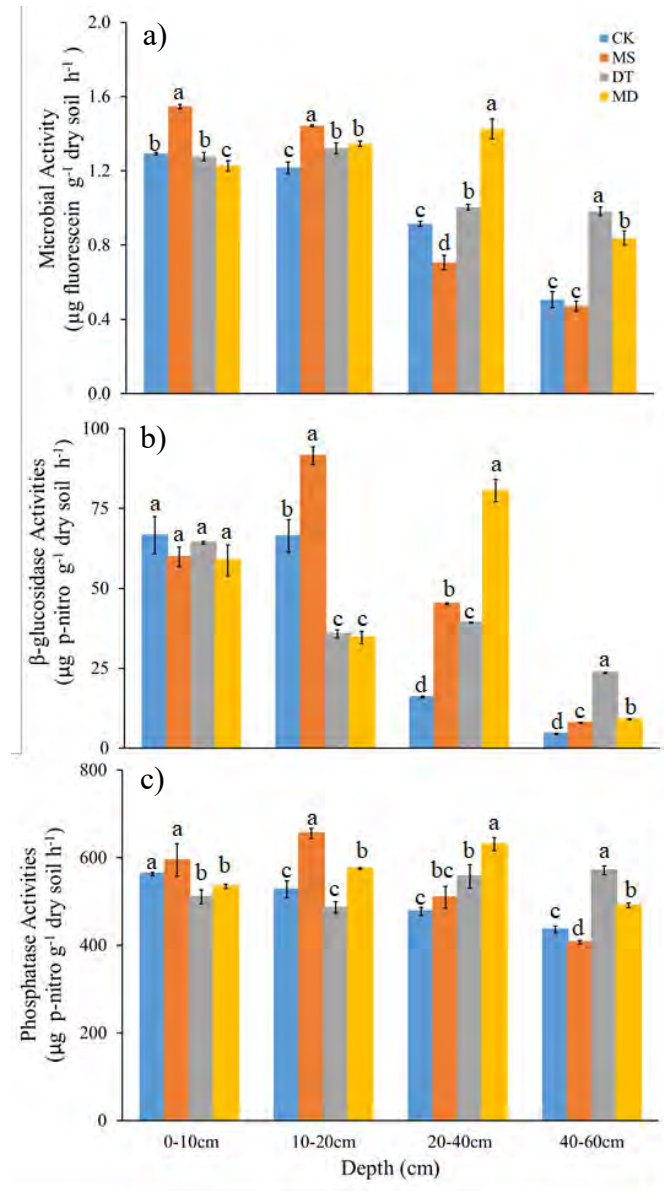


Fig. 6.3 Responses of soil microbial and enzyme activities to different mill-mud application in a compaction-affected sugarcane field. Control treatment (CK, without mill-mud); mill-mud shallow furrow (ca. 20 cm) addition treatment (MS, mill-mud shallow furrow); deep trench treatment (DT, deep trench without mill-mud); deep mill-mud application (ca. 40 cm) treatment (MD, deep trench with mill-mud application). The reported data are the means of six replicates. Means of the treatments by the same letter are not different at 5% level of significance.

6.3.7 Cane and sugar yield

Fig. 6.4 shows clearly that field management like mill-mud application, deep trench and deep trench applied mill-mud obviously increased plant cane yield. It is worth to point out that deep trench application (DT) increased plant cane yield (157.4 tonnes ha⁻¹) which is even higher than mill-mud surface application (MS, 152.1 tonnes ha⁻¹). As expected, field management also increased sugar yield however, there is only a slightly increase observed in mill-mud surface application (MS) while deep trench applied treatments (DT and MD) boosted sugar yield.

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

Soil bulk density was significantly negatively correlated with many biological properties such as microbial activity, enzyme activity, MBC and MBN ($r = 0.557-0.768$, $P < 0.01$) (Table 6.2). Soil pH was also negatively correlated to microbial activity, phosphatase activity, MBC and microbial C: N ratio ($r = 0.308-0.519$, $P < 0.05$) although there was no significant correlation between pH and β -glucosidase activity (Table 6.2). In addition, TC, TN and Total P were positively related to microbial activity, enzyme activity, MBC and MBN ($r = 0.285-0.880$, $P < 0.05$). As expected, soil Colwell P was positively correlated with microbial activity, enzyme activity, MBC and MBN ($r = 0.505-0.696$, $P < 0.01$). Soil NH_4^+ -N was positively correlated with microbial activity, enzyme activity, MBC and MBCN ratio ($r = 0.334-0.566$, $P < 0.05$) while NO_3^- -N only showed a positive correlation with enzyme activities ($r = 0.354-0.542$, $P < 0.05$). Notably, soil labile pools also showed positive correlations with microbial activity, enzyme activities, MBC and MBN and microbial C: N ratio ($r = 0.364-0.846$, $P < 0.05$) while labile N showed no correlation with microbial C: N ratio (Table 6.2).

The PCA results showed that principal components (PC) PC1 and PC2 explained 57.0% and 12.2% of the data variance, respectively (Fig. 6.5). Treatments were clearly separated from each other based on PC1 and PC2. At the 0-10 cm and 10-20 cm depths, surface mill-mud applied treatment (MS) was clearly separated from the other three treatments along PC2. The CK, DT and MD could be separated from each other along the PC2 at soil depths (Fig. 6.5). At the 20-40 cm and 40-60 cm depths, the MD treatment was separated from the other treatments along the PC1, while the DT treatment was separated from the CK and MS along the PC2 at these depths (Fig. 6.5). The parameters with the highest correlation coefficients (> 0.85) for PC1 were total C, labile C, MBC, microbial and β -glucosidase activities; and the parameter showing the highest correlation coefficient (> 0.85) for PC2 was pH (Table 6.3a and 6.3b).

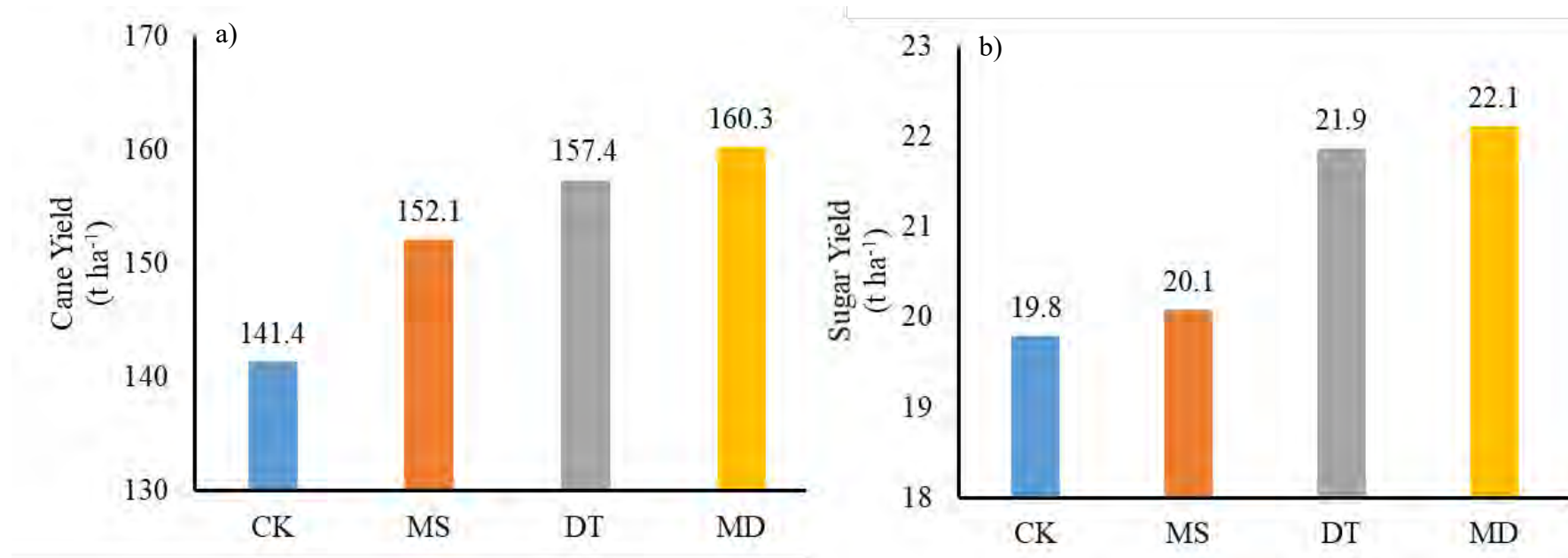


Fig. 6.4 Responses of cane yield (a) and sugar yield (b) to different mill-mud application in a compaction-affected sugarcane field. Control treatment (CK, without mill-mud); mill-mud shallow furrow (ca. 20 cm) addition treatment (MS, mill-mud shallow furrow); deep trench treatment (DT, deep trench without mill-mud); deep mill-mud application (ca. 40 cm) treatment (MD, deep trench with mill-mud application).

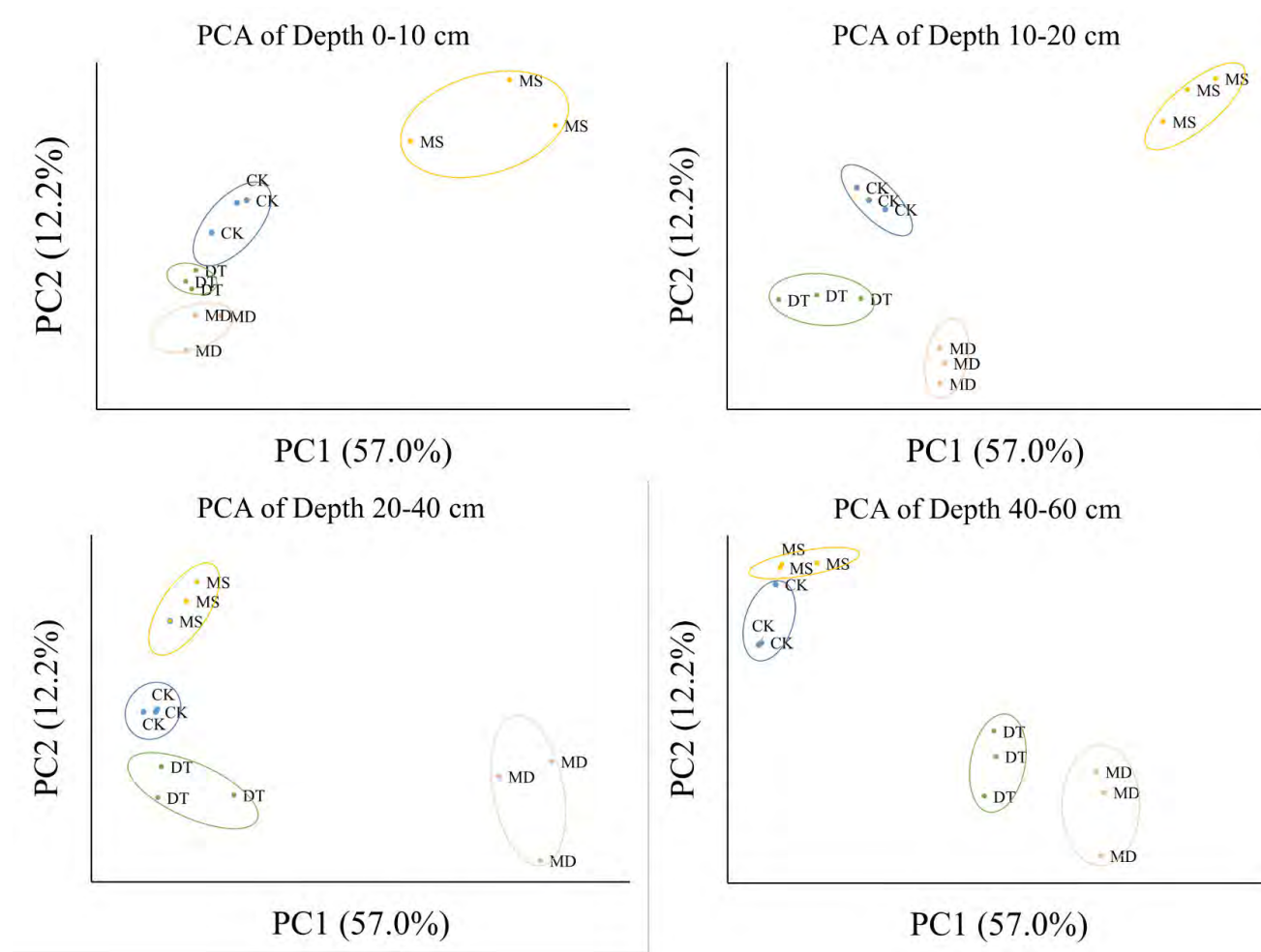


Fig. 6.5 Score plot of principal component analysis (PCA) showing the separation of soil samples collected under different mill-mud application and loading values of the individual soil parameters for PC1 and PC2 for soil samples. Control treatment (CK, without mill-mud); mill-mud shallow furrow (ca. 20 cm) addition treatment (MS, mill-mud shallow furrow); deep trench treatment (DT, deep trench without mill-mud); deep mill-mud application (ca. 40 cm) treatment (MD, deep trench with mill-mud application).

Table 6.2. Pearson correlation coefficients (r) between soil physicochemical and biological properties (n=96).

	BD	Moisture	Clay	Silt	Sand	pH	TC	TN	TP	Colwell P	NH ₄ ⁺ -N	NO ₃ ⁻ -N	HWEOC	HWETN	Microbial activity	β-glucosidase	Phosphatase	MBC	MBN	Microbial C:N	
BD	1																				
Moisture	-.459**	1																			
Clay	.527**	-.285*	1																		
Silt	0.129	0.125	.295*	1																	
Sand	-.323*	0.020	-.654**	-.916**	1																
pH	0.206	0.131	0.153	.356*	-.347*	1															
TC	-.714**	.569**	-.748**	-0.082	.379**	-0.082	1														
TN	-.574**	0.273	-.501**	-0.160	.337*	-.395**	.601**	1													
TP	-.608**	.341*	-.442**	-0.003	0.188	0.080	.666**	.595**	1												
Colwell P	-.593**	.629**	-.470**	-0.004	0.200	0.190	.697**	.477**	.822**	1											
NH ₄ ⁺ -N	-.466**	0.067	-.363*	-0.002	0.154	-0.066	.365*	.639**	.485**	.321*	1										
NO ₃ ⁻ -N	-.374**	0.221	-0.194	0.093	0.008	0.209	.326*	.307*	.445**	.389**	.710**	1									
HWEOC	-.664**	.329*	-.635**	-0.110	.354*	-.494**	.718**	.784**	.469**	.371**	.657**	.414**	1								
HWETN	-.562**	.318*	-.758**	-0.038	.348*	-0.260	.726**	.681**	.475**	.406**	.549**	0.260	.825**	1							
Microbial activity	-.768**	.287*	-.622**	-0.202	.421**	-.519**	.793**	.725**	.637**	.517**	.511**	0.273	.846**	.757**	1						
β-glucosidase	-.690**	0.164	-.510**	-0.139	.324*	-0.128	.734**	.589**	.764**	.505**	.566**	.542**	.690**	.605**	.775**	1					
Phosphatase	-.617**	0.248	-.331*	-0.090	0.210	-.308*	.508**	.539**	.737**	.546**	.334*	.354*	.494**	.364*	.683**	.683**	1				
MBC	-.557**	.377**	-.490**	-0.089	0.276	-.409**	.616**	.880**	.737**	.626**	.554**	0.254	.737**	.629**	.766**	.565**	.663**	1			
MBN	-.562**	.647**	-.522**	-0.065	0.271	-0.038	.812**	.285*	.492**	.696**	0.095	0.169	.458**	.486**	.615**	.490**	.409**	.399**	1		
Microbial C: N	-0.091	-0.142	-0.058	-0.074	0.083	-.416**	-0.022	.661**	.338*	0.066	.485**	0.115	.380**	0.214	.289*	0.172	.335*	.687**	-.355*	1	

BD, bulk density; TC, total C; TN, total N; TP, total P; HWEOC, hot water extractable organic C; HWETN, hot water extractable total N; microbial C:N ratio, microbial biomass C and N ratio.

*indicates significant correlation at the 0.05 level (2-tailed).

**indicates significant correlation at the 0.01 level (2-tailed).

Table 6.3.a

PCA loading factors of physicochemical properties of sugarcane field soil under different field mill-mud application.

	BD	pH	C%	N%	total P (mg kg ⁻¹)	Colwell P (mg kg ⁻¹)	NH ₄ (mg kg ⁻¹)	NO ₃ (mg kg ⁻¹)	HWEOC (mg kg ⁻¹)	HWETN (mg kg ⁻¹)
Principal Component 1	-0.81	-0.28	0.86	0.82	0.81	0.72	0.65	0.49	0.86	0.78
Principal Component 2	-0.06	0.87	0.16	-0.30	0.38	0.52	-0.01	0.40	-0.36	-0.22

BD, bulk density; TC, total C; TN, total N; TP, total P; HWEOC, hot water extractable organic C; HWETN, hot water extractable total N.

Table 6.3.b

PCA loading factors of biological properties of sugarcane field soil under different field mill-mud application.

	Microbial Activity (µg fluorescein per g dry soil per hour)	Phosphatase Activities (µg <i>p</i> -np per g dry soil per hour)	β-glucosidase Activities (µg <i>p</i> -np per g dry soil per hour)	MBC (mg kg ⁻¹)	MBN (mg kg ⁻¹)
Principal Component 1	0.92	0.74	0.85	0.85	0.64
Principal Component 2	-0.25	0.03	0.13	-0.21	0.28

p-np, *p*-nitrophenol.

6.4 Discussion

In the present study, it is assumed that adjacent soils (under different treatments) within a paddock were similar in their origin and parent materials. This assumption has been generally accepted and applied as the basis of many paired-site studies (Chen et al., 2004). We acknowledge the limitation of this study, as for many other paired-site studies, conducted using pseudo-replication. Soil compaction and the decrease in soil organic matter are key issues which limit the continuous increase of sugarcane yield (Liu et al., 2018). The compaction is brought about by the overuse of heavy machinery in harvesting while lower organic matter results from long-term monoculture and cultivation. To address sugarcane yield decline due to deterioration of soil health (Garside et al., 2005), mill-mud has been widely applied to the surface soil of sugarcane fields, but this application is limited to within 20 km of the sugar mill due to the freight cost. Mill-mud application to soil can enhance soil health and fertility through an increase in soil organic matter and supply of nutrients (Orndorff et al., 2018), improved soil physical structure and retention of water (Fang et al., 2020), increased soil microbial activity (Ishak and Brown, 2018), and enhanced soil pH buffering capacity (Medina et al., 2015).

It has been reported that deep trench application of composted municipal-biosolid to sugarcane fields can help plant growth and increase sugarcane yield (Viator et al., 2002). However, soil nutrient pool and microbial activity responses to compaction removal may vary with different depths of mill-mud application, soil type and environmental conditions such as soil temperature and moisture content. The PCA results showed a clear separation of soil samples under different treatments (Fig. 6.5), indicating that overall, mill-mud application methods had diverse and significant impacts on soil

properties and function. Changes in the key soil parameters (including soil pH, total soil C, labile C, MBC, microbial activities and β -glucosidase) induced by the different treatments contributed to the separation of samples.

6.4.1 Impact of different compaction removal approaches on soil physiochemical properties

Mill-mud application significantly reduced soil bulk density at targeted levels (ca. 20 cm soil depth in MS and ca. 40 cm soil depth in MD) compared to the control treatment. In addition, deep trench (DT) also reduced bulk density down to the 60 cm soil depth (Table 6.1). This indicated that deep trench and mill-mud application could be considered compaction removal methods to reduce soil compaction stress. Early research conducted on a clay soil showed that deep trench field practices would reduce soil bulk density down to 102 cm depth to improve soil water infiltration and cotton root growth (Heilman and Gonzalez, 1973). In the present study, deep trench did not have a significant impact on soil pH while mill-mud application slightly increased soil pH which concurs with results of other studies (Liu et al., 2018; Morris et al., 2007). Notably, increased soil pH via mill-mud application will provide better environmental conditions for soil nutrient turnover which would further increase crop yield (Morris et al., 2007).

Labile pools of soil organic matter are key fractions in terms of soil quality as they are available for microbial consumption and play an important role in soil nutrient cycling (Hu et al., 1997). Chen and Xu (2005) suggested that the hot water extractable soil organic C and total N measured as soil labile C and N pools are some of the best indicators of the impact of field management practices on soil fertility. It has been reported that compost application can increase soil organic matter content (Luo et al.,

2010; Pankhurst et al., 2002; Rahman et al., 2007) and provide the bioavailable organic C and N for soil microbial growth and for stimulating soil nutrient recycling (Mendham et al., 2003). In this study, deep trench application of mill-mud significantly increased concentrations of HWEOC and HWETN in the deep soil layer due to the inputs of labile organic matter from mill-mud (Table 6.1). On the other hand, deep trench without mill-mud application (DT) also increased HWEOC concentrations throughout the soil profile compared with the CK, which might be ascribed to the increased mineralisation of organic matter because of the disturbance effects induced by deep trenching. As expected, mill-mud application substantially increased soil total C and total N concentrations in the present study due to the presence of high C and N in the mill-mud. This result indicates that application of mill-mud increases soil C and N stock, and with the deep trench application method, mill-mud can significantly increase soil total C and N concentrations in deep soils (40-60 cm soil depth). Eghball et al. (2004) suggested that soil mineral N concentrations increased following organic amendment application to soil. Our study also showed an increase in concentrations of soil NH_4^+ -N and NO_3^- -N following mill-mud application, however, this occurred at different depths with different application methods (MS, MD). The mill-mud surface application (MS) slightly increased mineral N in the 10-20 cm soil layer while the deep trench mill-mud application slightly increased NH_4^+ -N and NO_3^- -N in the 20-40 cm soil layer.

Mill-mud application significantly increased soil P concentration (MS and MD) at the applied layer mainly due to the presence of a substantial amount of P in the mill-mud (ca. 0.73% P of dry matter). Increased P availability (Cowell P) following the mill-mud application could also contribute to (i) enhanced competitive sorption between low molecular weight aliphatic acid (via mill-mud application) and P for soil sorption sites and (ii) increased metal complexation and dissolution reactions by organic matter,

resulting in increased release of sorbed P (Bolan et al., 1994; Hue et al., 1994; Maurice, 1995). Additionally, mill-mud application may improve soil moisture conditions (Parmar and Sharma, 1996) and cause structural shifting of soil microaggregates which decrease the number of potential P sorption sites (Wang et al., 2003). Importantly, mill-mud application increases soil mineralizable C which boosts microbial incorporation of P resulting in increased available P (Chen et al., 2000; Guppy et al., 2005). In the present study, we also found that mill-mud application increased soil labile C and available P simultaneously. However, the deep trench method without mill-mud addition (DT) had no effect on soil P content.

6.4.2 Impact of different compaction removal approaches on soil biological properties

Mill-mud application to sugarcane fields can supply available organic C and reduce compaction stress for microbial activities. Liu et al. (2018) indicated that organic amendment application might not only increase soil MBC, but also enhance soil MBN content. In the present study, soil MBC contents at the mill-mud applied layers (0-10 cm in MS and 20-40 and 40-60 cm in MD) were significantly higher in comparison with other treatments. This may be ascribed to the organic C inputs to the targeted and adjacent soil layers in the mill-mud applied treatment, and subsequently more litter, which may increase MBC contents in the soil profile. (Lima et al., 2009) confirmed that presence of carbohydrates from organic amendment influenced synthesis of humic substances and biological properties in general. Higher concentrations of MBN in MS at the 0-20 cm depth were observed compared with other treatments, which was similar to the trend in MBC. However, the DT treatment had the highest MBN at deep layers (20-60 cm), which may contribute to leaching and improved porosity due to decreased bulk density. Leaching of soluble organic C and N provides an available energy source

for utilization by soil microbial communities, leading to an increase in soil microbial activity (Mendham et al., 2003). This is supported by the positive correlations between soil microbial biomass content and the HWEOC and HWETN contents in the present study. (Li et al., 2002) reported that decreased bulk density increased microbial pools as it is negatively correlated to bulk density. This statement supports our finding that the deep trench field method (DT) increased MBC and MBN content in deep soil layers (20-60 cm) which suggests that compaction removal would increase soil microbial pools. In the present study, we found that MD had the highest MBC concentration of all the treatments which indicates that the deep trench method could help mill-mud boosting of soil MBC content.

Field application of soil organic amendments has been reported to significantly increase soil enzyme activity (Albiach et al., 2000). In the present study, the overall microbial activity, β -glucosidase and phosphatase activity tended to be higher in the mill-mud application treatments than the other treatments (10-20 cm in MS and 20-40 cm in MD). This was likely due to the higher organic inputs from the mill mud as shown by the significant and positive correlations between enzyme activities and soil total C and N as well as soil HWEOC and HWETN contents. (Phalke et al., 2016) showed that soil organic C content had a significant correlation with soil β -glucosidase activity after organic amendment of a soybean-maize cropping system. In addition, it has been reported that the increase in soil enzyme activities can be related to a decreased soil bulk density (Kaiser and Heinemeyer, 1993). In this study, microbial activity showed a significant negative correlation with soil bulk density and thus the improved enzyme activities in the mill-mud and deep-trench applied treatments suggest a partial alleviation of constraint due to soil compaction removal practices.

6.4.3 Impact of different compaction removal methods on sugarcane yield

Organic amendment field application can significantly improve crop yield (Goswami et al., 2017). Tejada and Gonzalez (2003) argued that the significantly increased grain yield after organic amendment application could be attributed to increased soil organic matter. Liu et al. (2018) demonstrated that a significant increased cumulative sugarcane yield over 4 years was observed in organic amendment applied treatment which linked improved soil chemical properties to the resulting yield. In the present study, we found that increases in plant cane yield and labile C took place in deep trench and mill-mud applied treatments. This indicated that soil labile C from slow decomposition of mill-mud plays a vital role in increasing sugarcane production. A possible explanation could be lignin-like products from decomposition of plant tissue benefiting the formation of soil humus which further benefits plant growth (Tan, 2009) and carbohydrates released from mill-mud being a very active soil organic component and ready energy source for improving microbial activities (Pascual et al., 1999). Ghimire et al. (2017) also suggested that soil labile C pools played important roles in maintaining soil fertility. In addition, soil labile C, which is extracted by hot water, is biologically active and highly related to soil microbial activity (Chen et al., 2004). Mill-mud would also increase soil P and mineral N to further improve crop yield by overcoming P deficit (Balemi and Negisho, 2012) and balancing the soil N: P ratio (Dai et al., 2020). In the present study, demonstrated that labile C, mineral N, total P and Colwell P were significantly positive correlated to MBC, MBN, enzyme activity and microbial activity. Therefore, the labile organic C and nutrients derived from the mill-mud decomposition enhanced soil microbial activity and nutrient cycling and ameliorated soil compaction stress for sugarcane growth. This subsequently improved soil chemical and physical conditions and increased the plant cane yield. In the present study, surface applied mill-mud

slightly increased plant cane yields but there was no significant increase in soil labile C stores and microbial activity in comparison to CK. Interestingly, the deep trench (DT) treatment significantly increased plant cane yield and moderately increased soil labile C store and microbial activity. As there is no organic matter input in the DT treatment, the significantly reduced soil bulk density resulting from this practice increased soil aerobic condition (Drew, 1983), availability of nutrients (Jusoff, 1991) and decomposition of native organic matter which may increase nutrient cycling (De Neve and Hofman, 2000), labile C store, microbial activity (Li et al., 2002) and crop yield (Wallace and Terry, 1998). However, this field compaction removal practice may not be good for sustainable farming as huge soil organic C loss would happen in a long run without extra organic matter input. This study clearly demonstrated that mill-mud deep trench application would simultaneously increase plant cane yield and maintain soil health by removing soil compaction, increasing soil labile C store and boosting microbial activity.

6.5 Conclusions

It is clearly demonstrated that the application of mill-mud improved soil physiochemical properties including bulk density, nutrient availability, labile organic C and N (HWEOC, HWETN) contents, thereby stimulating nutrient cycling, soil health and crop yield. Deep trench application of mill-mud stratified nutrients supplied the soil microbial community in all soil layers, enhanced nutrient cycling processes, and thus increased soil health and sugarcane productivity. In contrast, surface applied mill-mud only increased surface nutrients and organic matter content with a limited crop yield improvement. Surface mill-mud application would also result in carbon loss from soil. It is worth noting that the deep trench (DT treatment) practice unexpectedly increased sugarcane yield which was mainly attributed to increased mineralization of native

organic matter, increased nutrient availability and subsequent ameliorated soil compaction, improved soil structure and redistribution of soil organic carbon through the entire soil profile. Further research should focus on soil C and N cycling functional genes in response to compaction removal to further explore the mechanisms responsible for soil microbial regulation of nutrient cycling in sugarcane farming systems. In addition, it is also important to adapt these findings into different soil types and other environmental stressors.

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Chapter 7 Summary, conclusions, and recommendations for future work

7.1 Summary

There is an increasing concern that climate change and human activities are altering the environment by increasing the atmospheric CO₂, seasonal temperatures, and frequency of extreme weather events (high intensity rainfall incidences or drought). High intensity rainfall during prolonged parts of the growing season can result in long periods of limited oxygen availability in the crop root zone, which may consequently result in a significant decline of crop yield. On the other hand, uneven distribution of rainfall may lead to periodic drought, despite high annual rainfall, and result in low crop yield. All these phenomena could be contributed to the decline of soil health. To be specific, global decline of soil health becomes a severe consequence of the combination of climate change, overused agricultural machinery, and intensive application of chemical fertilizers. However, currently used indicators in soil health monitoring framework are relatively latency, insensitive and expensive. Therefore, it is essential to predict soil health condition with a cheap, easy-to-understand, and robust index. Soil microbial properties should be adopted into soil health assessment as soil microbial community is sensitive to environmental stresses and respond rapidly to environmental disturbance in comparison to traditional soil physicochemical properties. In addition, how soil microbial community responses to environmental disturbance remains largely uncertain. Therefore, it is important to generate new knowledge to fill this research gap by investigating how soil microbial community respond to environmental disturbance and

use this information as an index to represent soil health condition. The findings of four experimental chapters are summarized below:

Information collected from compaction and drought study revealed that the Nitisols had a higher resistance index of microbial C use efficiency, while the Planosols had a higher resilience index of microbial C use efficiency to compaction and moisture (drought or waterlogging) stress. This could be attributed to the greater amount of finely textured particles and higher diversity of the microbial community in the Nitisols which provide a higher functional stability to resist environmental disturbance, while fast drainage and higher adaptation of microbial communities in the Planosols would provide a faster recovery from applied stresses.

Data collected from compaction and management history study showed that soil with improved management practice history has higher resistance against compaction stress and higher resilience following the removal of compaction stress. In addition, surface application of plant residues would not improve soil resilience against compaction stress, due to the rapid loss of labile C through soil microbial respiration process. Also, incorporation of plant residues would improve soil microbial activities and their recovery from compaction stress.

Result from incubation study of drought and management history indicated that soil biological functions are reliable indices for assessment of soil health and resilience to drought stress. Also, this outcome indicated a significant possibility to apply this lab-based index into field trial level in monitoring soil health condition under drought condition. Additionally, the response pattern of soil microbial community to drought stress is highly related to the applied stress levels rather than the history of field management practices. Soils under crop rotation practice are more resistant and resilient

to drought stress than soils under monoculture practice due to their higher organic matter content and nutrients bioavailability.

Information collected from field management experiment revealed that the application of mill-mud would increase soil nutrient pools, including total and labile pools. Shallow furrow application and deep trench application would lead to different distribution patterns of organic C and nutrients at different depths in the soil profile. Mill-mud application and removing soil compaction stress would increase the size of the soil microbial community and microbial activity, which further increase soil health condition and sugarcane yield.

7.2 Conclusions

The major conclusions of this study are as follows:

- 1) The response of soil microorganisms to compaction is regulated by moisture status, thus microbial responses manifest a potential robust indicator in soil resilience monitoring as it is sensitive to changes in the soil water filled pore space.
- 2) Higher aggregation potential and microbial diversity in clayey soils would build a higher resistance to environmental stresses, while higher adoption of microbial community in sandy soils would provide a faster recovery from environmental stresses.
- 3) Improved field management could help to build soil resistance to compaction, which shown as a faster stabilization of microbial properties after compaction stress and following the removal of stress, due to increasing soil organic matter content and diversity of soil microbial community.

- 4) Plant residue application increased the concentration of soil microbial biomass and enzyme activity regardless of soil management history. However, surface application of plant residue would not improve soil resilience against compaction stress due to a rapid loss of labile C through respiration.
- 5) Improved field management could help to build soil resilience to drought stress, which was shown as the tolerance to moderate drought and resistance to severe drought. However, soils under conventional field management would have a lower resistance, but higher recovery rate to drought stress.
- 6) Application of mill-mud improved soil physicochemical properties including bulk density, nutrient bioavailability, labile organic C and N contents, thereby stimulating nutrient cycling, soil health and crop yield.
- 7) Deep trench application of mill-mud improved nutrients supply to soil microbial community in all soil layers, enhanced nutrient cycling processes, and thus increased soil health and sugarcane productivity. In contrast, surface applied mill-mud only increased surface nutrients and organic matter content with a limited crop yield improvement.
- 8) Deep trench practice increased sugarcane yield, which was mainly attributed to increased mineralization of native organic matter, increased nutrient bioavailability and subsequent ameliorated soil compaction, improved soil structure and redistribution of soil organic C throughout the soil profile.

7.3 Future work

This work has greatly improved the understanding of the response of soil microbial community to compaction and drought stresses in sugarcane and wheat cropping systems and explored the reliability of using this information as an index of soil resistance and resilience in monitoring soil health decline.

However, further detailed studies are required to extend the findings of this study to other cropping systems and environmental stressors. Improved understanding of the mechanisms and key factors regulating soil microbial activities will help farmers making a wise informed decision in adopting the best management practices in order to reverse soil health decline in agricultural lands. There is a particular need for future investigation in the following important areas:

- The assessment of the responses of different key functional genes involved in C and N cycling following different stress scenarios may provide more insights into these complicated interactions.
- Understanding of shifts in microbial community composition involved in C and nutrients cycling in response to the applied stress and following its removal would further explore the mechanisms responsible for soil microbial regulation of nutrient cycling in agricultural lands.
- To provide more accurate predictions of soil health condition, equations of soil resistance and resilience status, which used in this study, need to be further simplified and incorporated with appropriate statistical approaches such as structural equation modelling and multivariate distance-based linear modelling.

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