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Variation in soil microbial properties and their contribution to soil carbon storage under long-term fertilization and organic amendments

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Variation in soil microbial properties and their contribution to soil carbon storage under long-term fertilization and organic amendments

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Résumé

La séquestration de carbone organique dans le sol (SOC) est reconnue comme l'une des stratégies efficaces pour contribuer à atténuer l'effet de serre et contribuer au développement durable de l'agriculture mondiale. Le plateau du loess semi-aride chinois représente un tiers des terres arables de Chine et fournit de la nourriture à plus de 100 millions de personnes à l'heure actuelle. Par conséquent, la fertilisation et l'amendement organique sont les stratégies de gestion cruciales sur ce plateau. Cependant, le recours depuis une longue période aux engrais chimiques a progressivement conduit à une baisse de la qualité des sols et à l'épuisement de la matière organique du sol (SOM).

En tant que moteur clé de multiples processus biogéochimiques, les communautés microbiennes du sol peuvent non seulement réguler la santé des sols, mais aussi faciliter la séquestration du carbone dans l'agriculture intensive.

L'objectif de cette thèse était d'évaluer l'impact à long terme de la gestion de la fertilisation sur les caractéristiques physico-chimiques du sol, le stockage du SOC et la contribution des activités de la communauté microbienne à la séquestration du SOC. Dans cette étude, nous avons exploité les résultats d'une expérience de terrain de 27 ans avec quatre traitements de fertilisation (CK: pas de gestion de la fertilisation; NP: application d'engrais inorganiques seuls; NPS: fertilisation inorganique plus incorporation de paille de maïs; NPM: fertilisation inorganique plus incorporation de compost de fumier de vache) et nous avons mené une expérience d'incubation (300 jours) d'agrégats classés (> 5 mm, 2–5 mm, 1–2 mm, 0.25–1 mm ou < 0.25 mm) avec application de paille de maïs marquée au ^{13}C.

Les résultats ont montré que la biomasse microbienne avait des relations étroites avec les propriétés chimiques et physiques du sol, et les propriétés chimiques du sol expliquaient une plus grande proportion de variation de la biomasse microbienne que les propriétés physiques. Les différentes gestions de la fertilisation pourraient entraîner des changements dans l'explication des propriétés chimiques et physiques du sol à la communauté microbienne. Les variables fongiques (fongique, biomasse AM et rapport F/B) et les activités enzymatiques (BXYL et LAP) étaient significativement corrélées avec la teneur en SOC dans les macro-agrégats (> 0.25 mm), mais pas dans les micro-agrégats (< 0.25 mm). De plus, l'amendement organique a considérablement réduit l'impact des engrais inorganiques sur la croissance de la communauté microbienne. Les champignons et les bactéries G– ont grandement contribué à augmenter l'accumulation de C dans les agrégats de > 2 mm, tandis que les bactéries G+ étaient plus importantes dans les agrégats de < 2 mm. Dans l'ensemble, les activités des micro-organismes dans les agrégats indépendants ont contribué à l'accumulation interne de C sous application de paille.

Par conséquent, les résultats ci-dessus ont montré que l'amendement organique peut aider à améliorer les propriétés physico-chimiques du sol et la communauté microbienne et à améliorer la contribution du processus microbien au stockage du

SOC à l'échelle globale par rapport à la fertilisation chimique.

Ces résultats ont indiqué que l'amendement organique peut contribuer à la III durabilité de l’agriculture dans le plateau chinois du Loess.

Abstract

Soil organic carbon (SOC) sequestration is well known as one of effective strategies for mitigating atmospheric greenhouse effect and sustainable development of global agriculture. Chinese semiarid Loess Plateau accounts for one-third of the arable land in China and provides food for more than 100 million people at present, therefore, fertilization and organic amendment are the crucial management strategies in Loess Plateau accounts for sustainable farming systems. However, long-term reliance on chemical fertilizers has gradually led to a decline in soil quality and soil organic matter (SOM) depletion. As the key driver of multiple biogeochemical processes, soil microbial communities can not only regulate soil health but also facilitate carbon sequestration in intensive agriculture.

The objective of this dissertation was to evaluate the long-term impact of fertilization management on soil physicochemical characteristics, SOC storage and the contribution of microbial community activities to SOC sequestration. In this study, we performed an 27-year field experiment with four fertilization treatments (CK: no fertilization management; NP: inorganic fertilizers application alone; NPS: inorganic fertilization plus the incorporation of maize straw; NPM: inorganic fertilization plus the incorporation of composted cow manure), and an incubation experiment (300 days) of classified aggregates (> 5 mm, 2–5 mm, 1–2 mm, 0.25–1 mm or < 0.25 mm) with ^{13}C-labeled maize straw application.

The results showed that microbial biomass had close relationships with both soil chemical and physical properties, and soil chemical properties explained a larger proportion of variation of microbial biomass than physical ones. The different fertilization managements could cause changes in the explanation of soil chemical and physical properties to the microbial community. Fungal variables (fungal, AM biomass, and F/B ratio) and enzyme activities (BXYL and LAP) were significantly correlated with SOC content in macro-aggregates (> 0.25 mm), but not in micro-aggregates (< 0.25 mm). In addition, organic amendment significantly reduced the impact of inorganic fertilizer on the growth of the microbial community. Fungi and G– bacteria contributed greatly to increasing C accumulation in > 2 mm aggregates, while G+ bacteria were more important in < 2 mm aggregates. Overall, the activities of microorganisms in independent aggregates contributed to inner C accumulation under straw application, which was closely associated with aggregate sizes under the straw application.

Therefore, the results above showed that organic amendment can help improve soil physicochemical properties and alter microbial community composition, and improve the contribution of the microbial process to SOC storage at aggregate scale compared with chemical fertilization. Those results indicated that organic amendment can contribute to agricultural SOC sequestration and sustainable farming in Loess Plateau.

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Jinjing Lu
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Chapter I

General introduction

This chapter presents the general context for conducting this research, the thesis frame work, thesis objectives and the outline.

1. Background

As the third largest global carbon (C) stock, terrestrial soil is estimated to store 1500 Pg C in the upper layer of soil (FAO 2015), approximately three times the stock of C of the atmosphere. Small changes in the soil C pool might affect the global C balance significantly, hence the rising atmospheric CO_2 levels have been assigned to the sink potential of soil organic carbon (SOC) (Murugan et al. 2019). In agroecosystem, SOC plays a central role in evaluating soil stability, fertility, and sustaining agricultural production by regulating many biological, chemical and physical soil functions (Lehmann and Kleber 2015). Due to the growing pressure for food production, long-term and large-scale excessive use of soil by humans is widespread in the world. Several decades of continuous cultivation have been confirmed to accelerate SOC degradation significantly, with consequent reduction of soil nutrients, soil aggregation, and biodiversity (Celik 2005). In this sense, enhancing SOC storage is one potential and critical approach to improve soil fertility and sequestrate atmospheric C (Somasundaram et al. 2017).

1.1 SOC status in agroecosystem

The soil C stock is determined by the balance between C inputs, as fresh organic matter (OM), and losses of C from the soil, as autotrophic and heterotrophic respiration in soil (Guenet et al. 2012) (Figure 1-1). Forest and grasslands systems tend to have large input of C all year round to the soil, while agroecosystem has the smallest input of C due to crop residue removal and excessive tillage (Smith 2008). Soils of many agroecosystems are strongly depleted of SOC stock, and loss of SOC

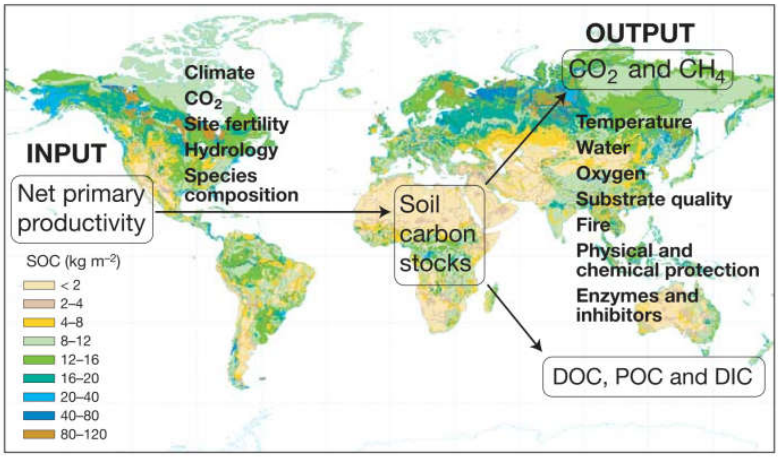

Figure 1-1. A view of factors controlling the main inputs and outputs of soil carbon at global scale (Davidson and Janssens 2006).

with agricultural cultivation is receiving more and more attention. Studies show that soils have lost 40–90 Pg C globally through cultivation and disturbance which perturbs the global C cycle (Norton et al. 2012; Smith 2008). In the western United States, agricultural cultivation is thought to induce 20 to 40% decline in SOC (Norton et al. 2012; Yu et al. 2012). In China, an area of 130 M ha was used for cropland. It was estimated that there is a C sequestration potential of 25–37 Tg C yr^{-1} in cropland (Yan et al. 2011), which plays a critical role in SOC stock. Therefore, many agricultural ecosystems are generally suggested having the potential to enhance C sequestration in the soil (Nakicenovic and Swart 2000).

1.1.1 SOC sequestration

Generally, SOC sequestration refers to capture atmospheric CO_2 through plants, plant residues, and other organic solids and store C in the form of soil organic matter (SOM) in the soil (Lal et al. 2015). The area of agricultural land, where SOC sequestration is feasible, is about 4,900 M ha, and the rate of SOC sequestration is approximately 0.3–1.0 (Mg C ha^{-1} $year^{-1}$) in croplands, while the global technical potential of SOC sequestration is 1.5–3.4 Pg C $year^{-1}$ (Lal 2018). Therefore, SOC sequestration in agricultural land is gradually recognized as a strategy to regulate the global C cycle.

There are three main mechanisms controlling SOC sequestration (Tivet et al. 2013): (i) physical isolation of SOC within aggregates to avoid being decomposed during the biological process; (ii) selective preservation through biochemical recalcitrance, and (iii) chemical interaction between organic matter and mineral matrix (Lützow et al. 2006; Wang et al. 2014).

1.1.2 The different SOC fractions

SOM is the core of soil quality, and the C sequestration in SOM mainly comes from dead biological residues and organic materials such as livestock manure and green manure. Generally, SOM mainly consists of labile organic matter (Strosser 2011) and humic substances (fulvic acids, humic acids, and humins). Humic substances have been found to be a stable material that remain unchanged for decades (Stevenson 1994).

SOC is composed mainly of very heterogeneous compounds with turnover times ranging from a few days to several centuries. According to the solubility, hydrolysis and chemical reactivity of SOC in the extractant, dissolved organic C (DOC), acid-hydrolyzed organic C, and easily-oxidized organic C (ROC) are commonly studied as the representative indicators of chemical SOC fractions. According to the duration of the turnover time, SOC pools can be divided into different pools (Parton et al. 1987), such as stable organic C with long turnover time and labile C pool with short turnover (Guenet et al. 2012). Studies on different SOC fractions have revealed the mechanism of SOC change (Christensen 2001; Huo et al. 2013). Within organic C, the transformation and decomposition of various C fractions directly influence the SOC pool. Labile SOC fractions, i.e., DOC, ROC, and microbial biomass C (MBC), are important components of SOC that are readily oxidized or decomposed by microbial biomass (Bastida et al. 2013;

Fischer et al. 2010). DOC, an important and significant component of SOC, is the main source of energy for microbial activity. MBC is known as a small fraction of SOC and derived from the living component of the SOM in soil. Current studies generally regard it as sensitive indices to monitor longer-term trends in SOM, due to its significant influence on many microbial-driven processes (Joergensen and Wichern 2018). As part of the labile C pool, ROC is more sensitive and responds quickly to the variation of soil physical and chemical properties (Xu et al. 2020). Therefore, ROC could be used as a common indicator of early SOC dynamic changes (Jiang et al. 2014; Song et al. 2021). Labile SOC fractions contain about 10% of SOC (Sainepo et al. 2018; Sun et al. 2021), and they are the most important readily available energy sources for microbial communities (Phillips et al. 2011). Due to their instability, they have been emerged as standardized indicators to reflect the changes in early SOC trends over time (Ramírez et al. 2020).

1.1.3 SOC status in aggregate

A number of previous studies on SOC at different scales have been well documented in order to interpret SOC dynamics (Hoffmann et al. 2017; Meersmans et al. 2009; O'Rourke et al. 2015; West and Marland 2002) (Figure 1-2). Most of the studies focus on the assessment of C storage and distribution at each scale with emphasis on stabilizing SOC, especially on the regional scale. However, assessing changes in soil C is imprecise only based on the total amount of C, it is more important to know the specific location where SOC is stored in the soil. Over several decades, greater attention has been given to aggregate scale in SOC science.

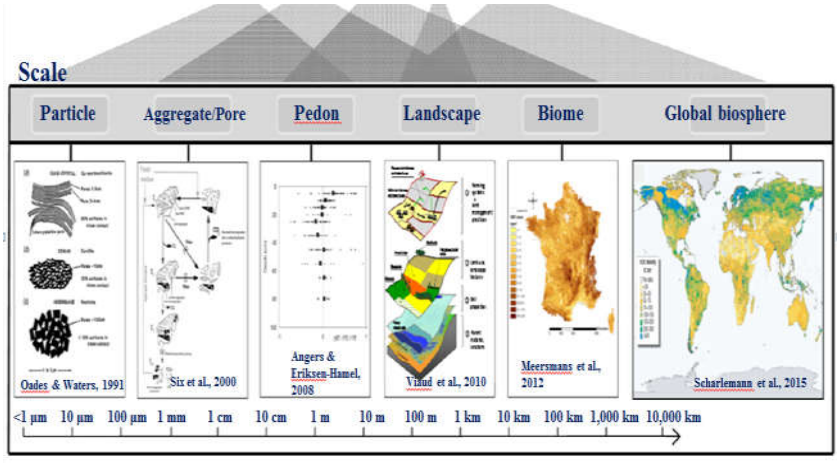

Figure 1-2. Soil organic carbon at different scale (O'Rourke et al. 2015).

As a basic unit of soil, aggregates are considered as a stable SOC pool (Blanco-Canqui and Lal 2004), which accounts for approximately 90% of topsoil C (Jastrow 1996). Hence, soil aggregate is considered to play a significant role in C storage in terrestrial regions (Stockmann et al. 2015). Soil aggregates play

important role in influencing the physicochemical and biological properties of soil. Generally, soil aggregates are classified as micro- (< 250 μm) and macro-aggregates (> 250 μm), respectively (Edwards & Bremner 1967; Tisdall & Oades 1982). Aggregate sizes also could be classified differently depending on study designs (Jastrow 1996; Plante & McGill 2002).

During the formation of aggregate, plant roots and residues are the primary organic skeleton to enmesh the soil particles together and build aggregates (Blanco-Canqui and Lal 2004), and there have been several conceptual models proposed the process of soil aggregate formation. It is proposed that micro-aggregates (< 250 μm) are typically formed by binding agents (clay, OM, and polyvalent cations) and mineral particles, whereas macro-aggregates (> 250 μm) are typically formed by temporary binding agents, i.e., fungal hyphae, plant roots and coarse organic fragments (Oades 1984; Tisdall & Oades 1982). Jastrow (1996) found that some stable micro-aggregates (< 250 μm) can also be bond with the newly formed particulate organic matter within macro-aggregates (Jastrow 1996; Six et al. 2004) (Figure 1-3). Therefore, soil aggregates are considered important agents or containers of SOC retention (Haile, Nair, and Nair 2008). It is critical to understand the contribution of aggregate to SOC sequestration (Blanco-Canqui and Lal 2004).

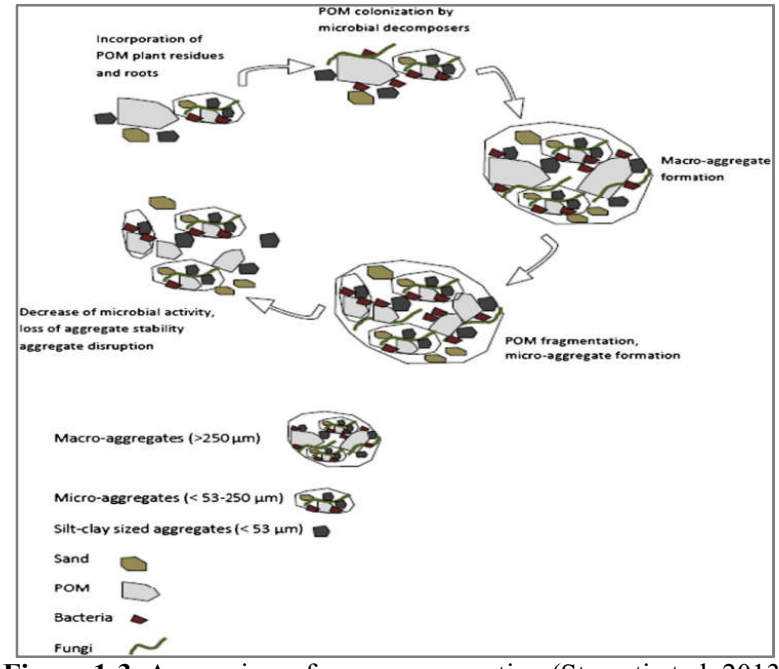

Figure 1-3. A overview of macro-aggregation (Stamati et al. 2013).

1.1.3.1 SOC and aggregate size

It is well known that SOC is stored within different sized aggregates. Aggregate size classes differ in many soil functions, such as microbial and enzymatic

activities (Drażkiewicz 1995) and mineralization of organic matter (Nie et al. 2014; Sey et al. 2008). The heterogeneity of soil structure, resulted from complicated composition and spatial arrangement of different sized particles, was considered to be the main factor for heterogeneous distribution of microorganisms and SOC among different aggregates (Jiang et al. 2011). Therefore, the transport of aqueous substances and diffusion of gases inside vary with aggregates sizes, resulting in different C accessibility and persistence (Christensen 2001; Monrozier et al. 1991).

Recent studies showed that soil aggregate size partly determined the microbial activity under climate change, which may result in potential implications for C cycle through microbial mediation (Dorodnikov et al. 2009). Thus, the location and dynamics of SOC could vary with aggregate size. Puget et al. (2000) showed that macro-aggregates contain more organic C but are more vulnerable to microbial degradation than in micro-aggregates, due to higher substrate accessibility to microbes (Sun et al. 2016; Tian et al. 2015). In general, micro-aggregates also play a key role in SOC sequestration (Gale 2000) by protecting SOC from microbial degradation within macro-aggregates (Coleman and Elliot 1988). In some studies, the highest SOC and MBC were found in 1.0–2.0 mm aggregates, which presented the highest microbial activity in the present ecosystem (Jiang et al. 2011). In addition, Buyanovsky et al. (1994) suggested that the dynamics of the new C varied with aggregate size. Six confirmed that the contribution of straw to new C in macro-aggregate would be higher than small size aggregate due to the faster decomposition (Six et al. 2006).

These studies emphasize the need to consider the soil aggregate size and the location of SOC when studying the turnover and sequestration of SOC in soil (Angers et al. 1997).

1.1.3.2 SOC and pore structure

Soil structure, with pores between and within aggregates (Coleman and Elliot 1988), play a vital role in various soil functions and processes, including soil respiration, nutrient transportation, and decomposition of organic substrates by microorganisms (Fan et al. 2020; Ranjbar et al. 2016; Six et al. 2000). The structure of solid and pore space determines intra and inter aggregate porosity and controls the composition and transport of gas and water in soil (Blagodatsky and Smith 2012). The intra-aggregate pore structure is quite complex, accompanied by high heterogeneity in the spatial distribution of soil water, oxygen, and nutrients at micro-scale (Ruamps et al. 2011), and varies significantly among different soil aggregate sizes (Zhao et al. 2020).

Despite the relatively small space, the intra-aggregate pore structure provides physical micro-environments for microbial activities and plays a key role in SOM stabilization and dynamics (Christensen 2001; Negassa et al. 2015). For example, the release of CO_2 and CH_4 from soil has been confirmed to be related to soil pore characteristics, such as pore size and total porosity (Mangalassery et al. 2013). As the microhabitats of microorganisms (Upton et al. 2019), the intra-aggregate pore structure largely controls the soil microbial activities which strongly depend on the

availability of organic substrate, oxygen, and water in aggregate. It was suggested that the pore structure, such as the pore size, connectivity, and porosity, may partly control SOC bioavailability to microorganisms under different tillage systems (Chevallier et al. 2010; Guo et al. 2020) (Figure 1-4).

Large pore volumes (15–60 μm), which were associated with greater microbial activity (Ruamps et al. 2011), led to faster decomposition of C in soil compared to micropores (Strong et al. 2004). Therefore, more than 75% of the SOC was protected within the micropores (10–1000 nm), due to the exclusion of digestive enzymes by pore network geometry and delays in the flow of solubilized C to microorganisms (Chevallier et al. 2010; Mayer et al. 2004). There was also a mesopore (2–50 nm) protection mechanism (Pierotti and Rouquerol 1985) by some studies in view of preferential sorption of mesopores by SOC (Mayer 1994). Dungait et al. (2012) suggested that the accessibility of SOC to decomposer is the main factor in governing SOC turnover, and they thought that soil structure and aggregation affect the connectivity between SOC inside and potential decomposers through controlling moisture and gas, thus influence the SOC turnover (Dungait et al. 2012; Kuka et al. 2007).

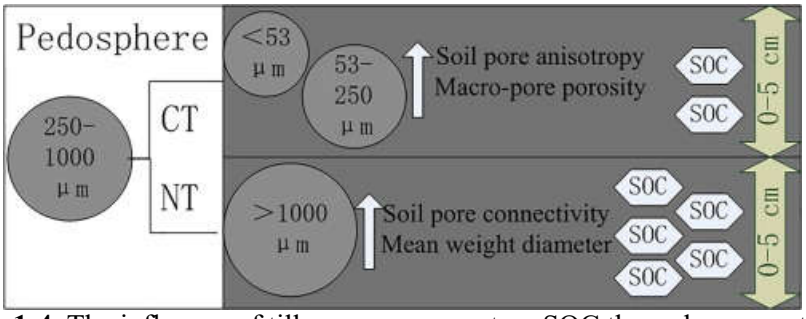

Figure 1-4. The influence of tillage management on SOC through aggregate pore structure (Guo et al. 2020).

1.2 Processes controlling SOC sequestration

SOC pools are extremely dynamic, and their dynamic accumulation and distribution are greatly influenced by biotic and abiotic factors in a belowground environment (Schnitzer and Monreal 2011). Biotic factors such as plant inputs, which provide the main source of food and energy for the survival of soil microorganisms, are critical regulators of SOC. Abiotic factors include management, climate, mineralogy, and fires, which induced differences in soil physical, chemical, and biological properties making SOC storage vary drastically (Jackson et al. 2017). Regardless of these factors, they ultimately affect the SOC sequestration by influencing three main processes, i.e., the physical isolation of SOC, chemical protection, and biological controls (Six et al. 2002; Tivet et al. 2013).

1.2.1 Biological processes

Among biological controls, the formation and decomposition of soil organic

matter (SOM) are mainly microbially-mediated (Lal et al. 2015). It is known that microorganisms are widespread in various soil environments and have strong adaptability relying on their activity and populations (Griffiths et al. 2000). In general, microorganisms play a critical role in controlling the SOC pool through their catabolic activities, not only promoting the biodegradation of easily degradable organic matter and release of gaseous C, but also stabilizing C into new organic metabolites (Schimel and Schaeffer 2012; Singh et al. 2019). Previous studies have shown the C dynamics in different fractions, such as labile OC, are strongly correlated to the microorganisms (Song et al. 2021).

In addition, more and more studies have identified that inputs of substrates could accelerate the decomposition of SOC through biological priming mechanisms (Guenet et al. 2018). In general, old SOM substrate are less decomposable by microorganisms than exogenous fresh organic matter (Jastrow et al. 2007). It was reported that microorganisms can continue to decompose old SOM to meet nutritional demands in the case of strong substrates limitation (Joergensen and Wichern 2018). However, the addition of fresh substrates leads to an increase in the decomposition of old SOM, which is named the priming effect, mainly due to the regulation and mediation of microorganisms (Kuzyakov et al. 2000). The growing body of literature on priming effects during recent years reflects the key role of biotic mechanisms on the SOC cycle in soil. Blagodatskaya and Kuzyakov (2008) divided priming into apparent priming (changes in C originated from microbial biomass turnover after the addition of easy-available substrates) and real priming (change in SOM decomposition) (Blagodatskaya & Kuzyakov 2008; Kuzyakov 2010).

Fresh substrates inputs could cause a greater nutrient demand by microorganisms, which leads to the increase of SOC mineralization to satisfy this demand (Guenet et al. 2018). During the process, a variety of dormant microorganisms are activated and higher enzyme production is induced, which enhance the degradation of SOM. Previous studies reported that the intensity of the priming strongly depended on the rate of exogenous C input (Blagodatskaya & Kuzyakov, 2008): low input causes a microbe biomass turnover, higher input could induce negative priming due to the preferential use of the added substrate rather than SOC, suggesting that substrate addition could affect the duration of SOC storage (Derrien et al. 2014).

Therefore, the potential of SOC sequestration is strongly dependent on the quality and quantity of substrate and the age of the SOC utilized by microbes (Derrien et al. 2014; Jagadamma et al. 2014). Thus, both apparent and real priming actions are mediated by the microorganisms community (Blagodatskaya & Kuzyakov 2008). Therefore, soil C pool dynamics can be driven by shifts in microbial community composition and activity (Jiang et al. 2019; Lehmann & Kleber 2015; Liang et al. 2017).

1.2.1.1 Soil microbial biomass

As an important ecological indicator, soil microbial biomass is responsible for the decomposition of SOM and mineralization of nutrients (Marinari et al. 2006), and

responds more quickly to external interference than other soil properties (Araújo and Melo 2010). Previous studies have shown that the turnover of SOC is closely associated with the changes in microbial biomass (Drum et al. 2019), especially Gram-positive (G+) bacteria (Kindler et al. 2009). In general, soil microbial biomass is not only limited by the availability of organic substrates in soil, but also be activated by exogenous decomposable organic C-containing compounds from a dormant state to an active state (Zhang et al. 2013). During these processes, soil microbial biomass varies drastically and is accompanied by the decomposition of SOC (Hamer and Marschner 2005). Additionally, the latest studies found that microbial biomass may be the most important factor for affecting SOC stability (Song et al. 2021). Therefore, microbial biomass acts not only as a C pool but also as an active driver of C turnover (Kuzyakov 2010), and any changes in microbial biomass may directly impact C in soil.

Microbial-derived C components account for about 2–3% of total organic C (Anderson and Domsch 2010) and play a critical role in the SOC pool (Li et al. 2021; Ludwig et al. 2015). On one hand, living microbial biomass, the living part of SOM, is an important component of SOC. On the other hand, microbial residues, the legacy of considerable microbial-derived constituents (Shao et al. 2017), have been confirmed to be an important source of stable SOC (Amelung et al. 2008; Zhang et al. 2018). It was reported that the total microbial biomass is composed of approximately 98% of the total dormant and dead microbial biomass (Blagodatskaya & Kuzyakov 2013). Ludwig found microbial products and necromass contributed greatly to the formation of arable SOM, through the chain of proliferation, metabolism, and mortality (Ludwig et al. 2015). It is noteworthy that glomalin-related soil protein, considered to be the only glycoprotein derived from arbuscular mycorrhizal fungi (AMF), is an important and stable component of SOC. Its well-known function is the contribution to soil aggregation and structure (Rillig et al. 2002), and it has been used to indicate the influence of AMF on soil C sequestration (Wang et al. 2017). Studies on subtropical forest succession found that more SOC was enriched in surface soil where the largest amount of microbial residues accumulated, than in deeper soil (Shao et al. 2017). Despite recent progress in the understanding of the responses of soil microbes to soil C, the mechanism of soil microbial properties on soil C turnover remains poorly understood.

1.2.1.2 Diversity of microbial communities

Previous studies have investigated the relationship between the diversity of microbial communities and soil C turnover during the C decomposition process (Baumann et al. 2013; Wertz et al. 2006). Same as soil microbial biomass, the diversity of microbial communities is demonstrated to play a crucial role in influencing the SOC pool (Baumann et al. 2013; Zhang et al. 2013). Bonkowski and Roy (2005) demonstrated reduced respiration at low microbial diversity and confirmed the potential effect on C mineralization. Baumann et al. (2013) found that lower microbial diversity was accompanied by higher C derived from wheat sugars, meaning the reduced decomposition of wheat sugars resulted from

microbial diversity loss.

In soil, microbial community structure partly depends on the nature of organic substrates, because of the differences in substrate utilization by diverse microorganisms. With the most abundant in the soil system (Fierer 2017), soil fungal and bacterial species have been reported to facilitate the SOC turnover during their metabolic actions (Guo et al. 2016). The dominant roles of soil bacterial populations in utilizing available substrates and fungi populations in decomposing recalcitrant substrates were confirmed (Thoms and Gleixner 2013; Urbanová et al. 2015). This can also explain that fungi populations were more competitive than bacterial for utilizing substrates in the early stage after the addition of external fresh matter. Therefore, the SOC sequestration along with the processes of C turnover in soil possibly underwent different stages governed by various dominant microorganism populations. However, there are still a few studies observing the little effect of microbial diversity on C mineralization (Setälä and McLean 2004).

Diverse microbial community can be found due to soil spatial heterogeneity (Filho & Junior 2020). The environmental conditions at the aggregate-scale, including water, gas, and resource availability (Young and Ritz 2000), drastically vary with aggregate sizes and stimulate large variance microbial community structure, thus greatly influence the dynamics of SOC (Dorodnikov et al. 2009). Given the preference of available substrate utilization, a large diversity of bacteria population was observed in macro-aggregates (Blaud et al. 2014) that contain more labile C than micro-aggregates (Bronick and Lal 2005). There are also some findings showing that fungi contribute to the C turnover much faster in macro-aggregates than in small ones (Dorodnikov et al. 2009; Jiang et al. 2019; Six et al. 2004). Furthermore, a higher abundance of AMF was found to enhance C accumulation in > 1 mm aggregates, while a higher abundance of gram-positive bacteria in < 1 mm aggregates (Zhang et al. 2013). However, there are also some results showing that AM fungi is not important in C decomposition, which is inconsistent with above studies (Juan-Ovejero et al. 2020). Despite a growing number of studies focused on microorganism diversity, there is a dearth of information on microbial diversity regulating the C cycle at aggregate scale.

1.2.1.3 Soil enzyme activity

Soil enzyme, mainly produced by soil microorganisms, is known to be an active component during all biochemical processes in soil (Xu et al. 2021). Due to their high sensitivity to environmental stimuli, a series of enzymes have been widely used as indicators of soil quality in current researches (Bastida et al. 2006; Rutigliano et al. 2009). Compared to other indicators, soil enzyme activity can provide an early microbial response (Samuel et al. 2008) in soil, therefore a series of enzymes have been widely used as indicators for evaluating biological activity. According to resource allocation theory, microorganisms could produce the corresponding enzymes to mine relatively limited elements through consuming abundant elements (Allison et al. 2010). Therefore, enzyme activity is regarded to

regulate the balance between growing demand and resources during microbe metabolism (Sinsabaugh et al. 2015).

Simultaneously, the action of microbial enzymes on plant materials has been proven to be an important way to promote the decomposition of SOM (Liang et al. 2017). Therefore, organisms that rely on organic debris to survive need extracellular enzymes to break down polymers to obtain nutrients (Sinsabaugh 1994). During the process of decomposition of SOM, diverse microorganisms could induce and produce different series of enzymes in order to degrade different molecules (Romaní et al. 2006). Mooshammer et al. (2014) confirmed that soil enzymes participate in and regulate the processing of SOC decomposition through the depolymerization of macromolecular substrates or decomposition of different molecules. After depolymerized by extracellular enzymes, macromolecular substrates could be assimilated by microorganisms and participate in the formation of SOM (Mooshammer et al. 2014). Hence, the microbial enzyme was thought to be responsible for specific processes during SOC turnover (Zhou et al. 2020).

From aggregate scale, the distribution of enzyme activity in aggregate fractions was suggested to be primarily governed by the aggregate size (Liang et al. 2014) due to different contents and availability of organic substances (Allison and Jastrow 2006).

1.2.2 Non-biological processes

Physicochemical protection offered by soil structure and mineral surfaces is critical for building and maintaining soil C and N stocks.

1.2.2.1 Physical protection

A huge difference in turnover timescales of SOC pools at aggregate scale has been observed, which is partly due to physical protection. It is well known that soil aggregates provide effective physical protection of SOM (Chaplot and Cooper 2015) against rapid decomposition (Pulleman and Marinissen 2004), and this protection by aggregates has been widely studied (Six et al. 2000; Tisdall & Oades 1982).

It has been confirmed by several models (Edwards & Bremner 1967; Tisdall & Oades 1982; Tisdall & Oade 1982) that soil aggregation is the most potential way to retain OM inside the soil. During the process, the organic binding agents play a primary role in enmeshing the soil particles together and build different sized aggregates (Jastrow et al. 2007), which are classified as temporary, transient, and persistent agents (Oades 1984; Tisdall & Oades 1982). Persistent agents, including humic compounds, polymers, polyvalent cations, and other highly decomposed OM, are reported to stabilize micro-aggregates and contribute to long-term SOC sequestration. Temporary agents, including comprise fungal hyphae and plant roots, generally could enmesh the mineral particles to form or stabilize young macro-aggregates which contribute to short-term SOC sequestration. Transient agents, such as organic mucilages and polysaccharides, could also temporarily accentuate the aggregation. For instance, the contribution of polysaccharides to aggregation is reported to last for several weeks because polysaccharides are easily

decomposed (Liu et al. 2005) and rapidly released from organic residues.

At present, most studies believe that the anaerobic microenvironments inside macro-aggregates greatly reduce the decomposition of SOC by microorganisms to achieve physical protection (Lützow et al. 2006; Tivet et al. 2013), which is an effective way for SOC sequestration. In addition, micro-aggregates within macro-aggregates have also been found to be associated with strong stability and long-term sequestration of SOC (Denef et al. 2007; Six et al. 2000).

1.2.2.2 Chemical protection

In addition to biological processes and physical protection, the chemical interactions between SOC pools and inorganic components also partly determine the SOC sequestration (O'Brien and Jastrow 2013), such as chemical sorption to mineral surfaces (Baldock and Skjemstad 2000), polyvalent cation bridging between SOM and minerals (Muneer and Oades 1989), and layered chemical binding to mineral surfaces (Kleber et al. 2007).

Organic C in the soil is stored in numerous chemical compounds, such as amino acids, phenols, cellulose, proteins and lignin, with different types of chemical bonds and structural complexity (Cotrufo et al. 2013). Chemically, SOM can have inherent recalcitrance based on the functional group (e.g., alkyl, amide, aromatic) composition and molecular structure of these chemical compounds (Cotrufo et al. 2013). Specifically, the 'recalcitrance' of these chemical compounds is important for SOC cycling (Kleber & Johnson 2010).

There are now substantial studies showing that the concentrations of SOC are associated with clay- or silt-sized mineral particles. Clay-sized organomineral complexes are observed with greater accumulations but lower stability of C than in silt-sized particles (Christensen 1992; Post & Kwon 2000). The rate of C sequestration depends on the surface area, ionic charge, type, and chemical and geochemical composition of minerals (Blanco-Canqui and Lal 2004), and the rate and magnitude of C sequestration could be largely controlled by clay-organic complexes (Laird et al. 2001). Gonzalez and Laird (2003) determined that C-enriched humic compounds were preferentially attached to the surface of smectitic minerals. During mineralogical control of SOM accumulation, the chemical sorption of Fe-, Al-, Mn-oxides through large surface areas, micropores, and micro-aggregation, predominate in acidic soils, while polyvalent cations are mainly responsible for the chemical chelation between SOM and phyllosilicates in neutral and calcareous soils (Cotrufo et al. 2013; Jackson et al. 2017). Therefore, physicochemical associations between SOM and mineral particles are suggested to be one of the most important mechanisms in enhancing SOC sequestration (Huang et al. 2020; Kleber et al. 2015).

There is substantial evidence from many studies that hardly 5% of SOM decomposition is caused by abiotic chemical oxidation (Lal et al. 2015). For instance, O_2 limitation may lead to Fe reduction and release of physicochemically protected C, which could lead to microorganism priming and stimulate previously protected C mineralization (Huang et al. 2020). Sulman et al. (2014) concluded that

there is a balance between mineral-mediated soil C storage and microbe-driven C turnover under elevated CO_2.

1.3 SOC status under fertilization management

1.3.1 Chemical fertilization

Chemical fertilizer application is one of the most common agricultural management practices, and its effect on SOC sequestration thus receives extensive attention (Lou et al. 2011). Wide studies indicated that inorganic fertilization can increase SOC content (Gong et al. 2009; Halvorson et al. 2002; Purakayastha et al. 2008).

The increase of SOC with inorganic fertilization was partly attributed to enhancing C input with returned residue and rhizodeposition (Haynes and Naidu 1998; Purakayastha et al. 2008). Due to better shoot and root production of crops under fertilization, the input of residue and root biomass in soil increased (Zhao et al. 2009). Furthermore, the addition of fertilizer may strongly influence microbial activity, which can enhance C humification in residues (Fanin et al. 2015; Song et al. 2021).

During this microbial process, new C is greatly integrated into the soil and enhances the accumulation of SOC (Lange et al. 2015; Zhao et al. 2009). However, some studies found no significant effect of chemical fertilization on SOC (Abrar et al. 2020; Halvorson et al. 2002). Although the fertilization increased crop yield and residue returns, a notable decrease in SOC was observed with long-term NPK application (Abrar et al. 2020; Purakayastha et al. 2008). Greater losses of easily degraded C and recalcitrant organic C during the microbial process were observed after long-term NP application which led to lower C accumulation in soil (Li et al. 2021). The inconsistent results might depend on differences in the climatic and soil conditions, rate and balance status of fertilizer, tillage regime, and experimental duration (Lou et al. 2011).

There is an increasing trend of using organic rather than inorganic fertilizers due to concern of food safety, environmental pollution, and the urgency to dispose of animal and municipal wastes.

1.3.2 Organic modification

It is well known that organic amendment is an important management practice with the potential to reduce the dependence on inorganic fertilizers and maintain SOM content (Zhao et al. 2009). In China, the annual production of straw and livestock is estimated at about 1.00 billion tons and 3.80 billion tons (Zhao et al. 2017), respectively. With the promotion of sustainable development in agriculture, resource utilization of agricultural waste is encouraged by the government and has been one of the most important agricultural waste management in China in recent years (Zhang et al. 2008). Therefore, the proper application of organic waste to agricultural soil can not only respond to the call of national sustainable development policy, but also increase soil potential for SOC sequestration.

Assuming a mean C content of 45%, crop residue is estimated annually to contain

1.5 to 1.7 Pg C worldwide (Zhang et al. 2008). And annualized straw biomass was suggested to explain 80% of SOC in the topsoil layer (Sherrod et al. 2003). Previous researches also estimated that appropriate management such as leaving sufficient crop residue in place after planting can restore the C loss of croplands over a 50 year period at a rate of 24–40 Mt C $year^{-1}$ in the United States, 25 Gt C $year^{-1}$ in the world (Baker et al. 2007; Lal et al. 2003). Therefore, it has been considered to be one of the critical global strategies for SOC sequestration.

In addition, manuring is also confirmed to improve SOC pools and soil quality (Min et al. 2003), and it has been reported in pieces of literatures. Manuring increases the C sequestration in the form of POM and mineral-associated organic C in soil in tilled systems (Schjønning et al. 2002). In some cases, OM in manure entering the soil initially increased POM-C and then increased C storage in the form of mineral-associated C (Aoyama et al. 1999).

The increase of C storage and sequestration in organically fertilized treatments can be attributed to greater C inputs through organic matter and root biomass due to better crop growth (Ding et al. 2012). On one hand, crop residue provides the organic C within itself to regulate the activities of heterotrophic soil microorganisms and soil enzyme activity, which could influence microbial C source utilization. On the other hand, the addition of OM sources could improve soil aggregation due to a higher SOC content and a probable increase in root biomass (Bhattacharyya et al. 2009; Halvorson et al. 1999). Whalen et al. (2003) found that application of up to 45 Mg ha^{-1} $year^{-1}$ composted manure for 2 years led to an increasing proportion of water-stable macro-aggregates (> 2 mm) in conventional and no-tillage systems, which can protect SOC from degrading processes, thus increasing the SOC content (Holeplass et al. 2004; Su et al. 2006).

However, we should keep in mind that the application of high rates of manure could increase the labile C fraction and lead to potentially greater greenhouse gas emissions, which may increase environmental risks (Blair et al. 2006; Ding et al. 2012). Previous studies have reported that the application of high-level manure had induced more than two times of CO_2 emissions in soil compared with fertilizer treatment (Rochette and Gregorich 1998), and the released CO_2 is supposed to derive from both mineralization processes of autochthonous and exogenous soil organic matter as a consequence of stimulated microbial activity (Bastida et al. 2013). The data showed that 4.4 Mg C ha^{-1} emissions were occurred under 33.5 Mg ha^{-1} C of crop residues and manure inputs in 18 years, resulting from the SOC depletion by oxidation (Srinivasarao et al. 2014). Therefore, a proper inorganic and organic amendment is essential to maintain and increase SOC pools.

Considering the above facts, an understanding of the dynamics and the main controlling factors of soil SOC sequestration is essential. Therefore, evaluation of soil physicochemical, microbial community, and SOC characteristics under long-term fertilization regimes is very important and necessary to study the sustainable development of agriculture in the loess plateau.

2. Objective

Based on the above research, long-term reliance on chemical fertilizers has the potential to induce a decline in soil quality, nutrient imbalance, and soil organic matter (SOM) depletion under agricultural practices. The overall objective of this dissertation was to evaluate the long-term impact of fertilization on soil physicochemical characteristics, soil organic carbon (SOC) storage and the contribution of microbial community activities to SOC sequestration. Therefore, we explored the SOC and soil physical, chemical, and microbial characteristics under different fertilization regimes, and hypothesized that long-term organic amendment will improve soil physicochemical properties (reduce pH and increase stability of aggregate), alter microbial community activities, and increase SOC content in bulk soil and aggregate.

To address the above main objective, the specific objectives of this study are as follows:

(1) To explain how fertilization regimes affected microbial properties through altering soil physicochemical characteristics in loess plateau (Chapter 2).

(2) To evaluate the contribution of microbial community to SOC sequestration under long-term fertilization (Chapter 3).

(3) To identify the dominant microbe at aggregate scale and evaluate their impacts on SOC stock (Chapter 4).

3. Overview of the experimental design and study area

3.1 Description of the study site

The study site is located in the Dryland Farming Experimental Station in Shanxi province (112–113◦E, 37–38◦N) in northern China (Figure 1-5) and was initiated in 1993. The site is characterized by a continental monsoon climate with an elevation of approximately 1,100 m above sea level, annual rainfall of 520 mm and temperature of 7–8 °C. Spring maize is the main crop grown under the one-crop-per-year cropping system. Soils belong to a sandy clay loam cinnamon soil series which are characterized as Calcaric-Fluvic Cambisol (ISS-CAS, 2003; IUSS, 2006). At the start of the project, soil pH was on average 7.9, and SOC and soil organic N concentrations were 15.0 g kg^{-1} and 1.0 g kg^{-1}, respectively.

3.2 Experimental design

3.1.1 Long-term experiment

The long-term experiment had a randomized block design with three replicates, each plot was 6 × 6 m. The four treatments included in this study were as follows: no fertilization management (CK), inorganic fertilizers application alone (NP), inorganic fertilization plus the incorporation of 3,000 kg ha^{-1} maize straw (NPS), and inorganic fertilization in combination with 1,500 kg ha^{-1} composted cow

manure (NPM). Each plot of the treatments NP, NPS and NPM had nitrogen 105 kg ha^{-1} and phosphorus 105 kg ha^{-1} applied once a year, respectively, using urea (46% N) and calcium superphosphate (7% P) in a ratio of N to P of 1:0.44. The mean proportions of SOM, total nitrogen, total phosphorus and total potassium were 75%, 0.63%, 0.04% and 0.72% in maize straw, and 36%, 0.96%, 0.17% and 0.74% in cattle manure, respectively. All of the fertilizers and OM were applied with conventional tillage (plowing once each year at a depth of 20 cm) after harvesting. Seeding was done at the end of April without any tillage and harvesting at the beginning of October, with twice weeding during growth seasons every year.

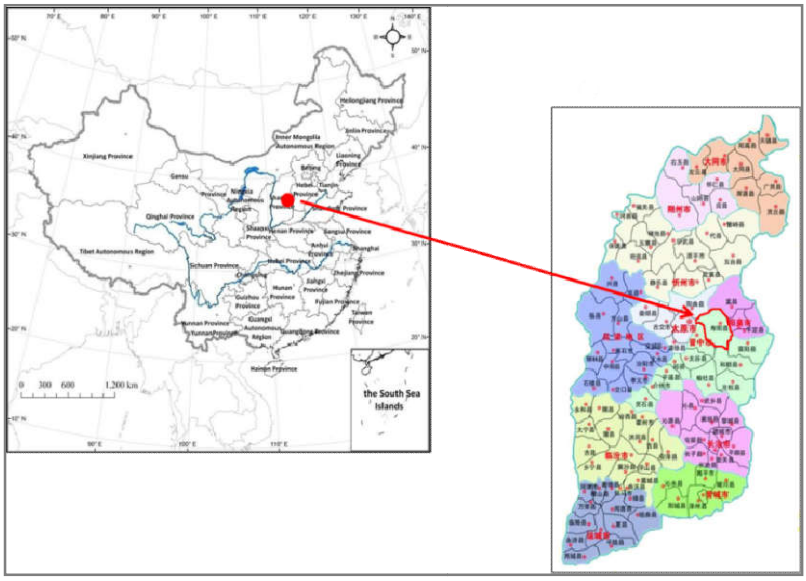

Figure 1-5. Map of the study site in Loess Plateau, China.

3.1.2 Incubation experiment

In order to determine whether the microorganisms derived from the initial aggregate promote C accumulation, soils were sampled at a depth of 0–10 cm from the field with no fertilization management (CK) and an incubation of classified aggregates (> 5 mm, 2–5 mm, 1–2 mm, 0.25–1 mm or < 0.25 mm) with ^{13}C-labeled maize straw application was conducted (Figure 1-6). Labeled maize residues were air dried at 40°C and then chopped into 1 cm. The C content of the straw was 40.01% and the $\delta^{13}C$ value was 690.50‰. All soil aggregate samples were adjusted to 50–60% of the water-holding capacity to reach maximum microbial activity. Rewetted soils were equilibrated by incubating for 15 days at 25° in the dark and at constant moisture. A total of 30 incubation microcosms were prepared in plastic containers without covers, and each microcosm was filled with 200 g dry weight equivalent of rewetted soil aggregates. Six microcosms were prepared per aggregate size treatment.

Three treatments with three replications were included: (1) CK, the original

non-incubated soil aggregates named the CK treatment were used as the control treatment for the incubation experiment; (2) NS, these soil aggregates were cultivated with no straw addition to limite the availability of organic material; (3) S, with labeled straw fully mixed into soil aggregates samples (Keep 1 g per microcosm, and manually shake these microcosms up and down until fully mixed). The incubation was carried out at 25°C and 50–60% water-holding capacity for 300 days maintaining moisture by periodical additions of distilled water. Soil moisture was controlled by weight twice a week.

After incubation, some of each sample was removed from each incubator and air dried to determine the organic C contents and analyze the amount of C derived from straw (C_{straw}) after picking out all visible debris. The remainder of the samples was stored at –20°C for biochemical analysis.

Figure 1-6. Short-term incubation experiment of different sized aggregates.

ChapterII

The influence of soil properties on microbial community under long-term fertilization in Loess Plateau

Microorganisms are vital in soil environments, given its important role in determining soil health and fertility and impacting plant productivity. This chapter aimed to evaluate the impact of physicochemical characteristics on microbial community under long-term fertilization in loess plateau.

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Chapter II The influence of soil properties on microbial community under long-term fertilization in Loess Plateau

Abstract

Clarifying the response of soil microbial communities under long-term fertilization is of great significance for preserving soil biodiversity in the intensive farmland ecosystem. However, the driving and relative contributions of soil physicochemical characteristics under long-term fertilization to microbial community variation are still unclear. Here, we examined soil microbial community biomass (via phospholipid fatty acid (PLFA) analysis) and composition (via 16S rDNA gene sequencing), soil physicochemical properties, and their relationships in a long-term fertilization experiment in China. The microbial communities differed dramatically across the four fertilization treatments. Microbial biomass had close relationships with both soil chemical and physical properties, and soil chemical properties explained a larger proportion of variation of microbial biomass than physical ones. The same result with variation of bacterial community composition. In addition, soil chemical properties are more suitable as indicators of variation in soil fungal community composition. The fertilization could cause the changes in the explanation of soil chemical and physical properties to microbial community. Overall, our results indicate that soil physicochemical properties are closely related to microbial community biomass and composition, which would be affected by fertilization regime. It is possible to evaluate or control directly soil microbial community via the regulation of main driving factors and farming management in future.

Keywords:

Soil physicochemical properties; Microbial community composition; Biomasses; Diversity.

1. Introduction

As the key driver of multiple biogeochemical processes, soil microbe have a critical role in maintaining soil ecological function, such as soil organic matter decomposition, nutrient cycling (Castrillo et al. 2017). Due to the opaque nature of the soil environment, the direct observation of soil communities is impossible (Strickland and Rousk 2010). The dominant method to observing soil microbial communities has been to analyze microbial properties, i.e., microbial biomass, diversity and community composition. Differences in soil microbial properties can drive enormous variations in many biogeochemical processes (Crowther et al. 2019; Kang et al. 2021). Fungi and bacteria are the most numerous in soil microbial community, and exhibits immense diversity in terms of structure. Generally, bacteria degrade the easily decomposed substrates more efficiently (Strickland and Rousk 2010), while fungi are more efficient colonizers of the recalcitrant substrates relying on powerful enzymatic capabilities (Six et al. 2006).

Microbial traits are extremely sensitive to changes in soil properties. Numerous studies have shown that both soil physicochemical properties are the dominating controls affecting soil microbial community (Kang et al. 2021; Pasternak et al. 2013; Tsiknia et al. 2014). For instance, the soil organic carbon (SOC), C:N ratio and pH have been confirmed to significantly affect the composition and structure of soil microbial communities (Arroyo et al. 2015; Moche et al. 2015; Shen et al. 2013a). In addition, soil texture were reported to be the most important factor after pH in shaping the soil microbial community (Xia et al. 2020). Consistent with Sessitsch' results, the silt and/or clay content significantly altered the relative abundances of some fungi and filamentous bacteria (Sessitsch et al. 2001). Moreover, soil aggregates harbor diverse microhabitats for microbes, and the aggregate stability causes variations in soil organic matter stabilities and bioaccessibilities (Najera et al. 2020; Johan Six et al. 2000), which directly induce variations of the microbial community structures (Wang et al. 2021). Based on previous single-factor analysis of soil physical and chemical properties on microorganisms changes, it can be expected that the impacts by which soil physical properties modulate the microbial community are expected to be not the same as those by chemical properties. Despite incomplete information on the linkage between soil physicochemical properties and microbial traits, all of these studies support the presence of the main drivers. Therefore, more work is needed for quantifying the relative contributions of these soil physicochemical properties to microbial community variations is indispensable in order to identify the main driving factors.

Fertilization and organic amendment are the crucial global management strategies for sustainable farming systems. The impact of fertilization and organic amendments on microbial community composition and structure has been extensively documented. For instance, long-term inorganic fertilizer application reduces bacterial biodiversity and abundance, while manure application increases bacterial abundance and diversity (Cui et al. 2018). Many previous studies have reported that fertilization managements affect soil microbial community structure

(Cui et al. 2018; Xiang et al. 2020) via influencing soil physical and chemical properties. The applications of both organic and inorganic fertilizers have been reported to induce alterations in soil physical properties, like bulk density, porosity, soil physical structural stability water content, and in soil chemical properties, like C/N ratio, substrate availability (Zhang et al. 2021; Zhao et al. 2021). All the changes caused by fertilization managements in the soil can further alter the microbial growth and microbial community composition and diversity (Lazcano et al. 2013; Li et al. 2021). It has been found that microbial community properties change with fertilization management (Wang et al. 2021) or main soil properties (Han et al. 2021). Indicators of soil properties vary in response to fertilization, depending on the original soil quality. For instance, nitrogen, phosphorus and potassium nutrients respond faster to fertilization than other indicators in poor soil (Li et al. 2020). However, the mediating effect of different long-term fertilizations on the dominant factors affecting microbial community properties, has not been thoroughly studied. Hence, there is still a knowledge gap regarding the regulation of various fertilization managements to microbial community composition on the Northeast Plain of China.

The objective of this study was to evaluate the contribution of soil physical and chemical properties to the variation of microbial community structure, including microbial community abundance and composition, and their response to different fertilization managements. To achieve these goals, we performed a 27-year field experiment with four fertilization treatments. The high-throughput sequencing and PLFA analysis were used to analyze microbial community composition and biomass. SOC content, C: N ratio and pH were determined as the main chemical properties affecting the microbial community. Soil water content, and aggregate stability determined by MWD, GMD, $R_{0.25}$ were measured as the main physical properties affecting the microbial community. We hypothesized that (H1) The main drivers of soil physicochemical properties would contribute differently to variations of the microbial community. (H2) Organic management would increase the diversity and abundance of microorganisms. (H2) The application of organic management would increase microbial biodiversity and abundance through more regulation of physical indicators, .

2. Materials and methods

2.1. Description and soil sampling

The experimental field site was located at the Dryland Farming Experimental Station, Shouyang county, Shanxi province, northern China (112–113 °E, 37–38 °N). The experimental site was started in 1993, and it has a continental monsoon climate with an elevation of approximately 1100 m above sea level, the average temperature of 7–8 °C, and the annual rainfall of 520 mm. The main crop in this region is spring maize with one-crop-per-year cropping system. The soil belongs to sandy clay loam cinnamon soil (Calcaric-Fluvic Cambisol; ISS-CAS, 2003; IUSS, 2006). At the start of the project, SOC content was 15.0 g kg^{-1}, and

soil pH was on average 7.9.

Four treatments arranged in a randomized complete block design with three replications in 12 experimental plots (each plot was 6 × 6 m). These four treatments were: (1) CK, no fertilization; (2) NP, chemical NP fertilizers; (3) NPS, the NP fertilizers plus maize straw at 3000 kg ha^{-1}; (4) NPM, the NP fertilizers plus composted cow manure at 1500 kg ha^{-1}. Nitrogen 105 kg ha^{-1} and phosphorus 105 kg ha^{-1} were applied using urea (46% N) and calcium superphosphate (7% P) with a ratio 1: 0.4 (N: P). The organic matter, total nitrogen, total phosphorus, and total potassium of maize straw were 75%, 0.63%, 0.04%, and 0.72% respectively, and 36%, 0.96%, 0.17%, and 0.74% in cattle manure. NP fertilizers and maize straw, cattle manure were plowed (depth of 20 cm) into the soil after harvesting in October every year. Seeding was done at the end of April without any tillage, and harvesting at the end of October.

Soil samples (0–15 cm) for each plot were collected after harvesting (before fertilizer application) in October, 2018. Five soil cores (10 × 10 cm in diameter) for each plot were collected randomly, then were placed on ice bag in soil box to prevent from the failure of aggregates and transferred to the laboratory within 24 h. The fresh soil was separated manually along the natural cracks of fracture with any remaining stones, plant material, and visible soil fauna removed by tweezers. Then, the cores for each plot were combined. 200 g dry weight equivalent of fresh samples were taken out in order to test aggregate stability using dry sieving method. After that, soil samples were immediately and gently sieved through a 2-mm mesh sieve, and each sample was divided into two subsamples. One was stored at –20 °C for biological analyses (16S rDNA and PLFA), and the other one was air-dried for soil physicochemical analyses. In addition, undisturbed soil samples were collected using 100 cm^3 core samplers for measuring the soil bulk density and soil moisture content with three replicates per treatment.

2.2 Soil analysis

2.2.1 Soil physical and chemical analyses

The soil organic content (SOC) and total organic nitrogen content (TN) were determined from dried subsamples with an element analyzer (C/N Flash EA 112 Series-Leco Truspec). Dissolved organic carbon (DOC) content was obtained from soil extracted solution which made by distilled water (1:5 w:v) and determined by a C analyzer (Multi N/C 3100, Analytic Jena, Germany). Soil moisture (%) was measured by drying fresh subsamples to constant weight at 105 °C following the oven-drying method. pH were measured using a pH meter at a soil: deionized water ratio of 1: 2.5.

2.2.2 Soil aggregate stability

Soil aggregate stability was determined using dry-sieving method. The 200 g air-dried soil samples were mechanically sieved (shaken for 2 min at a rate of 30 times per minute) using Retsch AS200 Control (Retsch Technology, Düsseldorf, Germany), and divided into five aggregate sizes (> 5 mm, 2–5 mm, 1–2 mm,

0.25–1 mm and < 0.25 mm), then weighed separately to obtain the proportions of each size fraction of dry soil aggregate.

The index of soil aggregate stability was expressed by the mean weight diameter (MWD), geometric mean diameter (GMD), and the percentage of > 0.25 mm aggregates ($R_{0.25}$). MWD and GMD have been used to quantitatively describe the stability characteristics of soil aggregates, and $R_{0.25}$ is used for aggregates distribution. Higher values of MWD, GMD and $R_{0.25}$ represent greater stability of soil aggregates (Dabin et al. 2019). They were calculated as following equation:

$$MWD = \sum_{i=1}^{n}(xi * wi) \quad (1)$$

$$GMD = exp(\sum_{i=1}^{n} wi * lnxi) \quad (2)$$

$$R_{0.25} = (1 - M_{0.25}/M_T) \quad (3)$$

where *xi* is the mean diameter of the *i*-th aggregate size (mm) and *wi* is the percentage of aggregate mass in the *i*-th size, *n* is the number of sieves, $M_{0.25}$ is the mass of > 0.25 mm aggregates (g), and M_T is the total mass of all aggregates (g).

2.2.3 High-throughput sequencing

DNA was extracted from 0.25 g dry weight fresh soil samples (12 subsamples of soil with 3 repetitions) using the NanoDrop One (Thermo Fisher Scientific, MA, USA), and then was determined using a UV–vis spectrophotometer. 1% agarose gel electrophoresis was used to evaluate DNA quality. The primer 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) were used to amplify the V4 region of the bacterial 16S rRNA gene. The primers ITS3-F (GCATCGATGAAGAACGCAGC) and ITS4-R (TCCTCCGCTTATTGATATGC) were used for the variable ITS2 region of fungi. Then, the Taq DNA polymerase with a universal eubacterial primer set was used to amplifying of the 16S rDNA gene of the isolates. The PCR conditions used were 94℃ for 5 min, 30 cycles of 30 s denaturation at 94℃, 52℃ for 30 s (annealing), and 72℃ for 30 s (extension), followed by 10 min final elongation at 72℃. The PCR products were extracted from 1% (w/v) agarose gel and purified with E.Z.N.A. Gel Extraction Kit (Omega, USA). Then the purified amplicons were sequenced on an Illumina Nova 6000 platform and 250 bp paired-end reads were generated (Guangdong Magigene Biotechnology Co., Ltd. Guangzhou, China).

After that, the raw fastq files (Version 0.14.1, https://github.com/OpenGene/fastp) need to be quality-filtered and merged. The primers were removed by using cutadapt software (https://github.com/marcelm/cutadapt/) according to the primer information at the beginning and end of the sequence to obtain the paired-end Clean Reads

Any site with average quality score longer than 16 bp were truncated, and then

they were merged according to their overlap sequence. RDP Classifier algorithm with a confidence threshold of 70% was used to determine the taxonomic provenance of all sequences through searching the UNITE databases and SILVA. The obtained high-quality sequences were merged as operational taxonomic units (OTUs). To process the taxonomic classification of OTUs, the representative sequences of each OTU were generated and aligned against the Greengenes database, RDP databases, and Sliva database for bacterial OTUs and Unite database for fungal OTUs, respectively.

2.2.4 PLFA extraction and analysis

The microbial biomass of the soil was determined through phospholipid fatty acids (PLFA) to study the living microbial response to the addition of exogenous organic matter. PLFA are major constituents of the membranes of all living cells, and phospholipids from different groups of microorganisms contain a variety of different fatty acids. Thus some PLFAs can serve as "bio-signatures" to observe changes in living microbial biomass and microbial community structure. In brief, soil samples (3 g freeze-dried) were extracted twice in 7.6 mL one-phase system at a 1:2:0.8 ratio of chloroform/methanol/citrate. Thus, the total lipid extract was fractionated into phospholipids, neutral lipids, and glycolipids with silica acid columns (Supelco Inc., Bellefonte, PA, USA). After methylation, the polar lipids were esterified to the fatty acid methyl esters (FAMES) which could be analyzed by Gas Chromatograph Agilent Series (GC 6890, Agilent Technologies, Wilmington, DE, USA) with MIDI microbial identification system (MIDI, Inc., Newark, DE, USA). Methyl nonadecanoate (19:0) was used in the method as an internal standard.

Total extractable PLFA were determined to represent the total microbial biomass. Bacterial PLFA were divided into gram-positive bacteria (G+), gram-negative bacteria (G−), and general bacteria (16:0, 17:0, 18:0, 20:0). Cyclopropyl and monounsaturated fatty acids were used to be the biomarkers for G–bacteria, and iso- and anteiso-branched fatty acids were the indicators for G+ bacteria. Fungal PLFA were divided into general fungi (18:2ω6c) and arbuscular mycorrhizae (AM, 16:1ω5c).

2.3 Statistical analysis

In this study, all results were showed using the mean ± standard deviations (SD). All statistical analyses were conducted in SAS 9.4 in Windows 10. One-way analysis of ANOVA followed by the least significant difference (LSD) ($p < 0.05$) was used to determine the effect of the fertilization on all of the soil physicochemical and microbial indicators. Spearman correlations with p-value lower than 0.05, 0.01, and 0.001 were considered to determine the relationship between microbial communities and soil properties. The significance standard of microorganism at the taxonomic level was LDA > 3 and $p < 0.05$. Additionally, redundancy analyses (RDA) were used to elucidate the relationships between the soil microbial community composition and soil properties using CANOCO 5.0 for Windows. Variation partitioning analysis (VPA) was performed to quantify the variation in microbial community composition or biomass explained by soil physical and chemical properties in the Vegan package of R.

3. Results

3.1 Variation in soil characteristics

Fertilization had a significant effect on environmental variables (Table 2-1). Specifically, inorganic fertilization had a significant ($p < 0.05$) effect on soil SOC, pH, MWD, GMD and $R_{0.25}$. Combined application of organic and inorganic fertilizers had significant effects on soil SOC, DOC, TN, pH, RZ, MWD, GMD.

As shown in Table 2-1, the higher SOC, total N, DOC contents, and lower soil pH were observed with NP (chemical fertilizer), NPS (straw plus chemical fertilizer) and NPM (manure plus chemical fertilizer) treatments. Relative to the CK treatment (no fertilization), the MWD, GMD and $R_{0.25}$ in NP treatment were significantly ($p < 0.05$) decreased by 15.4%, 16.9% and 8.1% respectively (Table 2-1), while in organic fertilizer-applied treatment were increased ($p < 0.05$) by 10.3–16.7%, 11.9–17.1% and 3.2–6.5% respectively. In addition, combined application of organic and inorganic fertilizers significantly ($p < 0.05$) reduced soil bulk density.

Table 2-1 Soil physicochemical properties under each treatment.

Treatments	CK	NP	NPS	NPM
SOC(g/kg)	12.4±0.8c	16.3±1.9b	18.2±0.9a	16.0±1.1b
TN(g/kg)	0.8±0.2b	0.9±0.1ab	0.9±0.1ab	1.1±0.1a
C/N	16.1±2.3b	17.5±1.5ab	19.3±1.6a	15.3±0.8b
DOC (g/kg)	0.1±0.02c	0.1±0.04bc	0.2±0.04a	0.1±0.02b
pH	8.2±0.01a	8.0±0.02b	7.9±0.01d	7.9±0.01c
Moisure	0.1±0.03ab	0.1±0.01b	0.1±0.02a	0.2±0.01a
RZ	1.3±0.01a	1.3±0.01a	1.3±0.01b	1.2±0.01c
MWD	1.6±0.1b	1.3±0.03c	1.7±0.07a	1.8±0.03a
GMD	0.6±0.04b	0.5±0.02c	0.7±0.04a	0.7±0.03a
$R_{0.25}$	0.6±0.02a	0.6±0.02b	0.7±0.02a	0.6±0.02a

Note: RZ, Bulk density.

3.2 Microbial biomasses

The analyses in Figure 2-1 showed that the individual group microbial biomass differed significantly between among the four treatments. Especially, inorganic fertilization reduced the total microbial biomass, including bacterial by (14.2%) and fungal biomasses by (26.2%), compared to CK. All soil microbial biomasses were significantly ($p < 0.001$) higher with OM treatment compared with NP treatment (by 12.6–14.5%). No differences of microbial biomasses were observed between NPS and NPM.

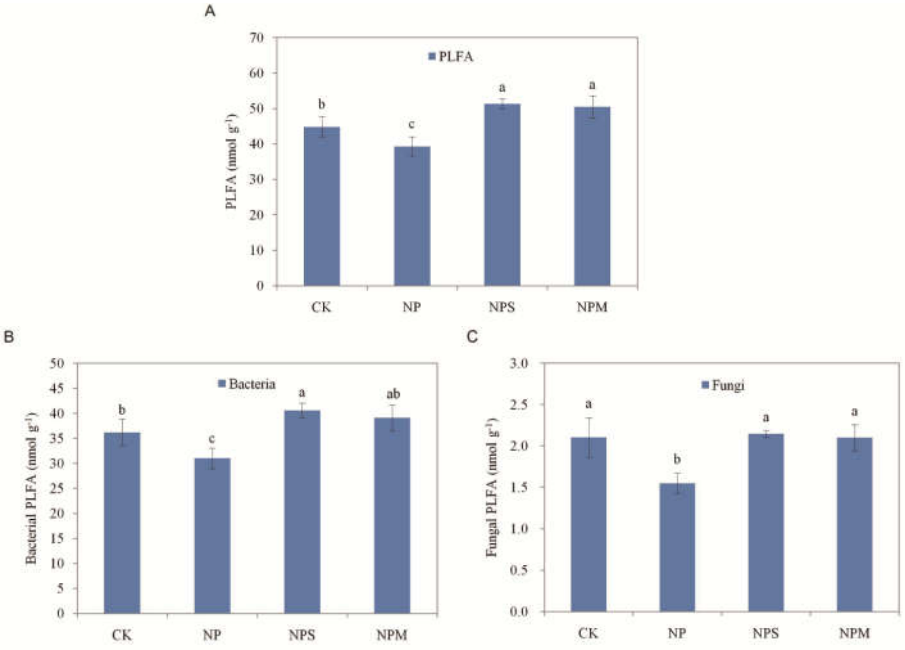


Figure 2-1. Total microbial biomass (A), bacterial biomass (B) and fungal biomass (C) in the four fertilization treatments. CK: the control treatment without fertilization management; NP: the treatment with only inorganic fertilizer; NPS: the treatment with inorganic fertilizer and maize straw addition; NPM: the treatment with inorganic fertilizer and cattle manure. Different lowercase letters indicated significant difference at $p < 0.05$ among the different treatments.

3.3 Bacterial and fungal community composition and species with significant difference

Approximately 841512 and 852084 sequences (24 samples, average 70,126 and 71,007 sequences) were obtained for the bacterial and fungal community, respectively. The results showed that the composition of bacterial and fungal community structures differed among the four treatments (Figure 2-2).

Obviously, the relative abundance of dominant phyla showed significant differences between bacterial and fungal communities among the four treatments (Figure 2-2). The dominant phyla of bacteria across all the treatments soils were

Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes, accounting for more than 88% of the bacterial sequences from each of the soils (Figure 2-2). In addition, Tenericutes, Cyanobacteria, Fusobacteria, and Verrucomicrobia were also present in these soils but at low abundances. The dominant phyla of fungi were Ascomycota, Mortierellomycota, and Basidiomycota, which together accounted for more than 85% across all soils. In addition, Chytridiomycota, and Glomeromycota were present in relatively low proportions.

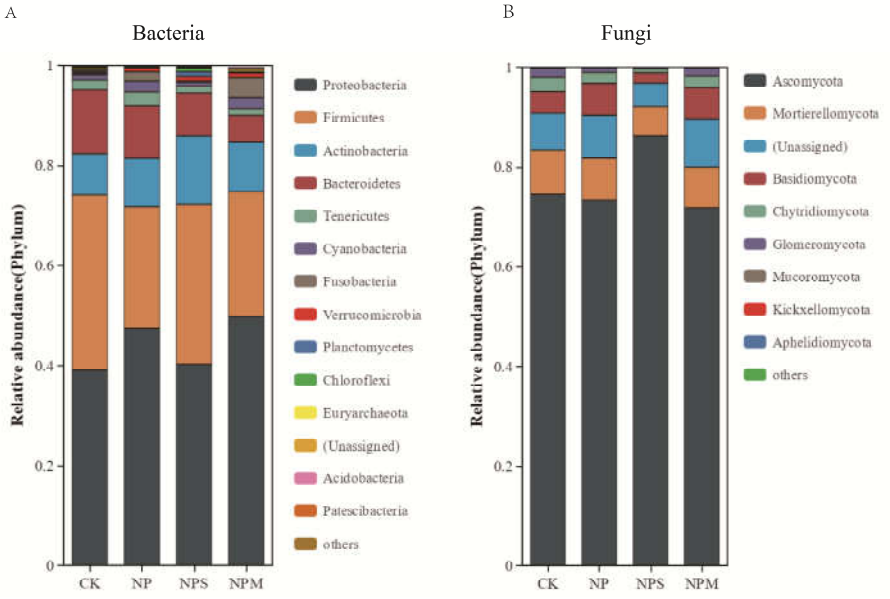


Figure 2-2. Relative abundances of bacterial (A) and fungal (B) phyla in soils separated according to fertilization treatments.

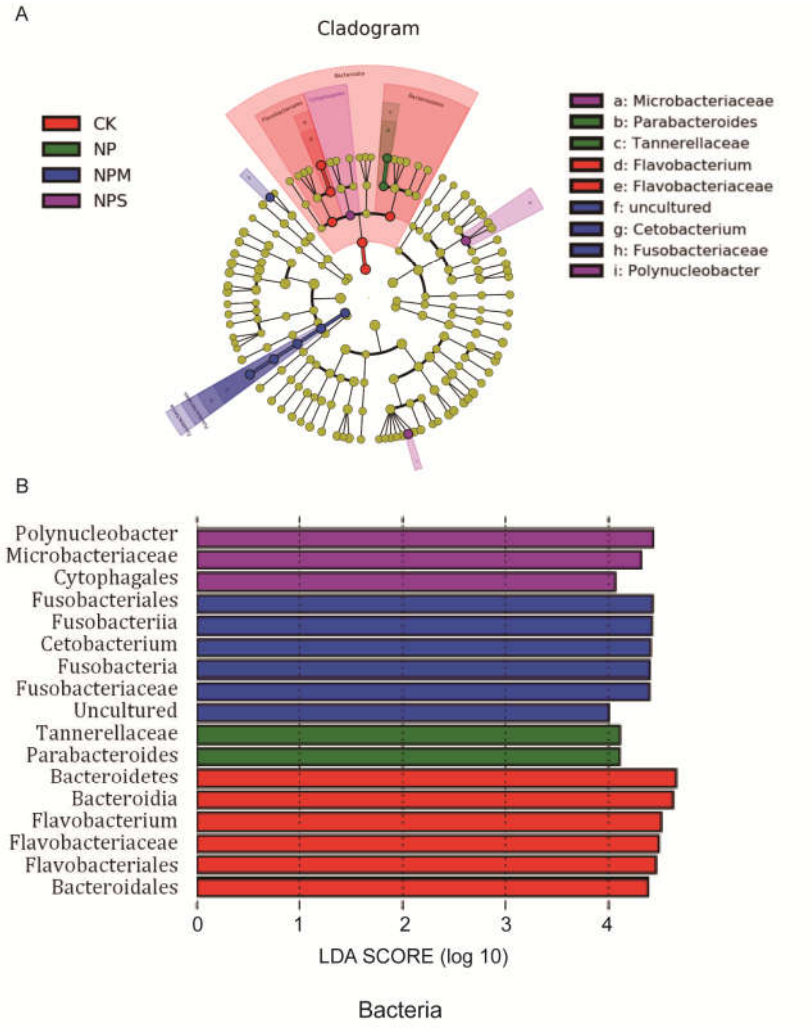
The LEfSe analysis showed that the abundance of some taxa differed among the CK, NP, NPS, and NPM samples, respectively (LDA > 3, $p < 0.05$). According to the linear discriminant analysis (LDA, Figure 2-3) of phyla bacteria, the LDA value of phyla Bacteroidete was 4.7 in CK, which were greater than the default value 2.0, meaning that the relative abundances of these two groups were significantly higher in CK than in other treatments. The LDA value of Tannerellaceae and Parabacteroides was 4.1 under NP treatment, other enriched taxonomic groups could also be the biomarker in the respective treatment group. The addition of organic fertilizers also enriched Polynucleobacter and Microbacteriaceae in NPS, Fusobacteria in NPM, respectively.

In fungal communities (Figure 2-3), the Conocybe_crispa_SH179180 group (Basidiomycota) had the LDA value of 4.6 in CK treatment. The application of inorganic fertilizer (NP) substantially enriched the Microascales (Ascomycota), Geopora (Ascomycota), Pyronemataceae (Ascomycota) and Conocybe_lenticulospora_SH220671 (Basidiomycota); in addition, the biomarker

phyla of NPS was Sordariomycetes (Ascomycota), while NPM significantly enriched the Tubaria (Basidiomycota) (Figure 2-3), suggesting that the relative abundance of these two phyla groups were significantly higher in NPS and NPM than in the other two treatments.

3.4 Bacterial and fungal α-diversity

We used Chao1 index and diversity (Simpson and Shannon diversity indexes) as indicators of α-diversity. Fertilization had a significant effect on the indicators of bacterial α-diversity with the exception of the Shannon index (Table 2-2), and on the all indicators of fungal α-diversity (Table 2-2). We found that Chao1 index of bacterial diversity in NPS and NPM were significant higher than in CK and NP treatments.



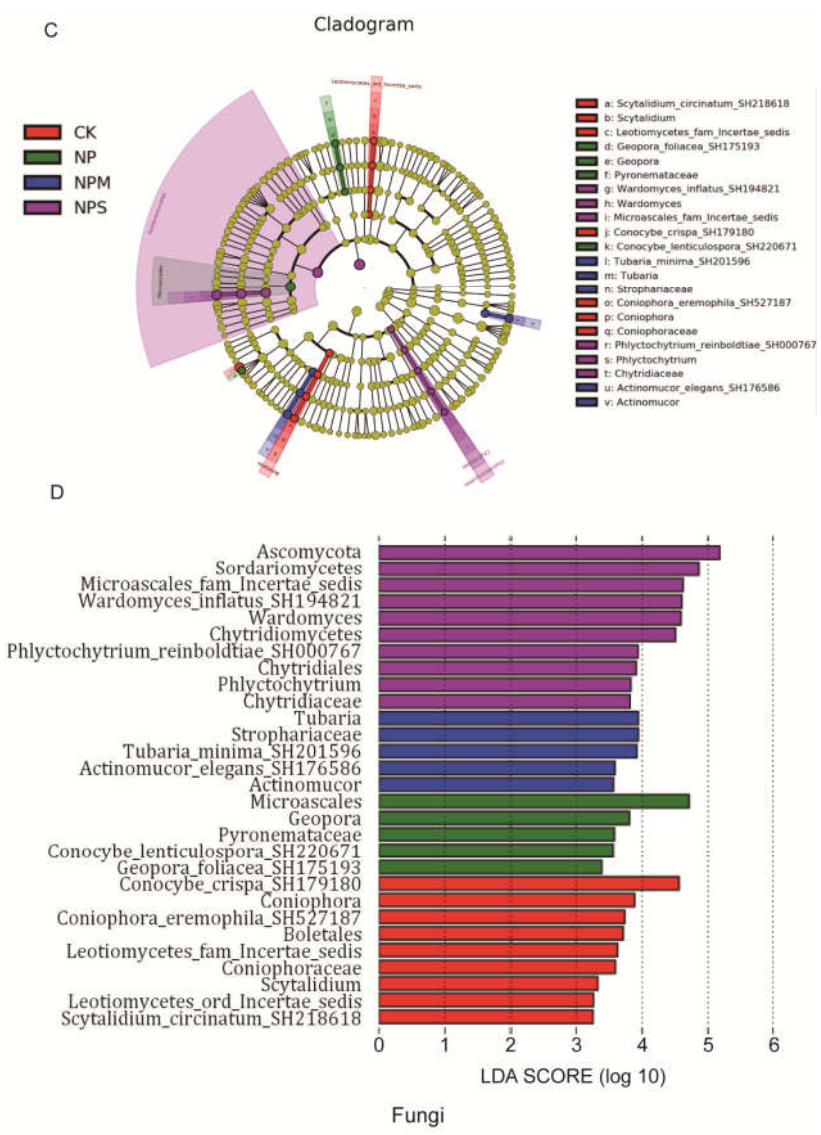


Figure 2-1. Cladogram shows significant differences between differential bacterial and fungal groups. Taxa with significant differences in abundance between different treatments are represented by colored dots (red indicate CK, green indicate NP, purple indicate NPS, blue indicate NPM, yellow indicating nonsignificant), and Cladogram circles represent phylogenetic taxa from phylum to genus. Only the LDA score > 4 for bacteria and > 3 for fungi were shown.

Table 2-2 Alpha diversity analyses of soil bacterial and fungal community in different treatments.

Treat	Bacteria			Fungi		
ments	chao	shannon	simpson	chao	shannon	simpson
CK	8.5±0.9b	1.4±0.1a	0.29±0.01c	6.04±0.1a	0.57±0.1b	0.93±0.1a
NP	8.4±0.6b	1.4±0.1a	0.31±0.01b	5.33±0.6b	0.56±0.1b	0.92±0.1a
NPS	14.0±1.5a	1.5±0.1a	0.29±0.01c	5.25±0.3b	0.76±0.03a	0.54±0.1b
NPM	11.7±3.0ab	1.4±0.02a	0.35±0.01a	6.17±0.3a	0.54±0.01b	0.99±0.04a
	$p < 0.05$	$p > 0.05$	$p < 0.001$	$p < 0.05$	$p < 0.01$	$p < 0.01$

Note: Different lowercase letters indicated significant difference at $p < 0.05$ among the different treatments.

3.5 Relationships of bacterial and fungal community with soil properties

Contributions of soil properties to soil microbial biomasses were analyzed separately by RDA (Figure 2-4). The amount of variability explained by all the redundancy axes was 99.8%. As shown in Figure 2-4, all soil microbial biomasses were significantly ($p < 0.05$) associated with soil physical indicators (MWD, GMD, and $R_{0.25}$), which is more prominently in NPS and NPM treatments.

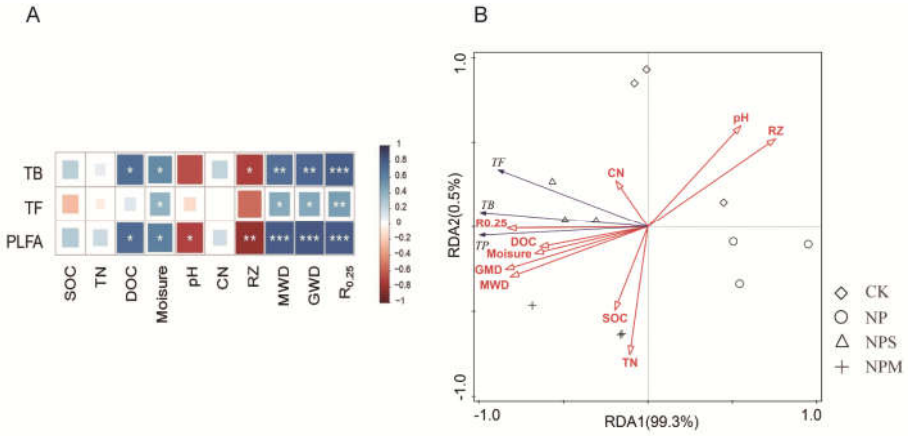


Figure 2-2. Correlogram (Pearson's correlation coefficient, A) and redundancy analysis (RDA, B) between the microbial biomass and soil physicochemical parameters in different treatments. * : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$ and ns: not significant. Different shapes represent different treatments.

From Table 2-3, we found that bacterial communities composition were significantly ($p < 0.05$) correlated with SOC, DOC, moisture, while fungal communities were correlated with most all of soil properties with exception of C/N, MWD and $R_{0.25}$. Of all the soil characteristics examined, SOC was the most important factors contributing to variation in soil bacterial community structure (r ¼ 0.677, $p < 0.001$) (Table 2-3), while C: N revealed no contributing to variation in both bacterial and fungal communities compositions (Table 2-3). These factors that significantly correlated with the bacterial and fungal community composition were selected to perform redundancy analysis (RDA), which showed that soil pH and DOC have stronger effects on bacterial community composition, C/N and DOC on fungal community composition (Figure 2-5). Thus, DOC is the fundamental factor that effecting bacterial and fungal community structure.

Results of the RDA (Figure 2-5) showed the adaptability of microbial phyla with differential treatments and the influence of soil physical and chemical factors on soil microbial community. In bacterial community, the relative abundance of dominant phyla Bacteroidetes showed significant correlation with TN, soil pH, RZ, MWD, and GMD, and Proteobacteria and Actinobacteria were observed to relate to TN and DOC (Figure 2-5), respectively. In fungal community, we found

a significant correlation between dominant phyla Ascomycota, Mortierellomycota and Chytridiomycota fungus with DOC, pH, and C/N. Overall, soil chemical properties were significantly correlated with the relative abundance of different dominant phyla.

Table 2-3 Differences in the influence of different environmental factors for all the treatments at operational taxonomic unit (OTU) level.

Characteristics	Bacterial				. Fungal			
	Sums Of Sqs	Mean Sqs	R^2	P. value	Sums Of Sqs	Mean Sqs	R^2	P. value
SOC	0.3	0.3	0.1	0.001	0.3	0.3	0.1	0.001
TN	0.2	0.2	0.04	0.1	0.2	0.2	0.1	0.002
DOC	0.3	0.3	0.1	0.003	0.2	0.2	0.1	0.001
Moisure	0.2	0.2	0.1	0.04	0.2	0.2	0.1	0.001
pH	0.2	0.2	0.03	0.3	0.3	0.3	0.1	0.001
C/N	0.1	0.1	0.03	0.7	0.1	0.1	0.02	0.5
RZ	0.2	0.2	0.04	0.2	0.1	0.1	0.1	0.01
MWD	0.1	0.1	0.02	0.8	0.1	0.1	0.03	0.004
GMD	0.1	0.1	0.03	0.5	0.1	0.1	0.2	0.001
$R_{0.25}$	0.2	0.2	0.03	0.3	0.1	0.1	0.1	0.002

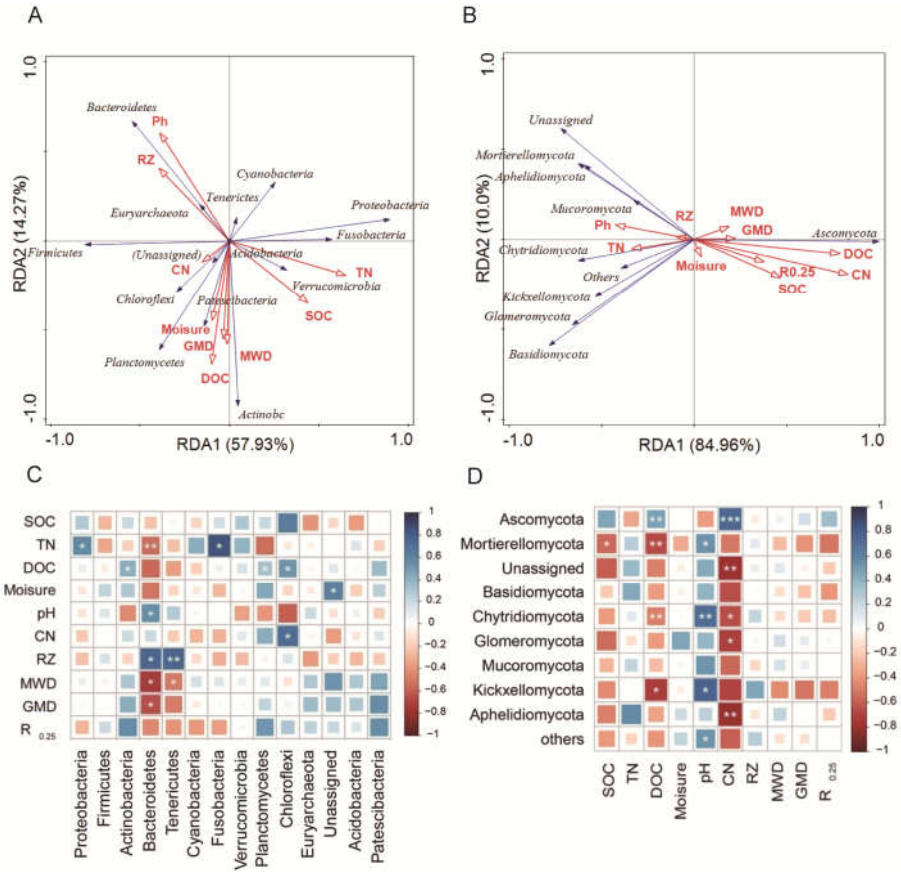


Figure 2-3. Redundancy analysis (RDA) and Correlogram (Pearson's correlation coefficient) between microbial community compositions and soil physicochemical parameters in different treatments, including bacterial phylum-level taxonomy, fungal phylum-level taxonomy. * : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$ and ns: not significant.

Results of the RDA (Figure 2-6) revealed that species with significant difference across the four treatments could be distinguished by soil properties. The first axis explained 64.02% of the variation in soil bacterial community composition, which was primarily associated with pH, and TN, and the second axis described 19.7% of the variation, which was mainly related to C/N, and DOC (Figure 2-6). SOC and pH had the largest effect on the fungal community composition, followed by DOC, and RZ. The first axis explained 81.58% of the variation in the soil fungal community composition, which was primarily associated with SOC, DOC, and pH, and the second axis explained 11.15% of the variation, which was mainly related to RZ and TN.

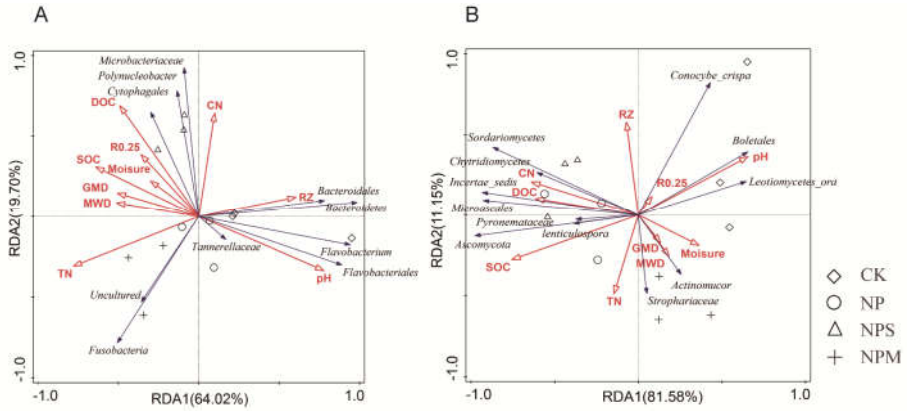


Figure 2-4. Redundancy analysis (RDA) between the bacterial (A) and fungal (B) phyla with significant difference and soil physicochemical parameters in different treatments.

3.6 Variation partitioning

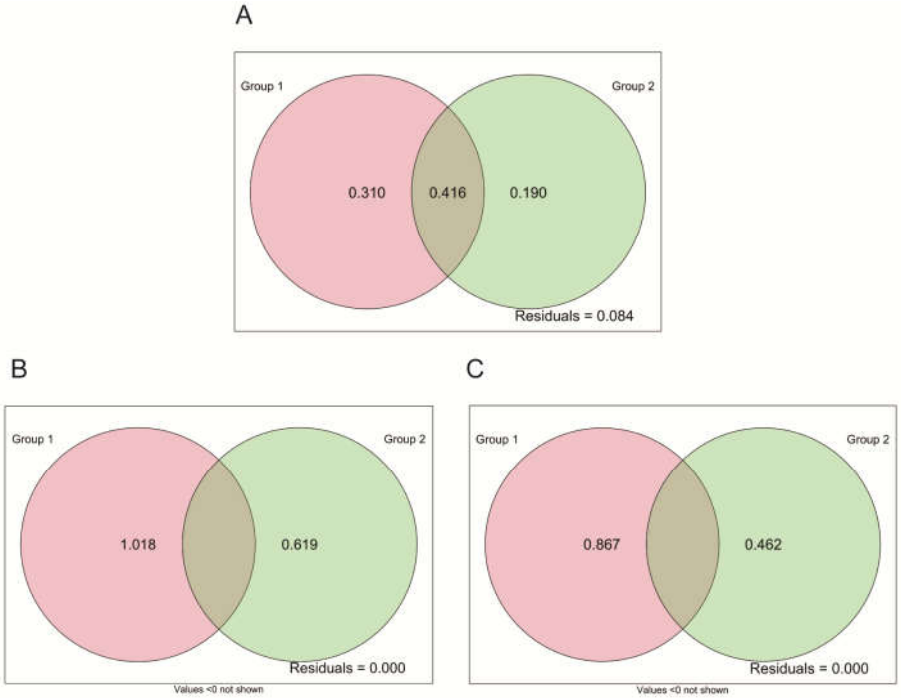


Figure 2-5. Variance partitioning analysis to determine the effects of soil physical and chemical properties on bacterial (A), fungal (B) compositions and microbial biomass (C). Group1：soil chemical properties, SOC, TN, DOC, C/N, pH; Group2：soil physical properties, moisture, RZ, MWD, GMD, $R_{0.25}$.

The results showed that the total explained variation in the microbial biomass was 91.6%, and the undetermined variation was 8.4% (Figure 2-7). The physical and chemical soil properties explained 31% and 19% of the variation in the microbial biomass, respectively. Significant proportions of the soil microbial biomass could be jointly explained by physical and chemical soil properties (Figure 2-7).

In addition, the chemical soil properties collectively explained higher portions of the variations in soil microbial communities and biomass, than physical soil properties. The variation partitioning results showed that the physical soil properties explained variation in the bacterial community was 101%, and the chemical soil properties explained 61.9% (Figure 2-7). In the fungal community, the physical and chemical soil properties explained 86.7% and 46.2% (Figure 2-7), respectively.

4. Discussion

4.1 Effect of different fertilizer regimes on soil microbial abundance and community composition

In intensive agricultural regions, the variations of soil environmental factors are mainly determined by farming practice, and strongly affect the composition and functioning of the microbial community. It is therefore necessary to explore how fertilization affects soil microbial community structures and what are the main control factors.

Our results showed that microbial biomass had significant differences across the four long-term fertilizations. In our study, inorganic fertilization strongly reduced microbial biomass, including bacterial and fungal biomasses, and organic amendments had weakened this trend, in agreement with Chao 1 of α-diversity (Table 2-2). There is no doubt that continuous organic matter addition could increase the availability of substrates (C and N), which imply the primary energy and nutrient sources for microbial survive and growth (Liu et al. 2021; Liu et al. 2018). Our results were consistent with previous studies that incorporating straw and manure into the soil contributed to the obvious variation in microbial biomass and diversity (Li et al. 2021).

Obviously, bacterial and fungal α-diversities were also affected by the fertilization regimes. Straw amendment was thought to be one of the most effective methods of regulating microbial beta diversity (Mengshuai Liu et al. 2020). Unlike straw, the manure amendment is beneficial to the growth of some copiotrophic bacteria (Wang et al. 2017) but not conducive to the growth of some potentially pathogenic fungi (Ding et al. 2017), that can explain the reduce of fungal diversity in NPM in our results. The application of different organic amendments not only promoted variation in soil microbial community structure, but also changed the status of functional microorganisms. In the process of OM decomposition, the variation of soil microbial communities depends on the

interaction of eutrophic and oligotrophic bacteria caused by the abundance of resources. Therefore, the compositions of the microbial communities under fertilization amendments in our study were changed due to different type and content of carbon.

Straw contains a large amount of lignin and humic acid that are not easily utilized by microorganisms, so its decomposition process is mainly dominated by microorganisms that decompose complex organic carbon. In our study, straw addition mainly and greatly increased the relative abundances of dominant phyla bacterial Proteobacteria, Firmicutes and Actinobacteria. As the dominant species among all treatments, Proteobacteria and Actinobacteria are regarded as the main bacteria for decomposition and transformation of organic matter (Yang et al. 2020).

For the dominant phyla fungi, our result was similar with previous study that Ascomycota is the most abundant phylum among soil fungi and also the key group that decompose organic matter. The change of dominant phyla fungal taxa is more obvious than that of bacteria. This indicates that in the process of OM application, the microbial community structure based on resource acquisition and nutritional status have changed due to high abundance of the dominant flora (Zheng et al. 2021).

In addition, affected by different organic amendments, the status of key functional microorganisms was changed. The taxa with significant difference obtained in our results do not have high relative abundance, but they affect the microbial community composition by interfering with the characteristics of other microbial community. During the organic matter degradation, Bacteroidetes, Basidiomycot and Ascomycota with significant difference, may be the key microorganisms that distinguish organic substrates containing different carbon sources (Yang et al. 2020). It is in line with other study, showing that Bacteroidetes and Basidiomycota were found to be the key microorganisms to distinguish broad-leaved forest and coniferous forest species (Yang et al. 2020).

In short, the fungal and bacterial phyla taxa with significant differences may cause the conversion of soil microbial community from a slow-growing oligotrophic group to a fast-growing symbiotic nutrient group under organic fertilization.

4.2 Response of microorganisms to environmental factors under fertilization

In intensive agricultural regions, the variations of soil environmental factors are mainly determined by farming practice, and strongly affect the composition and functioning of the microbial community. It is therefore necessary to explore how fertilization affects soil microbial community structures and what are the main control factors.

The factors driving the abundance and community structure of microbial

community were related to nutritional status in soil. Microbial biomasses, including bacterial and fungal PLFA, were associated with both physical and chemical properties (Figure 2-4). For instance, animal-derived organic fertilizer was found to regulate bacterial community through changing aggregates, pH and resource availability (Li et al. 2021). These physical properties, i.e., GMD, MWD were more strongly correlated with PLFA in OM treatments than in CK and NP. It indicated that the soil physical status would make a great impact on microbial community if there is no soil resources stress (Hueso et al. 2012). Similar results were also found in previous studies, that the aggregate stability was promoted by organic fertilization (Wang et al. 2021; Zhu et al. 2021), and was significantly and positively correlated with microbial biomass, growth, and activities (Wang et al. 2021; Zhang et al. 2021).

According to the RDA and the correlation heat-map analysis, TN, DOC, and pH were the main chemical factors, and the parameters of aggregate stability and moisture were the main physical factors driving differences in dominant bacterial community structures (Figure 2-5, Table 2-3). Bacterial community have the highest diversity and the most stable community structure in soil, but it would vary strongly even with slight changes in environment factors, i.e., pH, C content and soil bulk density (Li et al. 2021; Shen et al. 2013). Differently, dominant fungal community structures were showed only strongly depending on chemical properties. This conclusion is inconsistent with others, indicating not only soil organic carbon, nitrogen, phosphorus, but also moisture, and pH are driving soil fungal community structure variation in different soil environments (Yang et al. 2017).

With strong adaptability, microbial community responds to various agricultural fertilizations through enriching specific microbes that could efficiently utilize these nutrients (Wang et al. 2017). The bacterial taxa Bacteroidetes, fungal taxa Basidiomycot and Ascomycota with significant difference in this study were confirmed to play a key role in decomposing organic debris, that is why all of the microbes with significant difference were correlated with C: N or SOC in our study. Similar results were also found by other researchers (Haidong et al. 2021), that some specific bacterial taxa were also sensitive to nutrient variation.

Different from bacteria, the main fungal taxa with significant difference were shown no significant correlation with aggregate stability. The same result with dominant and specific fungi indicated that fungal community structure is more likely to be determined by chemical factors. We are not surprised by this result. The close relationship between fungus, especially arbuscular mycorrhizal fungi (AMF; Glomeromycotina) (Leifheit et al. 2014) and soil aggregates formation/decomposition has been widely confirmed in the literature (Lehmann et al. 2017; Rillig et al. 2015), and it may be connected through specific microbial metabolites (Řezáčová et al. 2021), not fungal abundance. In order to deeply understand their relationship, it is necessary to focus on their metabolites next time.

In conclusion, it means that these soil properties are suitable as indicators of soil

microbial community variations under long-term fertilization, and can be used as new tools to monitor soil microbial community variations.

4.3 Relative contribution of soil physical and chemical factors to microbial community

Soil abiotic properties are important factors that can lead to shifts in microbial community abundance and structure under long-term inorganic or organic fertilizations. Previous studies have shown that soil chemical and physical properties are the primary factors changing the microbial community structure (Wang et al. 2021). Comparing with soil physical properties, soil chemical properties captured a greater proportion of the variations in soil microbial biomass and community composition under different fertilization regimes, thus confirming our hypothesis (Figure 2-7). Similar to a previous study, spatial structure explained a high proportion of microbial community changes in the soil among sites at the regional scale (Liu et al. 2021). In detail, DOC, TN, ratio of C: N, and pH were the main chemical parameters correlating with both bacterial and fungal phyla taxa. This finding may suggest the importance of nutritional resources and balance in maintaining the stability of the composition and growth of the microbial community. Compared with other studies (Kang et al. 2021), physical properties had a relative smaller influence on microbial community structure (Figure 2-7), despite the shifts in the soil amendment practices applied.

Moreover, it should be noted that the influence of soil physical properties on microbial community biomass may have been underestimated, as the aggregate stability could not present all physical properties. These findings suggest that physical factors may also had an important influence on soil microbial community variation, and this influence may have further exacerbated by the capture and release of aggregate-organic carbon.

However, it needs to be explained that there was a limitation in our study: Only a few indicators were selected to represent soil physicochemical properties, and other important indicators were not involved, such as soil texture, pore structure, mineral content and other indicators. Therefore, the low correlation between the indicators involved in this research cannot represent that there is no correlation between the physical and chemical properties of the real soil, which has been confirmed in previous studies (Heuscher et al. 2005). The next step in this study is to take more indicators into account and further analyze their impact on the microbial community.

5. Conclusions

We found strong relationships among soil physicochemical properties and microbial community composition across different fertilization. That application of inorganic fertilization could possibly inhibit microbial growth, while organic amendment could promote the microbial biomass, especially the richness of bacteria. During the process, the contribution of soil chemical properties to

variation in microbial biomass was greater than it of soil physical properties under long-term fertilization, the same with in bacterial community composition. The application of organic amendments could possibly increase the decisive effect of aggregate stability on microbial biomass. In addition, soil chemical properties are more suitable as indicators of variation in soil fungal community composition. Overall, this research enhances our understanding of the drivers of bacterial and fungal diversity and community structure in different fertilization regimes, and confirms that it is critical that relevant indicators be accounted for in the understanding and prediction of microbial communities.

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ChapterIII

The influence of microbial community properties on protecting soil C under long-term fertilization at aggregate scale

The influence of long-term fertilization on the soil microbial environment can induce the transformation of SOC. This chapter aimed to evaluate the contribution of microbial community to SOC sequestration under long-term fertilization in loess plateau.

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Chapter III The influence of microbial community properties on protecting soil C under long-term fertilization at aggregate scale

Abstract

Long term fertilization alters soil microbiological properties and affects soil organic carbon (SOC) pool. However, the interrelations of SOC with biological drivers and their relative importance are rarely analyzed quantitatively at aggregate scale. We investigated the contribution of soil microbial biomass, diversity and enzyme activity to C pool in soil aggregate fractions (> 5 mm, 2−5 mm, 1−2 mm, 0.25−1 mm and < 0.25 mm) at topsoil from 27-year long term fertilization regime. Compared to CK (no fertilization management), NP (inorganic fertilization alone) decreased all of the microbial groups biomass, while NPS and NPM (inorganic fertilization plus the incorporation of maize straw or composted cow manure) significantly reduced this negative effect of NP on microbial biomass, and increased the microbial contribution to C pool. The results showed that microbial variables were significantly correlated with SOC content in > 0.25 mm aggregates rather than in < 0.25 mm aggregates. Fungal variables (fungal, AM biomass, and F/B ratio) and enzyme activities (BXYL and LAP) in > 0.25 mm aggregates explained 21% and 2% on C, respectively. Overall, organic matter addition could contribute to higher C storage by boosting fungal community and enzyme activity rather than by changing microbial community diversity in macro-aggregates.

Keywords:

Fertilization; Soil aggregates; Microbial properties; Enzyme activity; SOC

1. Introduction

Terrestrial soils contain approximately three times the stock of carbon (C) of the atmosphere, hence small changes in soil organic carbon (SOC) have a significant impact on climate change (Smith 2012). Among the numerous drivers that regulate the SOC pool, microorganisms are essential for SOC turnover (Kallenbach et al. 2016). Microorganisms have been reported to promote the formation of macro-aggregates to physically protect C (Six et al. 2004), and their residues are also considered to be an important source of stable C (Zhang et al. 2015). Simultaneously, microbe-driven soil C decomposition plays a critical role in C cycling (Crowther et al. 2019). It is reported that over half of the cumulative CO_2-C emitted from soil was induced by microbial community (Melillo et al. 2017). In addition, soil organic matter could be synthesized or degraded by soil enzyme activity (Burns et al. 2013), which were linked to CO_2 production (Li et al. 2012). As such, understanding the contribution of microorganisms and enzymes to the accumulation or consumption of SOC in soil is of utmost importance for regulating soil C and reducing the impact of CO_2 on the climate system.

Despite the direct microbe-driven in soil C cycling, the contribution of microorganism to C turnover is often overlooked in C cycle prediction (Bond-Lamberty et al. 2018). Maintaining high richness and diversity of soil microorganisms is critical to mediate C cycling. However, there is less consistency on the research regarding soil microbiological properties, such as soil microbial diversity, microbial community and enzyme activity. With the most abundance in soil system (Fierer 2017), microorganisms (e.g., bacteria and fungi) have been reported to facilitate the C cycling, through increasing metabolic actions (Guo et al. 2016) and bonding organic particles together or stimulating root secretion of OM (Kallenbach et al. 2016). Some studies found that bacteria contribute to the SOC storage more greatly than fungi in the rice and wheat system (Dhaliwal et al. 2020). Differently, arbuscular mycorrhizal (AM) fungi have been thought not to be very important in C decomposition (Juan-Ovejero et al. 2020). Both microbial biomass C and diversity are suspected to play a crucial role in influencing SOC pool (Baumann et al. 2013; Zhang et al. 2013). Another result showed that microbial biomass had a significant influence on soil C cycling rather than its community composition under manure application (Ma et al. 2020).

Additionally, soil enzymes, produced by soil microorganisms, are reported to regulate the overall processing of SOC through degrading different molecules (Romaní et al. 2006) or depolymerizing macromolecular substrates (Mooshammer et al. 2014). Some enzymes show a strong relationship with SOC content and are generally reported to be good indicators of soil biological change (Bandick and Dick 1999; Caravaca et al. 2002). It is remarquable that, even if the complexity of SOC-related mechanisms is widely recognized, most of studies focused on a single factor, less on multiple factors regulating SOC. Moreover, the potential mechanisms by which microbiological properties are linked to C regulation are ignored.

As the basic unit of soil, aggregate plays a key role in C cycling (Somasundaram et al. 2017). Containing more than 90% sequestered SOC, aggregate can be divided into macro- (> 0.25 mm) and micro- (< 0.25 mm) aggregates (Jastrow 1996; Six et al. 2004). Previous studies have shown that macro-aggregate (> 0.25 mm) contains more SOC content than micro-aggregate (0.053–0.25 mm), the same as labile SOC (Elliott 1986). As the basic elements in soil structure, aggregate provides spatially heterogeneous microenvironments for soil microorganisms (Liao et al. 2020). Large variance in environmental conditions in different sizes of aggregate, including water potential, oxygen concentration and resource availability (Young and Ritz 2000), could result in diverse biomass and community diversity for microorganisms (Briar et al. 2011; Larkin and Martiny 2017; Wisz et al. 2013) and affect their functions on C turnover (Dorodnikov et al. 2009).

It was reported that soil spatial heterogeneity could stimulate biodiversity by limiting these specific or individualized microbial communities (Griffiths and Philippot 2013; Larkin and Martiny 2017; Wisz et al. 2013). The distributions of microbial biomass and enzyme activity in aggregate fractions were reported to be primarily governed by the aggregate sizes (Liang et al. 2014), due to different availability of organic substances (Allison and Jastrow 2006). Furthermore, fungi were found to contribute to the C turnover more greatly and rapidly in macro-aggregates than in micro-aggregates (Six et al. 2004). There are many evidences that the aggregate sizes affect microbial community composition and enzyme activity. However, to the best of our knowledge, few studies have investigated the mechanisms linking aggregate size and multiple microbial properties with SOC turnover.

Regarding food demands, the application of inorganic and organic fertilizers in agricultural systems is necessary to increase crop productivity in the world (Inselsbacher et al. 2010; Li and Xiao 1992). As one of the most common organic amendments in fields, crop straw and manure application could increase the unstable C contents (e.g., dissolved organic carbon (DOC) and readily oxidizable organic carbon (ROC) contents (Yu et al. 2020)) that are the main C sources for microorganisms. Several studies revealed that the alterations in microbial activity could cause priming effects due to the addition of the substrate, which might simulate the turnover of nature organic matter in soil (Qiu et al. 2020). These practices have exhibited high impacts on soil microorganisms community structure and diversity (Wang et al. 2017). It also affected the enzyme activity through altering the habitat conditions for soil microorganisms. Some researchers also have reported that soil C cycling is stimulated by changing microbial biomass rather than its community composition under manure application (Ma et al. 2020). During this process, soil enzyme activities influenced and related to C cycling (Burns et al. 2013).

As the microhabitats for microorganisms, the aggregates are greatly changed in their physical conditions under fertilization (Tripathi et al. 2014). For example, soil moisture within aggregate was directly altered by fertilizer application, which

plays a key role in the survival of soil microorganisms (Tripathi et al. 2014). The application of manure increased the microbial biomass in the form of phospholipid fatty acids (PLFA) in macro-aggregates, e.g., bacterial, fungal, and AM fungal biomass, while not significantly in micro-aggregates (Dhaliwal et al. 2020). Additionally, soil enzymes are reported to react quickly to changes in most of soil managements (Bandick and Dick 1999). Thus, the aggregate size plays a significant role in the relationships between microbial properties and C cycling. Hence, a better understanding of the influence of microbial properties on C cycling at aggregate scale under long term fertilization regime is important, which aid to develop suitable management practices to better increase C accumulation, simultaneously maintain a healthier soil microbial environment.

The objective of this study was to investigate the effects of 27-year fertilizations (CK: no fertilization management; NP: inorganic fertilizers application alone; NPS: inorganic fertilization plus the incorporation of maize straw; NPM: inorganic fertilization plus the incorporation of composted cow manure) on soil microbial community and enzyme activity and their roles in influencing C at aggregate scale in the Loess Plateau of China. Here are three hypotheses: (1) Different fertilization managements can induce differences in the distribution of soil C and microorganism communities in aggregates; (2) the relationship between soil microorganisms and enzymes, and their contributions to C accumulation vary with aggregate size; (3) NPS and NPM could increase the contribution of microorganism to C accumulation through influencing the population of microorganism community at aggregate scale.

2. Materials and methods

2.1. Site Description

The study site is located in the Dryland Farming Experimental Station in Shanxi province (112–113 ◦E, 37–38 ◦N) in northern China and was initiated in 1993. The site is characterized by a continental monsoon climate with an elevation of approximately 1,100 m above sea level, annual rainfall of 520 mm and average temperature of 7–8 °C. Spring maize is the main crop grown under the one-crop-per-year cropping system. Soils belong to a sandy clay loam cinnamon soil series which are characterized as Calcaric-Fluvic Cambisol (ISS-CAS, 2003; IUSS, 2006). At the start of the project, soil pH was on average 7.9, and SOC and soil organic N concentrations were 15.0 g kg^{-1} and 1.0 g kg^{-1}, respectively.

The long-term experiment had a randomized block design with three replicates, each plot was 6 × 6 m. The four treatments included in this study were as follows: no fertilization management (CK), inorganic fertilizers application alone (NP), inorganic fertilization plus the incorporation of 3,000 kg ha^{-1} maize straw (NPS), and inorganic fertilization in combination with 1,500 kg ha^{-1} composted cow manure (NPM). Each plot of the treatments NP, NPS and NPM had nitrogen 105 kg ha^{-1} and phosphorus 105 kg ha^{-1} applied once a year, respectively, using urea

(46% N) and calcium superphosphate (7% P) in a ratio of N to P of 1:0.44. The mean proportions of organic matter, total nitrogen, total phosphorus and total potassium were 75%, 0.6%, 0.04% and 0.7% in maize straw, and 36%, 1.0%, 0.2% and 0.7% in cattle manure, respectively. Maize straw, cattle manure and inorganic fertilizers were broadcasted and incorporated into the soil with conventional tillage (plowing once each year at a depth of 20 cm) after harvesting in October. Seeding was done at the end of April without any tillage and harvesting in October, with twice weeding during growth seasons every year. Table 3-1 shows the chemical and physical properties of the soil in these treatments in 2018.

Table 3-1 Soil characteristics for CK, NP, NPS and NPM treatments in 0−15 cm layer in 2017–2018.

Indices	CK	NP	NPS	NPM
pH	7.80	7.83	7.76	7.77
Bulk density (g cm^{-3})	1.28	1.30	1.23	1.20
SOC (g kg^{-1})	12.35~13.11	15.96~16.3	17.01~18.1	15.18~15.9

Note: CK, the control treatment without fertilization management; NP, the treatment with only inorganic fertilizer; NPS, the treatment with inorganic fertilizer and maize straw addition; NPM, the treatment with inorganic fertilizer and cattle manure.

2.2. Soil sampling

Soil samples were obtained after harvesting (before fertilizer application) in October, 2017 and 2018. For each treatment, five soil cores (10 × 10 cm in diameter) were collected randomly at a depth of 0–15 cm in each plot and pooled together, and thereafter taken to laboratory immediately. The fresh soil (Samples obtained in 2018) was separated manually along the natural cracks of fracture to obtain aggregate sizes of < 6 mm. After removing stones, plant material and visible soil fauna, 100 g fresh soil samples were air-dried for the analyses of aggregates distribution using dry sieving method, and the rest were sieved (5 mm, 2 mm, 1 mm and 0.25 mm mesh sieves) immediately. Then, the classified aggregates (20 groups of aggregate samples in total) were divided in half: one half was immediately stored at −80 °C for biochemical analysis, and the other half was kept at 4 °C for the analysis of organic carbon contents. All samples were tested in 3 replicates.

2.3. Aggregate fractionation

The fractionation of soil aggregates was measured using the dry sieving method (Elliott 1986). After removing the visible impurities, 200 g samples (air-dried soil) were passed through a series of four sieves (5 mm, 2 mm, 1 mm and 0.25 mm) and divided into five aggregate sizes.

2.4. Soil analysis

2.4.1 Determination of organic C concentration of soil sample

The SOC (from dried soil) was estimated with an element analyzer (C/N Flash EA 112 Series-LecoTruspec). Dissolved organic carbon (DOC) content was measured through detecting soil extracted solution made by distilled water (1:5 w:v) using a C analyzer (Multi N/C 3100, Analytic Jena, Germany). Readily oxidizable organic carbon (ROC) content was analyzed by $KMnO_4$ oxidation. Briefly, after passing through 0.15 mm sieve, the air-dried soil (containing approximately 15–30 mg C) was added in 25 mL of 333 mM $KMnO_4$, then shook and centrifuged. The supernatants were diluted with deionized water (1:250) and detected by a UV spectrophotometer at 565 nm. Microbial biomass carbon (MBC) content was measured using the fumigation-incubation method (Brookes et al. 1985). In detail, four aliquots of freeze-dried soil samples (25 g each aliquot) were prepared, two aliquots being fumigated with ethanol-free $CHCl_3$ for 24 h in the dark at room temperature, while the other two was kept untreated as control. Then, these samples were mixed into 100 mL of 0.5 M K_2SO_4 solution, respectively, and shaken for 30 min at 200 r min^{-1}. The supernatants were diluted with deionized water and then detected by a total organic C (TOC) analyzer.

2.4.2 Analysis of enzyme activity

In this study, the four soil enzymes activities of β-Glucosidase (BG), β-Xylosidase (BXYL), N-acetyl-glucosaminidase (NAG), and leucine aminopeptidase (LAP) were estimated following the method of previous studies(DeForest 2009; German et al. 2011). 4-Methyllumbelliferyl (MUB) and 7-amino-4-methylcoumarin (AMC) were used as substrate to determine the activities of all enzymes (MUB for BG, BXYL and NAG; AMC for LAP). Firstly, 1 g of fresh soil was mixed in 125 mL of $NaHCO_3$ buffer (pH = 8) and stirred at 800 rpm for 2.5 min. Secondly, the slurry was transferred into 96-well microplate using an eight channel pipet, and substrates were quickly added. Thirdly, all the microplates were incubated in a dark for 3 h at 25 °C. Finally, the fluorescence of the supernatants was detected using a multilabel fluorescence reader (Tecan Infinite F200/M200).

2.4.3 PLFA extraction and analysis

Phospholipid fatty acids (PLFA) were measured to calculate the soil microbial biomass and diversity. The method details were described in previous research (Bossio et al. 1998). In brief, aliquots of 3 g (freeze-dried) aggregate samples were extracted twice in 7.6 mL chloroform/methanol/citrate buffer (1:2:0.8 v/v/v) system. After that, phospholipids were separated from neutral and glycolipids with silica acid columns (Supelco Inc., Bellefonte, PA). After methylation of the polar lipids, the fatty acid methyl esters (FAME) were identified by Gas Chromatograph Agilent Series (GC 6890, Agilent Technologies, Wilmington, DE) and calculated by MIDI microbial identification system (MIDI, Inc., Newark, DE). Nonadecanoic acid (19:0) was used as an internal standard.

PLFA were assigned to general bacteria (16:0, 17:0, 18:0, 20:0), gram-negative bacteria (G–), gram-positive bacteria (G+), general fungi (18:2ω6c), arbuscular mycorrhizae (AM, 16:1ω5c) and actinomycetes (16:0 10-methyl) (Aciego Pietri and Brookes 2009; Guo et al. 2016). Cyclopropyl and monounsaturated fatty acids were indicators for G-bacteria, whereas iso- and anteiso-branched fatty acids were biomarkers for G+ bacteria.

2.5. Data calculation and statistical analysis

MBC concentration was obtained by calculating the difference in OC between fumigated and non-fumigated samples with 0.45 (the proportion of soil biomass C extracted by K_2SO_4 after chloroform fumigation) using the equation 1 (Jenkinson, Brookes, and Powlson 2004):

$$MBC = (Fumigated - Unfumigated)/0.45 \quad (1)$$

where, Fumigated and Unfumigated were the OC extracted in K_2SO_4 from fumigated and non-fumigated soil samples per gram of soil.

In this study, the change of SOC was calculated according to equation 2, and organic C input was the sum of the C in the added straw, manure, stubble and root according to equation 3. The soil C sequestration efficiency was calculated as following equation 4:

$$\Delta SOC = SOC_{2018} - SOC_{2017} \quad (2)$$

$$Organic\ C\ input = C_{addition} + (Yc * C_r) \quad (3)$$

$$C\ sequestration\ efficiency = \Delta SOC * 100/\ Organic\ C\ input \quad (4)$$

where, SOC_{2018} and SOC_{2017} were the soil organic C contents under the four treatments in 2018 and 2017, $C_{addition}$ was the soil organic C in added straw and manure, Y_c was the biomass of stubble or root, C_r was the soil organic C in stubble or root. The proportions of graded aggregates with different particle sizes were calculated as following equation 5:

$$Dry - pi = (Wi * 100\%)/200 \quad (5)$$

where, Wi was the mass of i-th graded aggregates with different particle sizes, and Dry-pi was the proportion of i-th graded aggregates in total soil.

Microbial community diversities were evaluated using Shannon–Wiener diversity index (H'), Simpson evenness index (D) and Margalef richness index (M). They were generally calculated as follow:

$$H' = -\sum pi * ln\, pi \quad (6)$$

$$D = 1 - \sum (pi)\text{^}2 \quad (7)$$

$$M = (S - 1)/lnN \quad (8)$$

where H′, D and M were Shannon–Wiener, Simpson and Margalef indexes, respectively; p_i was the percentage of the peak area of i-th FAME to the total area in each sample; S was the total number of FAME in each sample; and N was the amount of total microbial PLFA.

Statistically, all data were carried out by SAS 9.4 in Windows 10. Two-way analysis of ANOVA was used to examine fertilization treatment and aggregate size on all of the soil physicochemical and microbial indicators. We have detected heterogeneity using Levene's test before carrying out ANOVA and the data for each variable met the heterogeneity of variance and criteria. Correlations with p-value lower than 0.05, 0.01 and 0.001 were considered. Spearman correlation was computed between microbial parameters and SOC under each aggregate size, and between soil physicochemical and microbial parameters in > 0.25 mm and < 0.25 mm aggregates. Additionally, principal component analysis (PCA) was performed to divide the microbial factors correlated with SOC into different groups in > 0.25 mm aggregates using Vegan package in R (Version 3.2.2) and variation partitioning analysis (VPA) was further applied to quantify how much variation in SOC was explained by fungal community, microbial diversity and enzyme activity in the Vegan package of R (Version 3.2.2).

3. Results

3.1. Soil organic C and moisture in aggregate and aggregate proportions under different fertilizations

The C sequestration efficiency after 27 years of experiment is presented in Table 3-2. Clearly, the lowest C sequestration efficiency is found in CK treatment, and the highest in NPS.

Table 3-2 The organic C input and C sequestration efficiency under the four fertilization treatments.

Indices	CK	NP	NPS	NPM
Stubble (kg ha^{-1})	739.3	1206.8	1718.8	1468.2
Root biomass (kg ha^{-1})	4223.8	2587.4	6015.6	5138.5
Organic C input (kg ha^{-1})	0.6	1.0	2.0	1.8
ΔSOC (g kg^{-1})	-0.8	0.4	1.2	0.8
C sequestration efficiency (%)	-128.2	42.4	60.3	44.9

From Table 3-3, SOC and DOC contents varied with fertilization and aggregate size, both of which were higher under NP, NPS and NPM than under CK in almost all aggregates ($p < 0.05$; Figure 3-1). MBC and ROC contents were significantly affected by fertilization and aggregate size, respectively ($p < 0.001$; Table 3-3). The four treatments had no significant differences in ROC and

MBC contents (Figure 3-1) in micro-aggregates (< 0.25 mm), and a similar observation was also found in the ratio of MBC to SOC content (Figure 3-2).

Table 3-3 Two-way ANOVA of fertilization treatment (T), aggregate size (A) and their interaction (T*A) on soil C, enzyme activity and microbial variables and aggregate properties.

Indices	Treatment (T)	Aggregate size (A)	T*A
	F P	F P	F P
SOC	19.1***	4.8**	0.6
DOC	57.2***	8.0***	4.3***
ROC	14.8***	0.4	7.6***
MBC	2.1	17.7***	4.5***
BG	35.3***	5.5**	2.7*
BXYL	39.3***	14.7***	2.1*
NAG	12.2***	17.4***	1.6
LAP	4.1*	55.0***	3.0**
Total PLFA	35.1***	17.6***	3.0**
Bacteria	22.4***	9.9***	3.2**
Fungi	23.1***	12.2***	2.4*
AM	46.3***	15.9***	2.2*
Actinomycetes	21.8***	12.4***	1.7
G+	20.7***	22.6***	1.0
G–	8.2***	5.2**	2.3*
G+/G–	5.9**	10.3***	1.9
F/B	10.5***	1.7	1.6
H′	1.0	6.0***	3.5**
D	0.4	5.2**	2.1*
M	1.9	2.1	2.6*
Moisture	54.9***	137.2***	4.9***
Dry-p	0	879.7***	14.8***

Note: SOC: soil organic carbon; DOC: dissolved organic carbon; MBC: microbial biomass carbon; ROC: readily oxidizable organic carbon. BG: soil enzymes activities of β-Glucosidase; BXYL: β-Xylosidase; NAG: N-acetyl-glucosaminidase; LAP: leucine aminopeptidase; AM: arbuscular mycorrhizal fungi; G+: gram-positive bacteria; G–: gram-negative bacteria; G+/G–: the ratio of G+ and G– bacterial PLFA; F/B: the ratio of fungal and bacterial PLFA; H′: Shannon–Wiener diversity index; D: Simpson evenness index; M: Margalef richness index; Dry-p: the proportions of aggregates using dry sieving method. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ns: not significant.

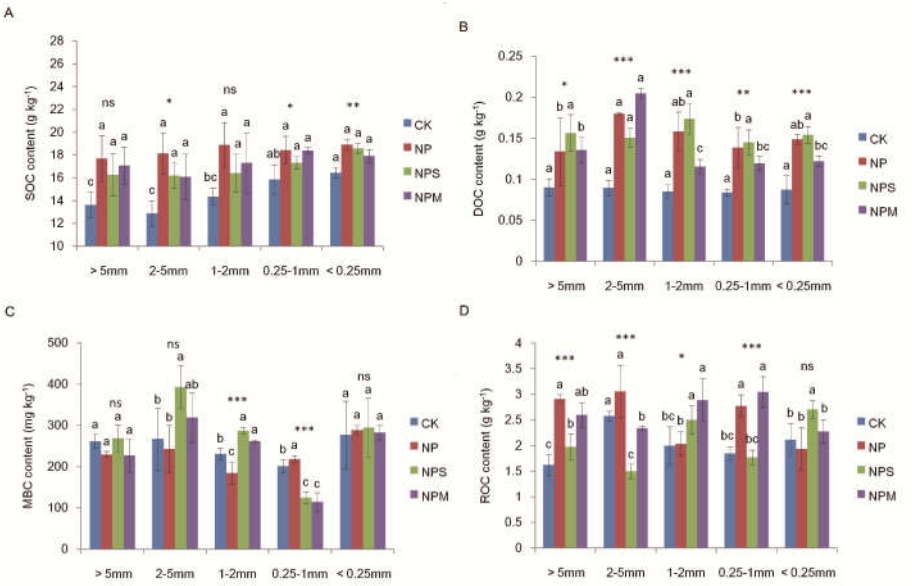

Figure 3-1. The distribution of SOC (A), DOC (B), MBC (C), ROC contents (D) in different aggregates under 4 fertilization managements (CK, NP, NPS and NPM). Different lowercase letters indicated significant difference at $p < 0.05$ among the different aggregate sizes. See Table 3-3 for abbreviations of some soil biological properties. The differences in C contents among the different fertilization managements were showed at * : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$ and ns: not significant.

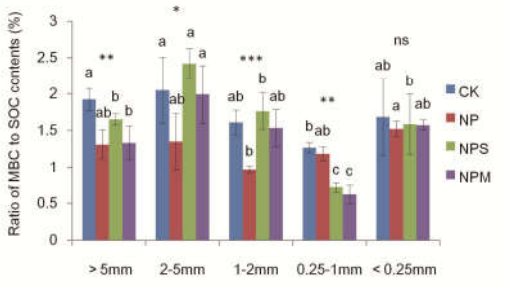

Figure 3-2. The ratio of MBC to SOC in different aggregates under 4 fertilization managements (CK, NP, NPS and NPM). Values in the same aggregate size followed by the same lowercase letters are not significantly different ($p < 0.05$) according to LSD test among the different aggregate sizes. The differences in the ratio of MBC to SOC among the different fertilization managements were showed at * : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$ and ns: not significant.

Fertilization, aggregate size and their interaction significantly affected the

moisture in aggregate ($p < 0.001$; Table 3-3). The moisture was lower in NP than in CK almost in all aggregates ($p < 0.05$; Figure 3-3). Compared to NP, NPS and NPM significantly increased moisture in all aggregates ($p < 0.05$). The moisture was higher in macro-aggregates (> 0.25 mm) than in micro-aggregates (< 0.25 mm) under the four treatments.

There was an extremely significant difference in aggregate mass proportions among aggregate sizes ($p < 0.001$; Table 3-3). Compared to CK, NP significantly ($p < 0.05$; Figure 3-3) reduced the mass proportions of macro-aggregates (> 2 mm) but increased the mass proportions of micro-aggregates (< 0.25 mm), while the results under NPS and NPM were opposite.

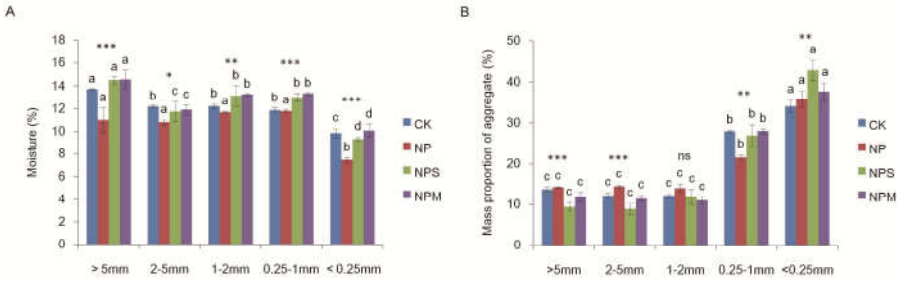

Figure 3-3. The moisture (A) and mass proportion (B) of different aggregates under 4 fertilization managements (CK, NP, NPS and NPM). * : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$ and ns: not significant.

3.2. Soil microbial biomass and diversity and enzyme activity

Soil microbial biomass was significantly impacted by fertilization and aggregate size ($p < 0.01$; Table 3-3). Compared to CK, NP decreased all of microbial indices (Figure 3-4), while NPS and NPM increased those (except fungi indices) in different sized aggregates (Figure 3-4). The increases in fungal biomass (including AM) and F/B ratio were observed only in micro-aggregates (< 0.25 mm) under NPS and NPM relative to CK. In micro-aggregates (< 0.25 mm), the bacterial and fungal biomasses were higher under NPS treatment than under CK. Most of the microbial groups biomass increased with the decreasing of aggregate size under NPS and NP, while there was no significant difference in microbial biomass (except bacterial biomass) among all aggregates under NPM. Additionally, the ratio of fungi:bacteria (F/B) and G+: G– (G+/G–) is also affected by fertilization (Table 3-3), and there were no significant differences in the two ratios among all aggregate sizes under NPM. The biomass of different microbial groups was lower in macro-aggregates (> 0.25 mm) than in micro-aggregates (< 0.25 mm) under fertilization. The total PLFA was significantly associated with moisture in macro-aggregates (> 0.25 mm).

As showed in Table 3-3, significant ($p < 0.05$) interactive effects between fertilization and aggregate size were observed in all indices of microbial diversity. The three indices were affected significantly by fertilization almost in 0.25–1 mm

and 2–5 mm ($p < 0.05$; Table 3-4), but not in micro-aggregates. Unlike CK and NP treatments, these three indices had no significant difference among variably sized aggregates under NPS and NPM. From Figure 3-5, the microbial diversities of Shannon-wiener (H′) and Simpson (D) were respectively correlated with bacteria (including G+ and G–) and actinomycetes in macro-aggregates (> 0.25 mm).

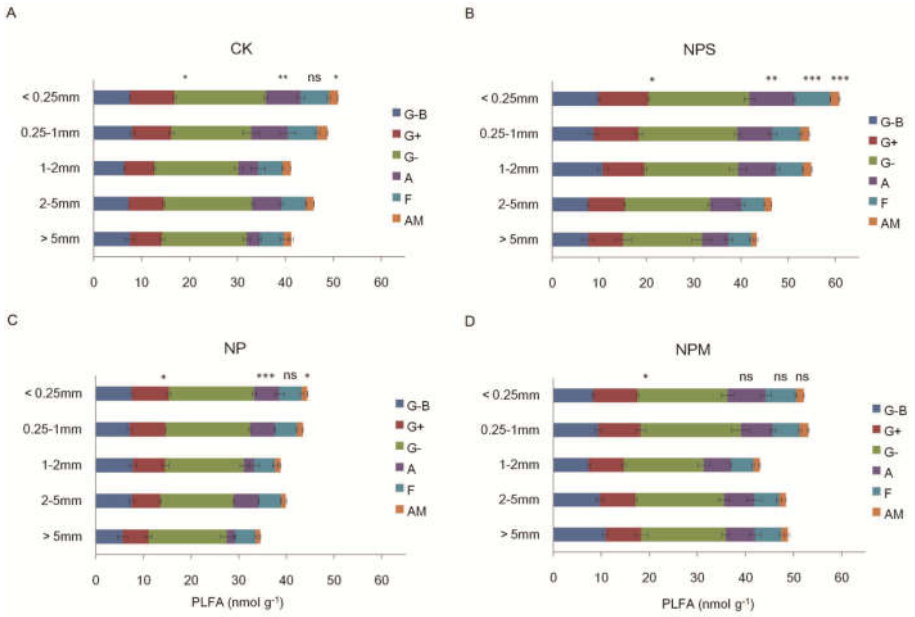

Figure 3-4. Microbial biomass and the phospholipid acid biomarkers in different sized aggregates under four fertilization managements, CK (A), NPS (B), NP (C), and NPM (D). See Table 3-3 for abbreviations of some soil biological properties.

The differences in total bacteria (the sum of G-B, G+ and G– bacteria), actinomycetes, general fungi and AM PLFA among the different sized aggregates were showed at * : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$ and ns: not significant.

All of the four soil enzymes activities varied with fertilization and aggregate size ($p < 0.05$; Table 3-3). In Figure 3-6, the four soil enzymes activities were higher under NPM and NPS than under CK in all macro-aggregates (except 1–2 mm). NP increased those only in 2–5 mm and 0.25–1 mm while decreased in other aggregate sizes, compared to CK. There were no differences on LAP and NAG between NPS, NPM and CK in micro-aggregates (< 0.25 mm). The four soil enzymes activities were higher in > 0.25 mm than in < 0.25 mm aggregates under NP treatment. The activities of NAG, LAP and BXYL were higher under NPS and NPM in > 0.25 mm aggregates (except 2–5 mm) than in micro-aggregates (< 0.25 mm). Soil enzyme activity was positively correlated with bacterial biomass and moisture in > 0.25 mm aggregates ($p < 0.05$; Figure 3-5).

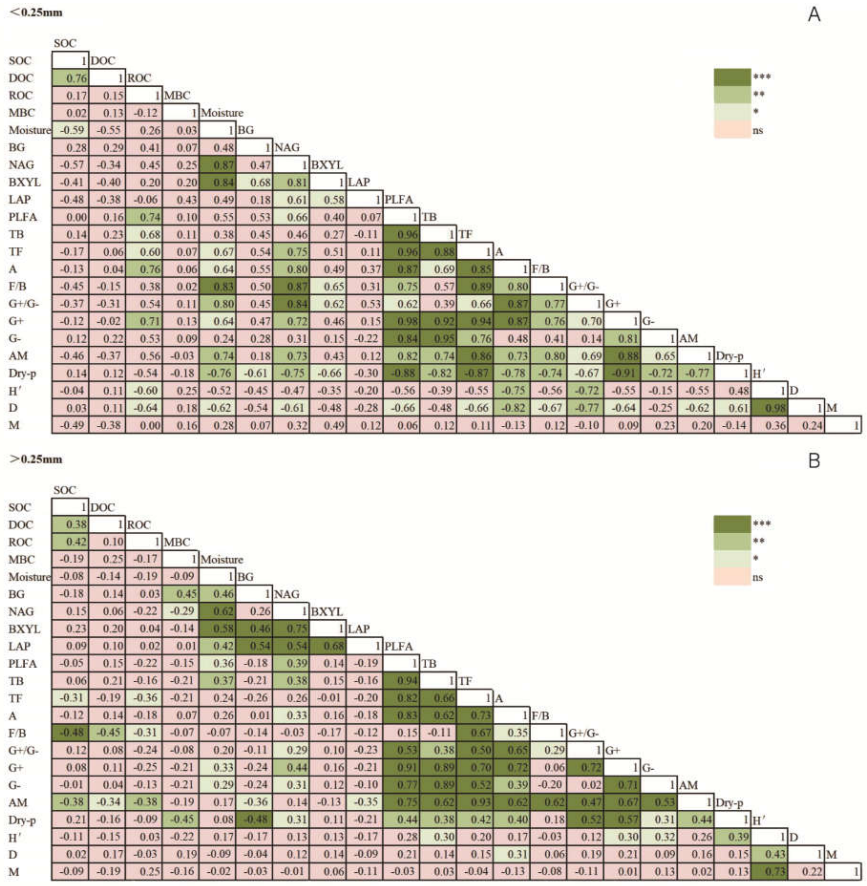

Figure 3-5. Correlative coefficients among the properties of soil organic C, microbial indices and enzyme activity in < 0.25 mm (A) and > 0.25 mm (B) aggregates, respectively. See Table 3-3 for abbreviations of some soil biological properties. * : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$.

Table 3-4 Microbial PLFA biomass and ratios of the phospholipid acid biomarkers and microbial diversities in different sized aggregates.

Microbial indices	Soil fraction (mm)	Fertilizer management				P value
		CK	NP	NPS	NPM	
F/B	> 5mm	21.7±2.2Ab	18.0±5.1Aa	18.8±0.5Ab	19.7±2.8Aa	ns
	2–5 mm	20.4±0.2Ab	19.7±1.2Aa	19.2±0.2Ab	18.6±2.1Aa	ns
	1–2 mm	22.5±0.8Aab	17.7±2.3Ba	19.1±1.5Bb	19.2±1.0Ba	*
	0.25–1 mm	25.2±1.2Aa	18.2±0.5Ba	19.8±0.6Bb	19.1±1.0Ba	***
	< 0.25 mm	21.7±1.0Ab	17.7±1.1Ba	22.9±0.9Aa	22.0±1.4Aa	**
	P value	*	ns	***	ns	
G+/G–	> 5mm	0.4±0.2ABb	0.3±0.04Bb	0.5±0.03Ab	0.4±0.1ABa	ns
	2–5mm	0.4±0.02ABb	0.4±0.02Bab	0.4±0.01Ab	0.4±0.02ABa	ns
	1–2mm	0.4±0.03Ab	0.4±0.1Aa	0.4±0.02Ab	0.4±0.1Aa	ns
	0.25–1mm	0.5±0.1Aa	0.4±0.01Aa	0.5±0.02Ab	0.4±0.02Aa	ns
	< 0.25mm	0.5±0.03Aa	0.4±0.02Ba	0.5±0.01Aa	0.5±0.03Aa	ns
	P value	**	*	*	ns	
Microbial diversity						
Shannon-wiener	> 5mm	3.2±0.1ABb	3.1±0.1Bc	3.3±0.1Aa	3.2±0.1ABb	ns
	2–5mm	3.4±0.03Aa	3.2±0.04Bc	3.2±0.1Ba	3.3±0.03ABa	*
	1–2mm	3.3±0.1Aab	3.3±0.1Ab	3.2±0.1Aa	3.3±0.1Aab	ns
	0.25–1mm	3.2±0.1Bb	3.4±0.02Aa	3.3±0.1Ba	3.4±0.1Aa	*
	< 0.25mm	3.3±0.1Aab	3.4±0.1Aa	3.3±0.1Aa	3.3±0.1Aab	ns

	P value	ns	***	ns	ns	
Simpson (D)	> 5mm	0.9±0.01Aa	0.9±0.01Bb	1.0±0.001Aa	0.9±0.01ABa	*
	2–5mm	1.0±0.001Aa	1.0±0.01Aa	1.0±0.01Aa	1.0±0.01Aa	ns
	1–2mm	1.0±0.01Aa	0.9±0.01Aa	1.0±0.004Aa	0.9±0.01Aa	ns
	0.25–1mm	0.9±0.004Ca	1.0±0.001Aa	1.0±0.01BCa	1.0±0.001AB	*
	< 0.25mm	1.0±0.01ABa	1.0±0.004Aa	0.9±0.002Ba	1.0±0.01Ba	ns
	P value	ns	**	ns	ns	
Margal (M)	> 5mm	10.8±0.5Ab	11.3±1.6Ab	12.0±0.8Aa	11.8±0.8Aa	ns
	2–5mm	13.6±0.1Aa	10.9±0.4Bb	11.1±1.7Ba	11.2±0.9Ba	*
	1–2mm	11.9±1.3Aab	12.0±0.7Aab	10.7±0.5Aa	12.1±2.1Aa	ns
	0.25–1mm	11.2±1.3Bb	13.3±1.0Aa	10.9±1.4Ba	13.5±0.8Aa	*
	< 0.25mm	12.9±0.6Aa	12.1±0.2Aab	12.3±0.5Aa	12.8±0.9Aa	ns
	P value	*	ns	ns	ns	

Note: See Table 3-3 for abbreviations of some soil biological properties. Capital and lowercase letters indicate significant difference among fertilization and aggregate size, respectively, at $p < 0.05$. ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$; ns: not significant.

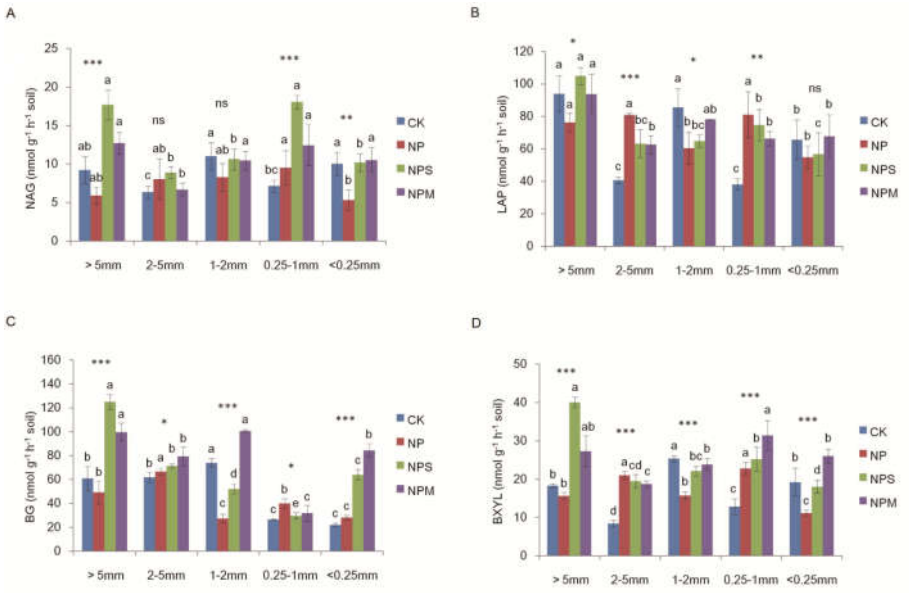

Figure 3-6. Histogram of N-acetyl-glucosaminidase (A), leucine aminopeptidase (B), β-glucosidase (C) and β-xylosidase (D) activities in aggregates in different treatments. NAG, N-acetyl-glucosaminidase; LAP, leucine aminopeptidase; BG, β-xylosidase; BXYL, β-xylosidase. Different lowercase letters mean signifcant differences among aggregate size fractions ($p < 0.05$). The differences in the four enzymes activities among fertilization managements were showed at * : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$ and ns: not significant.

3.3. Contributions of microbial community and soil enzyme to SOC storage

The correlations between SOC storage and indicators of microbial community and enzyme activity in different sized aggregates showed that microbial and enzyme indicators, i.e., fungal and AM biomass, F/B ratio, BXYL, LAP, H' and M, were significantly related to SOC content in > 0.25 mm aggregates, while no significant correlation in < 0.25 mm aggregates (Table 3-5). Soil microorganism and enzyme contributions to C storage under different fertilizations were analyzed by PCA and VPA, considering the differences in their compositions in > 0.25 mm aggregates (Figure 3-7).

The PCA revealed that the predictors explained 63.9% of the variation in > 0.25 mm aggregates, and all of these indicators were clearly divided into 3 groups by the first two principal components among all samples (Figure 3-7). The Venn diagram (Figure 3-7) revealed that the fraction of C storage variation explained by the fungal indices was 21% (fraction [a]; $p = 0.005$). The soil enzyme component explained a lower proportion the variation of SOC content in > 0.25 mm aggregates ([b] = 2%, $p = 0.013$). Meanwhile, the explanation from microbial

diversity was far less than fungal community and enzyme activity, and was not displayed in Figure 6B with a value of less than zero. Most of the SOC variation remained unexplained by the model variables (Residuals = 77%).

Table 3-5 Correlative coefficients between SOC and soil biological properties for CK, NP, NPS and NPM in different sized aggregates.

Index	> 5mm	2–5mm	1–2mm	0.25–1mm	< 0.25mm
Total PLFA	-0.1	-0.4	-0.2	0	0
Actinomycetes	-0.2	-0.2	-0.3	-0.2	-0.1
Bacteria	0	-0.4	-0.1	0.2	0.1
Fungi	-0.5	-0.4	-0.6*	-0.5	-0.2
AM	-0.7*	-0.6*	-0.7*	-0.6	-0.5
F/B	-0.8**	-0.1	-0.7**	-0.7*	-0.5
G+/G–	-0.2	0	0.3	-0.3	-0.4
H′	-0.3	-0.8**	-0.3	0.6*	0
D	-0.4	0.5	-0.4	0.6	0
M	0.1	-0.7**	-0.1	0.5	-0.5
BG	0.1	0.2	-0.3	0.6	0.3
NAG	0	0.5	-0.4	0.3	-0.6
BXYL	0.1	0.8***	-0.6*	0.7*	-0.4
LAP	-0.5	0.8**	-0.3	0.7*	-0.5

Note: See Table 3-3 for abbreviations of some soil biological properties. * : $p < 0.05$; ** : $p < 0.01$; *** : $p < 0.001$.

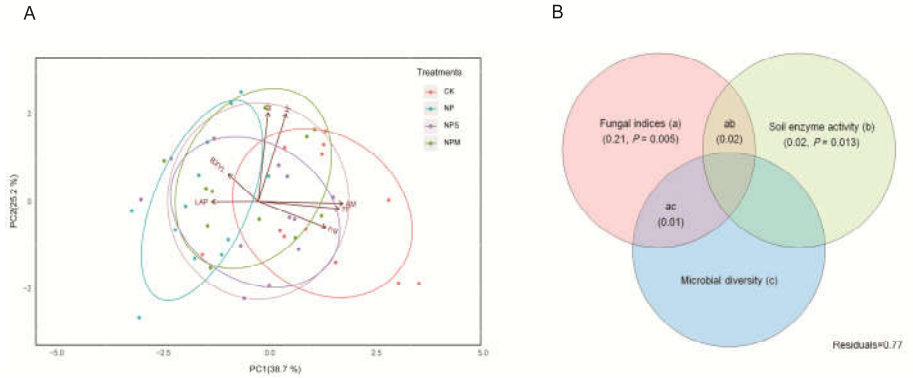

Figure 3-7. PCA analysis (A) and variation-partitioning (B) of SOC accumulation variance among fungal indices (a), soil enzyme activity (b), and microbial diversity (c) predictor matrices in macro-aggregates (> 0.25 mm).

4. Discussion

Fertilization can directly affect the soil C sequestration efficiency. Although the application of straw or manure increases organic C input, it also increases the soil C sequestration efficiency. During the process, the balance between C input and output is mainly determined by microbial activity. Long term inorganic and organic fertilization was considered to affect soil microbial community because they change the micro-environmental conditions and nutrients for microbial survival (Chakraborty et al. 2011; Yang et al. 2020). In this study, there was a remarkable reduction of the soil microbial biomass in all aggregates under NP compared to CK. Although inorganic fertilizer provided the nutrients such as nitrogen and phosphorus, it reduced the moisture values in aggregate, which affects the survival of microorganisms inside (Manzoni et al. 2012). However, the results showed that the effect of a single fertilization on microorganisms was greater than the interactions of fertilization and aggregate size (Table 3-3), indicating that fertilization was the main factor affecting the microbial biomass. Microbial community is sensitive to the increasing of available nutrients under organic matter application (Lin et al. 2019).

In comparison with chemical fertilization (NP), the long-term (27 years) organic matter incorporation resulted in an increase of microbial biomass (i.e., PLFA, bacteria and actinomycetes) in almost all of the aggregates, which vary with the aggregate size and organic matter type. It indicated that organic matter addition could alleviate this negative effect of NP on the microbial community, and the effect of fertilization on microbial biomass is associated with the soil aggregate size and nature of the organic matter. Notably, the amount of fungi was higher only in micro-aggregates under NPS and NPM than under CK, which indicates that fungi within macro-aggregate is more sensitive to environmental perturbation than in micro-aggregates because of the vulnerability of microenvironment in macro-aggregate (Trivedi et al. 2015). Especially, the amount of AM was slightly higher in NPS than in NPM, due to the high decomposition ability of AM in cellulose (Wei et al. 2019). This can also explain why lower F/B was found in macro-aggregates (> 0.25 mm) than in micro-aggregates (< 0.25 mm) under the application of organic and inorganic fertilizers. Therefore, the results supported that organic matter incorporation can build a more suitable environment for microbial surviving.

Among the aggregate sizes, significant differences in microbial diversity were observed under NP and CK, while no difference under NPS and NPM. Organic matter addition could promote higher bacterial richness or evenness among all fractions and enhance the microbial community resistance to disturbance relative to inorganic fertilization alone or no fertilization (Legrand et al. 2018). Based on these findings, the results suggested that the biomass and diversity of microbial community were changed with the application of fertilization, which were obviously ($p < 0.05$) associated with soil aggregate size (Liao et al. 2020). Relative to CK, NPM and NPS increased soil enzyme activities in almost all macro-aggregate sizes (except for 1–2 mm), due to the increasing substrates for

soil enzymes provided by the increasing of microbial population (Akhtar et al. 2018; Martens, Johanson, and Frankenberger 1992). Soil enzyme activities were suggested to be suppressed by the inorganic fertilization (Liu et al. 2010), however, they were favored in 2–5 mm and 0.25–1 mm aggregates under NP, in order to provide C or nitrogen nutrients for the survival of microorganisms (Caldwell 2005). In all of the aggregates, soil enzyme activities were correlated to the distribution of microbial community and moisture (Sardans and Peñuelas 2005). Therefore, our results indicated that the effects of fertilization regime on soil microbial community were associated with the balance between microbial nutrient requirements and secretion of enzymes, and varied with soil aggregate size.

It has been documented that microorganisms and enzymes affect C cycling (Dhaliwal et al. 2020; Romaní et al. 2006). As Table 3-3 showed, almost all soil microbial community and enzyme indices were associated with aggregate size. Meanwhile, it has subsequently been confirmed in the Table 3-5, that soil microbial and enzyme indices were significantly related to SOC in > 0.25 mm but not in < 0.25 mm in response to the different fertilization, supporting that micro-aggregates could protect SOC from being decomposed by microorganisms (Six et al. 2000), whereas macro-aggregates enhance SOC sequestration due to their greater stability mainly caused by the adhesion of microorganisms and secretions (Hurisso et al. 2013).

As a primary elemental energy source for microorganism, ROC was positively correlated with microbial biomass in < 0.25 mm aggregates, i.e., bacteria, fungi and actinomycetes. It indicated that the microbial activity may be limited by ROC, supported by the research that limited labile C is one of the main reasons restricting the growth of soil heterotrophic microorganisms (Demoling et al. 2007). The same trends were found on MBC content and MBC/SOC ratio, that the fertilization had no effect on them in < 0.25 mm aggregates. It indicated that microbial biomass C may have been saturated in micro-aggregates, supporting the findings that higher quality and protection of SOC in macro-aggregate are more conducive to the growth of microorganisms compared to micro-aggregate (Gupta and Germida 2015; Tisdall and Oades 1982). It was consistent with the results in Table 3-3 that aggregate size has a greater effect on MBC than the interactions of fertilization and aggregate size. However, our results that a significant increase of SOC occurred in micro-aggregates (< 0.25 mm) under the fertilization, indicated that more contribution to C sequestration comes from other pathways than microbial processes. For instance, the processes of chemical bonding to minerals or physical protection contribute to the mineral-associated organic C formation, which is one of the main components of organic C (Cotrufo et al. 2019; Kögel-Knabner et al. 2008).

In > 0.25 mm aggregates, the PCA analysis divided the indices that have a significant ($p < 0.05$) impact on SOC into three groups, and Venn models revealed that fungi-related factors were more important than microbial diversity and enzyme activities in affecting the SOC in macro-aggregates. This is largely due to

the structural heterogeneity of the macro-aggregate and the strong viability of fungal community (Guggenberger et al. 1999; Jasinska et al. 2006). Especially, as the dominant mycorrhizal type, AM fungi were reported to alter the C storage through enhancing litter decomposition (Cheng et al. 2012) or reducing the rhizosphere priming effect to increase C retention. Microbial diversity had no significant ($p > 0.05$) relationship with SOC in macro-aggregates (> 0.25 mm), the same trend as bacterial biomass. Some research reported that bacterial diversity increased more greatly than fungi in higher pH values (Shen et al. 2013), thus bacterial community plays a key role in microbial diversity. It was in line with our result that the microbial diversity of H′ was correlated ($p < 0.05$) with the bacterial biomass in > 0.25 mm aggregates. Inconsistent with our results, the abundance of bacteria was supported to contribute to the rapid decomposition of soil C (Wardle et al. 2004).

Similarly, soil enzyme activities were significantly and positively correlated with bacterial and actinomycetic biomass, which supported that the diversity and composition of bacterial community could be partially reflected by the soil enzymes (Ren et al. 2020). It also can explain why no significant contribution of enzyme activity to SOC was observed. The high unexplained residuals suggest that important aspects driving C dynamics were not included in this analysis, such as physical and chemical approaches. Our results confirmed that microbial variables had greater impact on SOC in > 0.25 mm macro-aggregates under different fertilizations, whereas no significant effect was found in < 0.25 mm micro-aggregates. The total effect of fungi-related indicators contribution was higher than other microbial indices (Baumert et al. 2018). More in-depth studies are needed to detect fungal reaction and variation resulting from fertilization and should be incorporated in the main causes of microbial approach that affect C accumulation.

Soil C redistribution and microbial habitat condition were altered under manure and crop residues inputs (Bossio et al. 2005; Six et al. 2004). Previous studies confirmed that different fertilization managements might affect the soil biological processes, through changing the soil environment, the nutrients and turnover of aggregate, which could directly or indirectly affect C storage (Dhaliwal et al. 2020). This study showed that NP made a significant reduction of the microbial biomass, i.e., fungal and bacterial biomass, and led to a stronger decrease in C decomposition by microorganisms than NPS and NPM. The main reason may be that long-term inorganic fertilizer inhibited the growth of microorganisms through affecting the soil pH or moisture condition (Cai et al. 2019; Shi et al. 2018; Tu et al. 2006), which lead to the weakened microbial decomposition of C. It also might be one of the reasons for the higher ROC content in NP than in CK.

The ratio of MBC/SOC and the mass proportions of macro-aggregates (> 2 mm) were lower under NP than CK, whereas the total SOC content was higher, indicating that the contribution of microbial process and physical protection to C reduced under inorganic fertilization (Six et al. 2000). Therefore, other important aspects should contribute more greatly to C accumulation than microbial process

under inorganic fertilization. For example, the chemical process of the interaction between C and soil minerals has been known as an important pathway of mechanisms of C sequestration (Cotrufo et al. 2019).

Simultaneously, the combined application of organic matter significantly reduced the impact of inorganic fertilizers on the growth of microbial community, due to the improvement of water condition and agglomeration. For dry-land agriculture, periodic rainfall causes soil to be in a long-term alternating state of dry and wet, causing periodic fluctuations in soil water contents. In this study, the application of organic matter alleviated the fluctuation of moisture and maintained it at a relatively high level, which is conducive to microbial activity (Li et al. 2020; Mondini et al. 2002). This is consistent with the result that MBC content was higher under NPM and NPS than under NP in macro-aggregates (except 0.25–1 mm). The fungal biomass were positively ($p < 0.05$) related to macro-aggregates proportion (> 0.25 mm), which promotes the physical protection of C sequestration (Six et al. 2000).

However, both of ROC and SOC contents have no significant differences between inorganic and organic fertilizations, which were negatively ($p < 0.05$) correlated with fungal biomass. This indicated that the C content was in dynamic equilibrium under the inorganic and organic fertilizations during the process of C sequestration and decomposition in macro-aggregate, in which fungi are key regulators. A similar observation was also found that fungi were affected more greatly by fertilization in macro-aggregates than in micro-aggregates (Liao et al. 2020), with the great ability to degrade complex C polymers (Thormann 2006). The lower F/B under fertilization than no fertilization in macro-aggregates suggested that bacteria were more able to adapt to fertile environment. Both of actinomycetes and bacteria, including G+, G– and G+/G–, had no significant relationship with C among all treatments, which was supported by the study that low net accumulation of C was found in fertile soils that are dominated by bacterial community (Wardle et al. 2004). Therefore, it indicated that the effect of fertilization management on the contribution of fungal community to C is more pronounced in macro-aggregates, compared to other microbial community.

5. Conclusion

After 27 years of long-term experiments, we studied the influence of microbial process on C accumulation and made several conclusions based on two years (2007-2008) of sampling. Microbiological properties play an important role in C reserves at aggregate scale under different fertilization regimes. We emphasized that the alteration of microorganism community (i.e., fungal and AM biomass and F/B ratio) and enzyme activities (BXYL and LAP), rather than microbial diversity, contributed greatly to C storage in macro-aggregates (> 0.25 mm). However, the influence of microbial factors in C storage was not significant in micro-aggregates. Compared to inorganic fertilization, combined application of organic and inorganic fertilizer increased the microbial contribution to C storages. Our study

indicated that the contribution of microbial processes to C accumulation not only depends on the aggregate size, but also on the variety of the microbial properties and their interrelationships under different fertilization regimes. Future research is needed to reduce the negative impact of inorganic fertilizers on soil flora by using organic matter application while maximizing the soil C accumulation in agroecosystems.

Chapter IV

The microbial community driving C accumulation in different sized aggregates under straw application

The studies on the relationship between soil C content and aggregate sizes can better illustrate the microbial mechanisms of soil C accumulation. This chapter aimed to identify dominant microbe in different sized aggregates and their contribution to soil C stock.

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Chapter IV The microbial community driving C accumulation in different sized aggregates under straw application

Abstract

Unraveling the influence of microbes on C content at aggregate scale is pivotal for promoting soil C accumulation under straw application. Previous studies on C accumulation at aggregate scale were based mainly on the mutual transformation process between aggregates, the links between the microorganisms in initial aggregates and inner C content and aggregate sizes were still unclear. In this study, the classified aggregates (> 5 mm, 2–5 mm, 1–2 mm, 0.25–1 mm, and < 0.25 mm) were individually incubated for 10 months under ^{13}C-labeled maize straw application to analyze the relationship between microbial community structure in independent aggregates and inner C accumulation under straw addition. The results show that the SOC content increased in independent aggregates under straw addition, with higher stable C accumulation in < 0.25 mm than in > 2 mm aggregates. Aggregates of > 5 mm were more capable of improving unstable C accumulation and C derived from straw (C_{straw}) than smaller aggregates. Fungi and Gram-negative bacteria (G–) were more important to increasing C accumulation in > 2 mm aggregates, whereas Gram-positive (G+) bacteria dominated in < 2 mm aggregates. The results indicate that the contribution of microorganisms within aggregates to inner C accumulation was associated with aggregate sizes under straw application.

Keywords:

C accumulation; soil aggregate; straw; microorganisms; bacteria; fungi.

1. Introduction

Soil organic carbon (SOC) is the largest terrestrial C reservoir and plays a key role in the global C cycle (Wan et al. 2019). Small changes in the soil C pool might affect the global C balance significantly (Murugan et al. 2019). Due to the long-term and large-scale use of soil by humans, the balance between soil and atmospheric C has gradually been affected. Hence, understanding the mechanisms of cycling SOC is essential to increase the accumulation of organic carbon (Sokol and Bradford 2019).

Aggregates, as the basic units of soil, are considered to be stable pools of SOC (Blanco-Canqui and Lal 2004) and contain nearly 90% of sequestered SOC (Somasundaram et al. 2017). Some researchers divide the aggregate sizes into micro- (< 0.25 mm) and macro- (> 0.25 mm) aggregates (Jastrow 1996; Six et al. 2004), and there is less consistency in the study of C in different sizes aggregates. In a previous publication, greater C content was found in macro-aggregates than in micro-aggregates in soils dominated by 2:1 clay mineralogy (Six et al. 2004), and micro-aggregates were suggested to contain the largest stable soil organic C pools (Kong et al. 2005). Similarly, some research found that occluded micro-aggregates inside macro-aggregates contributing greatly to C protection than macro-aggregates (Totsche et al. 2018). The research on the C content in different aggregates can provide more details for C dynamics, therefore, more studies on the relationship between soil C content and aggregate sizes should be illustrated for improving C accumulation at aggregate scale.

As is known, soil aggregates provide different micro-environments (Rabbi et al. 2016) and attract diverse microbial communities (Blaud et al. 2012) that can colonize them well, depending on the readily available carbon abundance, organic matter complexity, and other indexes (Mikha and Rice 2004; Upton et al. 2019). In general, microbial processes, such as microbe-triggered courses and microbial-originated polysaccharides, could promote the mutual transformation of macro- and micro-aggregates (Li et al. 2019). In the process, physically protecting SOC by macro-aggregate formation has been regarded to be an important process in SOC sequestration (Pulleman and Marinissen 2004). In addition, as drivers of the soil C cycling and sequestration (Lehmann and Rillig 2015), microorganisms contribute to the mineralization and biodegradation of SOC and the formation of new organic metabolites (Singh et al. 2019).

The effect of microorganisms on C at aggregate scale has been studied widely (Liu et al. 2020; Six et al. 2004). A qualitative difference in microbial communities was found within macro- and micro-aggregates (Miller and Dick 1995), and the microbial biomass in macro-aggregates was suggested to make a larger contribution to SOC storage than in micro-aggregates (De Gryze et al. 2005; Liu et al. 2020; Six et al. 2004). The presence of a large number of arbuscular mycorrhizal fungi (AMF) was considered to be related to C accumulation within > 1 mm aggregates, whereas Gram-positive (G+) bacteria and some nematodes increase C retention in smaller aggregates (Zhang et al. 2013). However, previous

studies looking at soil samples still contained all aggregate size classes together, which included the process of mutual transformation between aggregates. Currently, there are no studies focusing on initial or independent aggregates. The relationship between the microorganisms residing within independent aggregates and the inner C content, as well as the link between this and aggregate size, were still unclear. This study is instrumental for better investigating how the nature of the microbial community within macro- and micro-aggregates affects the SOC content within each size class.

Crop residue addition can affect SOC retention at aggregate scale and alter its distribution in aggregates (Ding and Han 2014; Wang et al. 2019). As a source of energy, organic addition can stimulate the decomposition of native SOM (Zhang et al. 2018). Therefore, adding organic substrates can alter SOC content during the process of substrate switching through microbial metabolism (Woolf and Lehmann 2019). Furthermore, AMF in the soil not only indirectly stimulates fresh residue decomposition, but also suppresses the decomposition of old C (Wei et al. 2019). As a result, soil C can be accumulated or lost, depending on the balance between the microbial decomposition of new inputs and the protection of new C (Kravchenko et al. 2019). To better understand the relationship between SOC dynamics and the microbes associated with residue addition, it is important to quantify the utilization and fixation of new C during microbial processes (Jiang et al. 2019).

Previous studies cannot distinguish in which group of aggregates the microbial processes affecting C accumulation occurred. We have added the ^{13}C-labeled maize straw to classified aggregate groups, and observed the variation of the inner C content. The difference in C accumulation between the straw addition and no straw addition can then be attributed to straw induced microbial community variations inside independent aggregates. The objective of this study was to determine the influence of straw addition on the relationship between microorganisms in independent aggregate groups and inner C content. We hypothesized that (i) straw application could promote the C accumulation in all independent aggregate groups, and (ii) more C content, including C_{straw}, would be sequestrated in macro-aggregates than micro-aggregates, and (iii) the dominant microbial groups associated with C accumulation vary with aggregate sizes.

2. Materials and methods

2.1 Soil sampling

Soil samples were collected from the Long-Term Residue Retention Experiment, initiated in 1993 at the Dry-land Farming Experimental Station in Shouyang, Shanxi (112–113 °E, 37–38 °N) in northern China. The area has a mean altitude of 1100 m above sea level and a continental monsoon climate with a mean annual rainfall of 520 mm and a mean annual temperature of 7–8 °C. The dominant crop grown there is continuous spring maize, which covers more than 50% of the total

crop-growing area in Shouyang. During the experimental period, maize was seeded at the end of April without any tillage and harvested in October. Plowing occurred once a year to a depth of about 20 cm. The area was weeded manually twice during the growth period. The study site had sandy clay loam soil, classified as a Calcaric-Fluvic Cambisol (ISS-CAS, 2003; IUSS, 2006).

Soils for this study were sampled at a depth of 0–10 cm from the field with no added fertilizer in August 2017, and soil pH was 7.8, and SOC and total N contents were 19.3 and 1.1 g kg^{-1}, respectively. Up to five soil cores (10 × 10 cm) were sampled and pooled. The fresh soil was immediately taken to the laboratory and then separated manually along the natural cracks to obtain aggregate sizes of < 6 mm. Large pieces of plant material, stones, and visible soil fauna were picked out with tweezers. Then, samples were sieved into five sizes (> 5 mm, 2–5 mm, 1–2 mm, 0.25–1 mm, and < 0.25 mm). Some of the "classified aggregates" were stored at –20 °C for biochemical analysis and some were air-dried for the analysis of the C contents, and the remaining samples were immediately subjected to the following experiment.

2.2 Incubation experiment

In order to determine whether the microorganisms derived from the initial aggregate promote C accumulation, an incubation of classified aggregates (> 5 mm, 2–5 mm, 1–2 mm, 0.25–1 mm, and < 0.25 mm) with ^{13}C-labeled maize straw application was conducted. The ^{13}C-labelled maize straw had a $\delta^{13}C$ value of 690.5‰, C content of 40%. A total of 30 incubation microcosms were prepared in plastic containers without covers, each with a 200 g dry weight equivalent of soil aggregates. Six microcosms were prepared per aggregate size treatment. All microcosms were adjusted to 50–60% of water holding capacity in an incubation chamber to reach maximum microbial activity and equilibrated for 15 d at 25 °C in the dark. Soil moisture was achieved by adding deionized water and determined gravimetrically every week and adjusted as necessary. Once soil samples had adapted to this optimal environment, ^{13}C-labeled straw (1 cm height, 1 g per microcosm) was applied to each of 15 microcosms (five sizes, three replicates) and named the S treatment during the incubation. The other 15 microcosms were cultivated without straw, limiting the availability of organic material, in the so-called NS treatment. In addition, the original non-incubated soil aggregates named the CK treatment were used as the reference for the incubation experiment.

The S- and NS- treated microcosms were incubated at 25 °C for 300 d. After incubation, some of each sample was removed from each incubator and air-dried to determine the organic C contents and analyze the amount of C derived from straw (C_{straw}) after picking out all visible debris. The remainder of the samples was stored at –20 °C for biochemical analysis.

2.3 Soil analysis

2.3.1 Determination of organic C content and δ^{13} C value of soil samples

The SOC content was analyzed using an elemental analyzer (C/N Flash EA 112 Series-Leco Truspec). Dissolved organic carbon (DOC) was extracted with distilled water (1:5 w:v). Samples were shaken for 2 h at 250 rpm, then centrifuged at 15,000 rpm for 15 min and filtered. The extracted solution was analyzed with a C analyzer (Multi N/C 3100, Analytic Jena, Germany). Readily oxidizable organic carbon (ROC) was tested with 333 mM $KMnO_4$ oxidation. The air-dried soil (passed through a 149 μm sieve) containing 15–30 mg C was mixed with 25 mL of 333 mM $KMnO_4$ and shaken for 1 h at 250 r min^{-1}, followed by centrifugation at 2000 r min^{-1} for 5 min. After dilution with deionized water (1:250), the supernatants were detected using a UV spectrophotometer at 565 nm and the ROC content was estimated by the variation in $KMnO_4$ concentration, assuming that 1 mmol $KMnO_4$ is equivalent to 9 mg C in the oxidation.

After ball-milling and acidifying with HCl (1 M), the $\delta^{13}C$ values of the dried samples were determined on an elemental analyzer (vario PYRO cube, Elemantar) coupled with an isotope ratio mass spectrometer (Isoprime 100, Germany). The C isotope value ($\delta^{13}C$) was referenced to the international Pee Dee Belemnite standard (R_{PDB} = 0.0112372) and expressed as $\delta^{13}C$.

$$\delta^{13}C = (R_{sample} - R_{PDB}) \times 1000/R_{PDB} \quad (1)$$

where R_{sample} is the ratio of $^{13}C/^{12}C$ in soil samples.

The proportion of C derived from straw (f; %) to SOC in soil aggregates with straw addition was calculated using the following equation (2). The contents of aggregate fraction C derived from straw and from native SOC can be calculated according to the following equation (3) and (4):

$$f = (\delta^{13}C_{mix} - \delta^{13}C_{soil})/(\delta^{13}C_{straw} - \delta^{13}C_{soil}) \times 100\% \quad (2)$$

$$C_{straw} = C_{mix} \times f \quad (3)$$

$$C_{native} = C_{mix} \times (1 - f) \quad (4)$$

where $\delta^{13}C_{mix}$ is the $\delta^{13}C$ value of the soil aggregate with added straw, $\delta^{13}C_{straw}$ is the $\delta^{13}C$ value in the straw residue, $\delta^{13}C_{soil}$ is the $\delta^{13}C$ value in the soil aggregate without added straw, C_{mix} is the SOC content in the aggregate with added straw, C_{straw} and C_{native} are the contents of SOC in the aggregate derived from straw and native SOC at the end of the incubation (300 d).

2.3.2 Analysis of enzyme activity

The activity of four soil enzymes, β-glucosidase (BG), β-xylosidase (BXYL),

cellobiohydrolase (CBH) and leucine aminopeptidase (LAP), was measured in all soil samples using a 96-well microplate method (DeForest 2009; German et al. 2011). In brief, 1 g of fresh soil was weighed into a beaker with 125 mL of $NaHCO_3$ buffer (pH 8.0 for alkaline phosphatase) and stirred at 800 rpm for 2.5 min with a stir bar. The slurry was then set to stir at 100 rpm for 3 min. The continuously stirred soil slurry was transferred into black 96-well plates using an eight channel pipet.

Every plate was set up for three soil analyses and included a blank well, reference standard well, quench well, negative control well, sample well, and sample control well. 4-Methyllumbelliferyl (MUB) was used as a substrate for BG, BXYL, and CBH activities, whereas 7-amino-4-methylcoumarin (AMC) was used for LAP activity. In detail, 250 μL of sodium acetate buffer were added to the blank wells; 50 μL of 10 mM MUB/AMC-linked substrate solution and 200 μL sodium acetate buffer were added to the reference standard wells; and 50 μL of a 200 mM MUB/AMC-linked substrate solution and 200 μL sodium acetate buffer were added to the quench wells. The sample control wells contained 200 μL soil slurry and a 50 μL sodium acetate buffer; while a 200 μL sodium acetate buffer and 50 μL of a 200 mM MUB/AMC-linked substrate were added to the negative control wells. Finally, the assay wells contained 200 μL soil slurry and 50 μL of a 200 mM MUB/AMC-linked substrate solution. All of the plates were then incubated for 3 h at 25 °C in a dark room/chamber. After incubation, 10 μL of 1 M NaOH were added to each well automatically to stop the reaction and enhance the fluorescence. After 1 min, the fluorescence was detected on a multilabel fluorescence reader (Tecan Infinite F200/M200) using l ¼ 355 nm excitation and l ¼ 450 nm emission filters.

2.3.3 Phospholipid fatty acid (PLFA) analysis

Soil microbial community composition was analyzed by measuring the profile of phospholipid fatty acids (PLFA), as described by Bossio (1998). The detailed methods are as follows: 3.0 g (3.0 g dry weight) aggregate samples were dissolved in 7.6 mL chloroform-methanol-citrate buffer (1:2:0.8 v/v/v) for 2 h, followed by centrifugation at 3000 rpm for 10 min. The liquid phase was then transferred to a new tube. The soil was vortexed with an additional 7.6 mL extractant and extracted again for 30 min. The liquid phase from the second tube was transferred to the first one. After extraction, citrate buffer (5 mL) and chloroform (5 mL) were added. The $CHCl_3$ layer was decanted and dried at 35 °C under N_2, before the phospholipids and glycolipids were separated on solid phase extraction columns containing 0.5 g Si (Supelco Inc., Bellefonte, PA). After methylation of the polar lipids, samples were dissolved in hexane (100 μL) and the fatty acid methyl esters (FAME) were detected using a gas chromatographer (Agilent 6890 Series, Agilent Technologies, Wilmington, DE) equipped with an automatic sampler and a flame ionization detector with ultra-high-purity nitrogen as the carrier gas. Nonadecanoic acid (19:0) was added as an internal standard to quantify the PLFA. FAME identities and relative percentages were automatically calculated using MIDI methods (MIDI, Inc., Newark, DE) (Buyer and Sasser

2012).

PLFA were assigned to general bacteria (16:0, 17:0, 18:0, 20:0), Gram-negative bacteria (G–; monounsaturated, cyclopropyl 17:0 and 19:0), Gram-positive bacteria (G+; iso and anteiso branched, a15:0, i16:0, a17:0 and i17:0), general fungi (18:2ω6c), arbuscular mycorrhizae (16:1ω5c), and actinomycetes (16:0 10-methyl), following previously described methods (Aciego Pietri and Brookes 2009; Guo et al. 2016).

2.4 Data calculation and statistical analysis

All data were statistically analyzed using SAS 9.4 for Windows 10. The contents of SOC, ROC, DOC and biomass of all microbial groups were expressed as the mean ± standard error. One-way ANOVA with Duncan's test was used to examine different aggregate size effects on SOC, DOC, ROC, enzyme activity, and microbial biomass. Two-way analysis of variance (ANOVA) with Duncan's test was used to examine straw treatment, soil aggregate size, and their interaction with all the organic C and microbial indicators. Significant differences are presented at $p < 0.05$, $p < 0.01$, or $p < 0.001$ levels. Pearson's correlations among all of the soil properties were calculated with the SAS 9.4 software. In addition, PCA and redundancy analyses (RDA) were performed to analyze the relationship between the soil microbial community composition and C-related indices in aggregates, and they were carried out using CANOCO 5.0 for Windows.

3. Results

3.1 Distribution of C content in aggregates following the incubation

Relative to the non-incubated treatment (CK), the SOC in NS treatment decreased greatly in > 5 mm than in other aggregates (Figure 4-1). Compared to CK, the SOC content in S treatment increased by 5.6–6.3% and 7.7%, respectively, in > 2 mm and < 0.25 mm aggregates. Conversely, the ROC and DOC contents significantly ($p < 0.01$) increased both in S and NS in all aggregate size classes after incubation (Figure 4-2).

For the S and NS treatments, the results showed that the effects of straw treatment, aggregate size classes, and their interaction on the SOC, ROC, and DOC contents were significant ($p < 0.05$; Table 4-1). Straw addition increased SOC, ROC and DOC the more in the > 5 mm aggregates, 17.9% (Figure 4-1), than in any other size classes. Increases in ROC and DOC contents varied drastically in different aggregate classes under straw addition: the ROC and DOC contents (Figures 4-2) were 3.4 g C kg^{-1} and 0.1 g C kg^{-1} in > 5 mm aggregates, respectively, which were significantly ($p < 0.001$) higher by 32.6% and 36.4% in S than in NS, while much lower in the other small aggregates. There was a significant ($p < 0.01$) increase in ratios of ROC to SOC content and DOC to SOC content (Figure 4-2) in > 2 mm aggregates under straw addition.

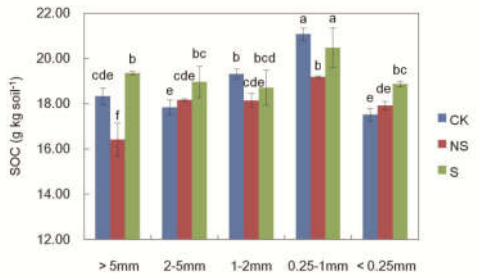

Figure 4-1. The distribution of SOC in different aggregates during the incubation. The same letter means that there are not significantly different (p> 0.05) between the three treatments according to LSD test.

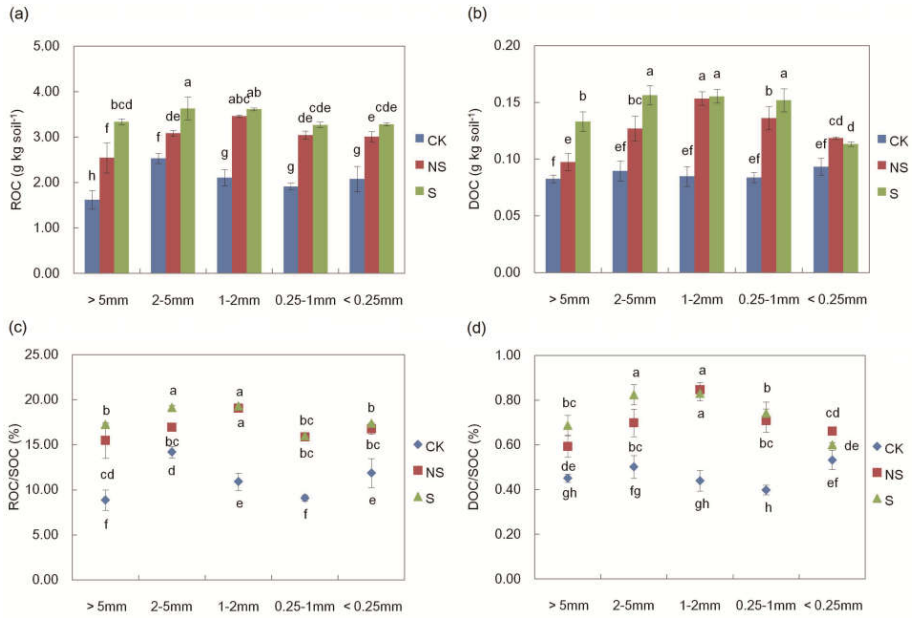

Figure 4-2. The distribution of ROC (a), DOC contents (b), ratios of ROC to SOC (c) and DOC to SOC (d) in different aggregates during the short-term incubation. Different lowercase letters indicated significant difference at $p < 0.05$ among the different sizes aggregates.

As shown in Figure 4-3, the higher C_{straw} content and proportion of C_{straw} to SOC were observed in all aggregate sizes under straw addition and it increased gradually as the aggregate size increased. On the contrary, the proportion of C_{native} to SOC in aggregates decreased gradually as the aggregate size increased (Figure 4-3).

Table 4-1 Two-way ANOVA of the effects of the predictors C = straw treatment, A = aggregate size and C * A = their interaction on all considered variables.

Indices	Straw treatment (C)	Aggregates size treatment(A)	C*A
	F P	F P	F P
SOC	44.8***	10.5***	4.1*
ROC	50.5***	10.2***	3.5*
ROC/SOC	8.7**	13.6***	2.5 ns
DOC	29.1***	31.1***	7.6***
DOC/SOC	3.3 ns	26.3***	8.3***
BG	69.5***	21.3***	2.9*
CBH	113.5***	59.9***	29.8**
BXYL	167.4***	29.0***	3.8*
LAP	5.2*	11.5***	2.4 ns
G+	88.6***	4.9**	9.2***
G–	1.3 ns	0.8 ns	27.9***
Actinomycetes	218.1***	4.9**	4.7**
Fungus	4.2 ns	12.2***	73.9***
Bacterial	277.0***	13.6***	48.0***
B/F	92.8***	19.5***	30.4***
G+/G–	67.8***	4.6**	1.7 ns
PLFAs	315.3***	2.7 ns	90.4***

Note: BG, β-xylosidase; CBH, cellobiohydrolase; BXYL, β-xylosidase; LAP, leucine aminopeptidase. B/F: the ratio of bacterial and fungal PLFA; G+/G-: the ratio of G+ and G- bacterial PLFA. ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$; ns: not significant.

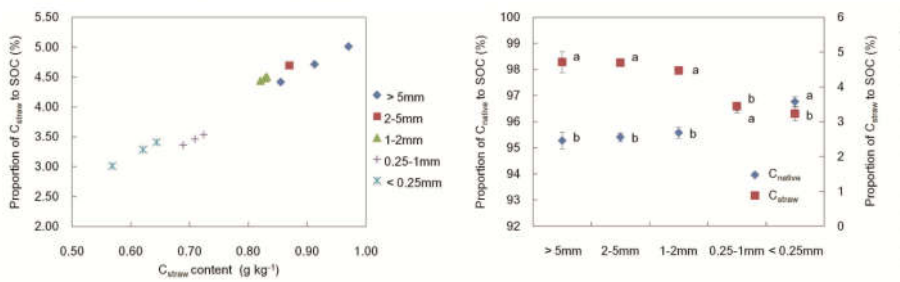

Figure 4-3. The distribution of C_{straw} content (a), proportions of C_{straw} and C_{native} to SOC in soil aggregates under straw addition after the incubation (b). Different lowercase letters indicated significant difference at $p < 0.05$ among the different sizes aggregates.

3.2 Soil enzyme activity in aggregates following incubation

Significant ($p < 0.01$) effects according to aggregate size were observed in the four enzyme activities (Figure 4-4). These activities increased almost in all sizes in S and NS treatments, relative to the CK. All of the four enzyme activities tended to be higher in S than in NS, being the highest in > 5 mm aggregates. After incubation, significant influences of aggregate size classes ($p < 0.001$) and straw treatment ($p < 0.001$) and their interaction ($p < 0.05$) on β-xylosidase (BG), cellobiohydrolase (CBH), and β-xylosidase (BXYL) activities were observed (Table 4-1).

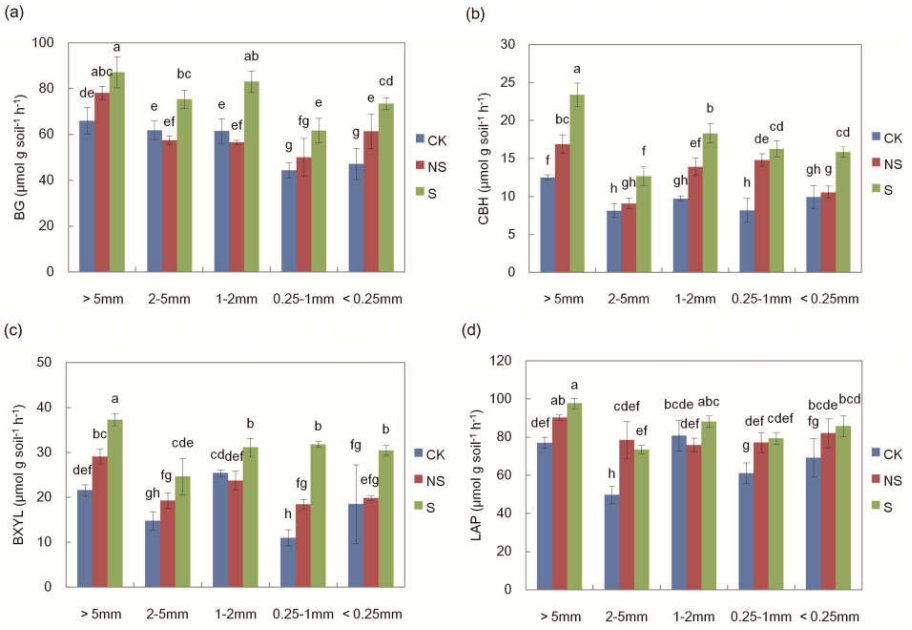

Figure 4-4. Histogram of β-glucosidase (a), cellobiohydrolase (b), β-xylosidase (c), and leucine aminopeptidase (d) activities in aggregates in different treatments. BG, β-xylosidase; CBH, cellobiohydrolase; BXYL, β-xylosidase; LAP, leucine aminopeptidase. Different letters mean signifcant differences among aggregate size fractions ($p < 0.05$).

3.3 The microbial biomass in aggregates after the incubation

Soil microbial biomass (measured as total PLFA concentration) in all aggregates was significantly ($p < 0.001$) lower in NS than in CK (Table 4-2). After incubation, microbial biomass varied greatly among aggregate sizes and between NS and S treatments. In S, the total PLFA significantly ($p < 0.001$) increased by 36.9–53.9% in > 2 mm aggregates, which was greater than that in < 2 mm aggregates (Table 4-2). Fungi were significantly ($p < 0.01$) increased (by 29.8–83.7%) in > 2 mm aggregates, but reduced (by 30.8–37.6%) in < 2 mm aggregates, showing the same trend as G– bacteria (Table 4-2).

Table 4-2 Content of microbial PLFA biomass and phospholipid acid biomarkers and the ratios of the biomarker in aggregate fractions in CK, NS and S treatments in the incubation.

Cultivation	Different aggregates sizes (mm)	Total PLFA (nmol g^{-1})	Bacterial PLFA (nmolg^{-1})			Actinomycetic PLFA (nmol g^{-1})	Total fungal PLFA (nmol	AMF PLFA (nmol g^{-1})	B/F	G+/G– (%)
			G+ bacteria	G- bacteria	Total bacteria					
CK	> 5	48.6bc	7.9b	19.2ab	35.8abc	5.3bc	7.5bc	1.9b	4.8cde	41.3cd
	2–5	49.2bc	8.2b	19.3a	36.0ab	5.9b	7.3bc	1.9b	4.9cd	42.4bc
	1–2	47.4c	7.4bc	18.5abcd	34.0de	5.9b	7.5bc	2.0b	4.5de	39.9cde
	0.25–1	50.1ab	8.1b	17.9bcd	34.1cde	7.7a	8.3a	2.1a	4.1e	45.3abc
	< 0.25	51.9a	9.5a	18.9abc	36.5a	7.6a	7.8ab	1.9b	4.7cde	50.0a
NS	> 5	32.2g	3.4i	14.1gh	25.9g	1.8g	4.2g	1.1d	6.7b	23.9g
	2–5	35.3f	4.2h	13.9gh	27.7f	1.7g	5.4f	1.1d	5.1cd	30.2f
	1–2	40.5e	5.7fg	16.1ef	32.7e	1.8fg	6.0ef	1.2d	5.5bc	35.5def
	0.25–1	42.0de	5.9efg	17.5cd	33.4de	2.0fg	6.6de	1.5c	5.1cd	33.7ef
	< 0.25	41.4de	5.3g	17.3de	33.5de	1.7g	6.2e	1.3d	5.4c	30.8f
S	> 5	49.6bc	7.0cd	17.7bcd	37.2a	4.7cd	7.8abc	1.4c	4.8cde	38.3cde
	2–5	48.4bc	6.8cde	17.1de	37.4a	3.9d	7.1cd	1.2d	5.3cd	39.7cde
	1–2	42.7de	6.3def	14.9fg	34.5bcd	4.0d	4.1g	——	8.4a	42.5bcd
	0.25–1	43.4d	6.9cd	14.3gh	36.0ab	3.8de	4.1g	——	8.5a	50.8ab
	< 0.25	41.0e	6.4def	13.2h	34.3cde	2.8ef	3.9g	——	8.4a	50.6ab

Note: Values within the same column followed by different lowercase letters indicate significant differences among the different aggregate size classes in the same incubation time at $p < 0.05$. See Table 4-1 for these soil properties abbreviations.

In particular, AMF could no longer be detected in < 2 mm aggregates after straw addition. In S, the greatest increases in fungi and bacteria biomass were observed in > 5 mm aggregates, where the fungi biomass increased by 83.7%, more rapidly than that of bacteria (which increased by 43.8%). The G+ bacteria and actinomycetes biomasses increased to varying degrees in all aggregates. The ratio of G+ to G– bacteria (G+/G–) was significantly ($p < 0.001$) increased in all aggregates after straw addition and was higher in < 2 mm aggregates than in those > 2 mm in size (Table 4-2). The trend in the ratio of bacteria to fungi (B/F) was exactly the opposite to that of G+/G– bacteria and was significantly ($p < 0.001$) higher in > 5 mm aggregates after straw addition but significantly ($p < 0.001$) reduced in < 2 mm aggregates. Overall, bacteria, actinomycetes, fungi, and the two ratios were all affected by aggregate size classes (Table 4-1).

3.4 Relationships between microbial community and organic C indicators in aggregates

The PCA revealed that the soil samples were divided into two groups (> 2 mm and < 2 mm aggregates), based on changes in microbial indices in S and NS (Figure 4-5). Considering the differences between > 2 mm and < 2 mm aggregates (Figure 4-5), soil microbial contributions to C accumulation were analyzed separately by RDA for the aggregates of > 2 mm and < 2 mm after the incubation (Figure 4-6).

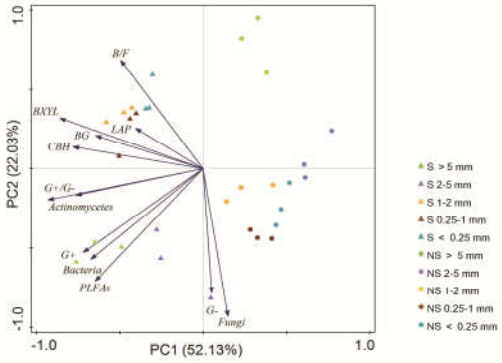

Figure 4-5. PCA analysis of soil microbial properties and enzyme activities in all aggregates after the incubation. See Table 4-1 for these soil properties abbreviations. Different colours represent different aggregate size classes, and different symbols indicate different treatments.

(a) > 2 mm (b) < 2 mm

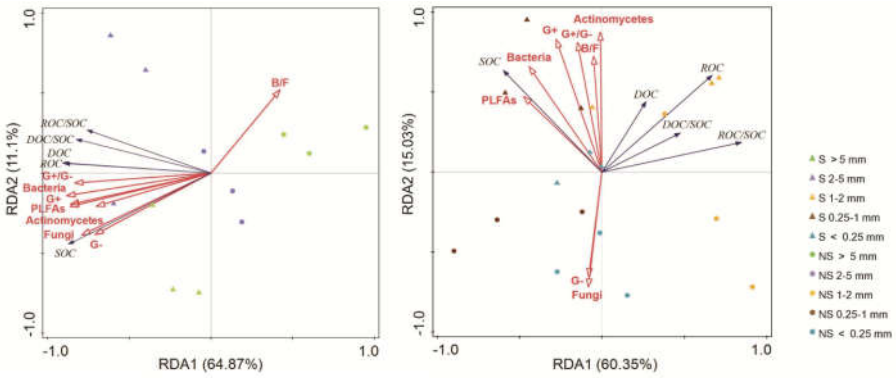

Figure 4-6. Redundancy analysis (RDA) of the effects of soil microbial properties on soil carbon in > 2 mm (a) and < 2 mm (b) aggregates. Different colours represent different aggregate size classes, and different symbols indicate different treatments.

The amount of variability explained by all the canonical axes in > 2 mm and < 2 mm aggregates were 76.1% and 75.5%, respectively. As shown in Figure 4-6, SOC content was significantly (p < 0.05) associated with microorganism indicators (microbial PLFA, bacteria, actinomycetes, B/F and G+/G– bacteria) in both > 2 mm and < 2 mm aggregates.

More specifically, the SOC was more strongly associated with both fungi and G– bacteria than other microbial indicators in > 2 mm aggregates (Figure 4-6), while was associated more with G+ bacteria in < 2 mm aggregates (Figure 4-6). In addition, DOC and ROC were positively (p < 0.05) correlated with the microorganism indicators (except for AMF and B/F) in > 2 mm aggregates, but not in < 2 mm aggregates (Figure 4-7). Meanwhile, the ROC was positively (p < 0.05) correlated with the C-related enzymes (BG, CBH and BXYL) in < 2 mm aggregates, but not in > 2 mm aggregate (Figure 4-7).

(a) > 2 mm

	SOC	DOC	ROC	BG	CBH	BXYL	LAP	PLFAs	Bacteria	Fungi	A	G+/G-	B/F	AMF	G+	G-
SOC	1															
DOC	0.81	1														
ROC	0.81	0.82	1													
BG	0.14	0.03	0.09	1												
CBH	0.18	-0.11	-0.01	0.90	1											
BXYL	0.16	-0.08	0.04	0.92	0.98	1										
LAP	-0.06	-0.50	-0.31	0.58	0.80	0.72	1									
PLFAs	0.84	0.75	0.80	0.52	0.42	0.44	0.08	1								
Bacteria	0.83	0.79	0.81	0.49	0.37	0.39	-0.02	0.99	1							
Fungi	0.86	0.71	0.73	0.39	0.37	0.36	0.10	0.94	0.91	1						
A	0.70	0.58	0.66	0.68	0.58	0.62	0.22	0.91	0.89	0.81	1					
G+/G-	0.75	0.74	0.80	0.41	0.28	0.33	0.02	0.90	0.87	0.88	0.83	1				
B/F	-0.60	-0.43	-0.42	0.00	-0.09	-0.08	-0.10	-0.54	-0.48	-0.79	-0.42	-0.62	1			
AMF	0.64	0.25	0.23	0.42	0.56	0.46	0.54	0.61	0.55	0.73	0.49	0.48	-0.62	1		
G+	0.84	0.76	0.80	0.51	0.41	0.43	0.08	0.99	0.97	0.95	0.88	0.95	-0.59	0.63	1	
G-	0.79	0.62	0.62	0.50	0.50	0.46	0.15	0.88	0.90	0.83	0.75	0.62	-0.39	0.71	0.84	1

***
**
*
ns

(b) < 2 mm

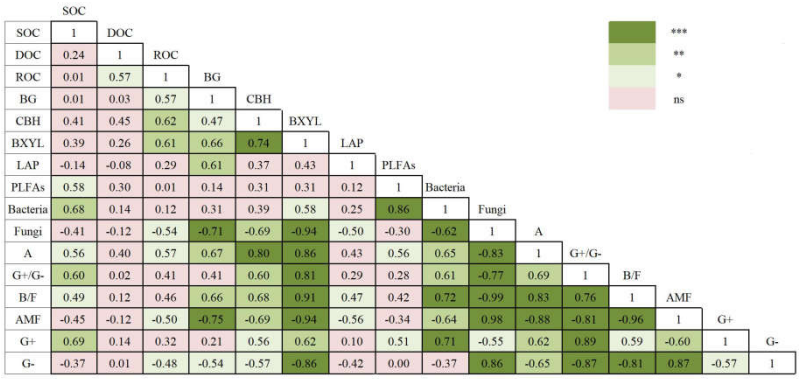

Figure 4-7. Correlation coefficients among the properties of soil organic C, microbial indices and enzyme activities after the incubation in > 2 mm (a) and < 2 mm (b) aggregates, respectively. * : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$.

4. Discussion

In the previous study, the SOC contents in most aggregates were found lower in NS than in CK, due to the mineralization of some C during the incubation process (Zhang et al. 2020). The C sequestered in soil could be turned over by exoenzyme activity or respiration processes of microorganisms, which lead to continuous C loss (Kindler et al. 2006).

During the mineralization process, DOC and ROC increased to provide C sources for starving microorganisms after the incubation. Comparing NS to S, significant impacts by aggregate size and straw addition were shown in the SOC content and C_{straw}, indicating that straw addition can promote C accumulation in aggregates. Straw addition stimulated microbial mineralization of native SOC in soil due to the priming effect (Figure 4-3), and it was also affected by the size and structure of aggregates (Juarez et al. 2013; Sarker et al. 2019). Meanwhile, the result in Table 4-1 showed that the effect of straw addition on SOC was much greater than that of interactions of fertilization and aggregate size, which may be determined by the C saturation of the substrate in aggregates (Xu et al. 2020).

Although most of the straw-derived C will be mineralized and released in the form of CO_2, part of the remaining C can be converted and retained in soil in the form of SOC by microorganisms (Xia et al. 2018). Therefore, an appropriate amount of straw addition can balance part of soil C losses. This is in line with the results presented by other studies (Li et al. 2020). Degradation of added straw and also of the native SOM maybe stimulated by microbial biomass (Blagodatskaya and Kuzyakov 2008), thus leading to the immobilization of C (Zhang et al. 2018).

The increases in DOC, ROC, and C_{straw} contents, in particular, were

significantly greater in > 5 mm aggregates than in smaller ones, implying that > 5 mm aggregates were more capable of improving unstable C and new C accumulation. Variations in the activity and distribution of microorganisms in different sized aggregates could lead to differences in CO_2 emissions and C sequestration during the biological process, which is related to the microenvironment and pore structure inside these aggregates (Jha et al. 2012). A previous study found a similar result: a SOC increase was observed in soil macro-aggregates, not in micro-aggregates and silty clay (Srinivasan et al. 2012), in the 0–10 cm layer with straw retention and macro-aggregates contained more easily decomposable organic C (Puget et al. 2000). In that study, maize residue C was enriched in the particulate organic matter located in macro-aggregates.

With good air permeability, the pore structure inside macro-aggregates can provide a convenient place for microbial activity under treatment with added organic substrates (Negassa et al. 2015). A great abundance of labile C can support faster growing microorganisms in macro-aggregates than in micro-aggregates (Bach et al. 2018; Lupwayi et al. 2001) which in turn accelerates the decomposition of straw. It was supported by a positive relationship between SOC, DOC, ROC and microbial PLFA in > 2 mm aggregates (Figure 4-7).

However, a higher mineralization rate of SOC, especially the native C, offsets a big increase in SOC in macro-aggregates (except for 2–5 mm aggregates). As a labile pool (Six et al. 2000), macro-aggregates were reported to be more highly susceptible to mineralization than micro-aggregates (Rabbi et al. 2014). In micro-aggregates, the highly recalcitrant SOC and limited oxygen supply (Kuka et al. 2007) were not beneficial to C mineralization. Therefore, higher stable C accumulation was observed in < 0.25 mm than in > 2 mm aggregates after straw addition, which did not support our second hypothesis. There was no significant difference in SOC content of 0.25–2 mm aggregates between the CK and S, indicating that the rates of C mineralization and sequestration were in balance. Nevertheless, it is unclear whether the trend for C accumulation in soil aggregates is the same as our results in different soil textures (Gao et al. 2017). Therefore, it will be worth exploring whether more C can be sequestrated in soil rich in differently sized aggregates in the future.

In order to obtain C for microbial survival, high activities of BG, CBH, BXYL, and LAP were found in all aggregates after incubation (Figure 4-4), which could explain why DOC and ROC increased during incubation. Straw treatment and aggregate size had significant ($p < 0.05$) impacts on all enzyme activities (Table 4-1). Moreover, the impact of straw addition on microorganisms was greater than the interactions of fertilization and aggregate size, indicating that the microorganisms community in this experiment could respond to straw addition more quickly than to the aggregate size. Straw amendment could cause rapid stimulation of soil microbes (Golchin et al. 1994; Liang et al. 2018). In order to accelerate the degradation of straw, microbes could produce the C-related enzymes (BG, CBH, and BXYL) and nitrogen-related enzyme (LAP) to regulate

the carbon-to-nitrogen ratio of the microenvironment (Tiemann and Billings 2011). The macro-aggregates could provide a suitable condition for rapid microorganism activities. This might be explained in part by the high soil enzyme activities in > 5 mm aggregates during straw residue degradation, especially of BG, which is the most easily detectable and abundant enzyme (Turner et al. 2002). Notably, the C-related enzyme activities were significantly ($p < 0.05$) related to ROC in < 2 mm aggregates (Figure 4-7), due to obtaining the available C source for the microbial growth through secreting the corresponding enzymes.

The microbial biomass was lower in NS than in CK (Table 4-2), because the original microbial habitat was disrupted, which in turn affected the growth of soil microorganisms after the classification of aggregates. After the incubation, PLFA profile analysis showed that the microbial biomass was greatly increased by the addition of straw (Rousk and Bååth 2007).

The increase in total PLFA biomass was positively ($p < 0.05$) correlated with SOC content (Figure 4-7), which indicated that the rapid degradation of straw in aggregates could also stimulate the growth of microorganisms (Negassa et al. 2015). Fungi and G– bacteria increased significantly in > 2 mm aggregates and decreased in < 2 mm aggregates (Table 4-2). However, only G+ bacteria and actinomycetes increased in < 2 mm aggregates, indicating that G– bacteria and fungi respond more strongly to straw addition in > 2 mm aggregates, while G+ bacteria and actinomycetes are more viable in < 2 mm aggregates. Since the nature and mass of the substrate can affect microbial metabolism (Madan et al. 2002), fungi and G– are more inclined to live in a resource-rich environment (Bahn et al. 2013; Margesin et al. 2009) inside macro-aggregates with more unstable C (Lehmann and Rillig 2015), while G+ bacteria compete better for limited environmental resources (Bahn et al. 2013; Margesin et al. 2009).

Due to the larger volume of mycelium, fungi communities stimulated by organic substrates tend to colonize macro-aggregate structures (Murugan et al. 2019; Six et al. 2006). This might explain why more AMF was observed in > 2 mm aggregates after straw addition, which was considered to be related to decomposition of the recalcitrant ligno-cellulose matrix by stimulating the activity of hyphosphere bacteria or producing a wide range of extracellular enzymes (De Boer et al. 2005) (Figure 4-4). This was also supported by the positive ($p < 0.05$) correlation between AMF and SOC in this study (Figure 4-7). In addition, it can also explain why G+/G– bacteria increase more in micro-aggregates (< 2 mm) than in macro-aggregates (> 2 mm) under straw application, while the B/F, as one of the indicators of ecosystem buffer capacity, displayed the opposite (Bardgett and McAlister 1999). In further research, the dominant microbial indices and strains that can affect C accumulation in different aggregates need to be deeply explored.

SOC content within all aggregates was closely ($p < 0.05$; Figure 4-7) related to the activities of inner microorganisms (microbial PLFA, bacteria, and actinomycetes). As the important components of C, DOC and ROC were significantly affected by microbial PLFA, which contributed to SOC in > 2 mm

aggregates, not in < 2 mm aggregates (Figure 4-7). This is in line with other research, that the DOC and macro-aggregate fractions (> 0.25 mm) were correlated with soil microbial diversity under straw addition (Bu et al. 2020). A plausible explanation could be that the macro-aggregates are more suitable for an efficient and rapid decomposition of the straw by microorganisms, thus stimulating the increase in labile organic C (Dhaliwal et al. 2020; Negassa et al. 2015).

As mentioned above, the significantly raised SOC level in > 2 mm aggregates was accompanied by a significant increase in the biomass of fungi and G– bacteria ($p < 0.01$), whereas the rise of SOC in < 2 mm aggregates was accompanied by G+ bacteria ($p < 0.01$). There are two possible explanations for this trend. The first is that fungi and G– bacteria strongly depend on C derived from straw residues (Bai et al. 2016), while G+ bacteria is more efficient with recalcitrant SOM decomposition (Waldrop and Firestone 2004), because resistant compounds are more readily degraded by their exoenzymes (Brant, Sulzman, and Myrold 2006). Fungi were observed to be able to absorb and utilize plant material more rapidly and efficiently than bacteria, especially in incorporating straw-derived C (Koechli et al. 2019), which was preferentially accumulated in the fungal biomass (Müller et al. 2016; Šťursová et al. 2012). The second is that fungi-dominated soils have more microbial residues in the SOM than those that are bacteria-dominated (Kallenbach et al. 2016). The contribution of total microbial residues to SOC is stronger in macro-aggregates than in micro-aggregates (Murugan et al. 2019), while the microbial residue C in soil is much higher than the living microbial biomass (Simpson et al. 2007).

Hence, we conclude that fungi and G– bacteria were more important to increase C accumulation in > 2 mm aggregates, while G+ bacteria dominated in that role for < 2 mm aggregates. Among all of the aggregates, the assistance of actinomycetes is indispensable during the decomposition of macromolecular plant materials (Dou and Wang 2011). This indicated that C in aggregates was closely related to its own microorganisms, and this relationship was directly affected by aggregate sizes. Future studies should consider that whether it is possible to assist the improvement of C accumulation at aggregate scale by selecting different microbial fertilizers.

5. Conclusions

This study aimed to evaluate the C accumulation in independent aggregates and the contribution of internal microorganisms to this under straw application. Our results showed that straw addition significantly increased SOC content in > 2 mm and < 0.25 mm aggregates, and greater stable C contents were found in < 0.25 mm aggregates than in > 2 mm aggregates. The activities of microorganisms in independent aggregates contributed to inner C accumulation under straw addition. Fungi and G– bacteria were more important to increase C accumulation in > 2 mm aggregates and G+ bacteria dominated in < 2 mm aggregates. Our results

imply that the specific microbes naturally occurring in soils could support an approach to increase C accumulation in soils that were regulated by aggregate sizes. Further studies about long-term experiments are warranted to better evaluate the potential of soils rich in differently sized aggregates to store SOC.

Chapter V

Final conclusion and future research

In this chapter, the importance and relevance of the research on the relationship between microorganisms and C accumulation under agricultural practices were delved into. We stated the answers to the main research question and provided suggestions about the potential improvements in future studies.

1. General discussion

The carbon (C) cyclic of transformation is thought to be the most important and basic material cycle on the earth's surface. As an important part of the terrestrial soil C pool, soil organic carbon (SOC) in agriculture has become the focus of global climate change research (Somasundaram et al. 2017). Generically, the rate of SOC turnover is moderated by the biological and environmental controls, rather than the molecular structural properties (Lal et al. 2015). Investigating the mechanisms and pathways of biological action in the process of soil C accumulation is deepening our understanding of the fundamental processes and the transformation mechanism of soil C in agriculture under intensive anthropogenic managements.

Among biological controls, the formation and decomposition of soil organic matter (SOM) are mainly microbially-mediated. Merely 13% of SOC energy is utilized by soil animals and only 5% is controlled by abiotic actions (Lal et al. 2015). Therefore, soil microorganisms are considered to be a key driver of material and energy flow in soil below-ground, especially of SOM turnover. Different soil microorganisms produce diverse enzymes to mediate SOM decomposition and formation via carrying out specific processes in agricultural ecosystems (Xu et al. 2021). The addition of exogenous organic matter largely controls the production of soil microbial biomass and directly contributes to the formation of stable SOM (Brant et al. 2006). The total microbial biomass was estimated to equivalent to 50-2000 μg C g^{-1} in soil (Anderson and Domsch 2010; Blagodatskaya and Kuzyakov 2013). Some results reported that microbial biomolecules have been thought to be the most important contributor to stable C pool. In addition, soil microbial community structure controls the decomposition pathway of SOM, which determines microbial carbon utilization, thereby affecting the formation of SOM (Brant et al. 2006). Therefore, further exploration of the link between microbes and soil C need to be established to better understand the basic mechanisms of SOC transformation under different agricultural practices.

Farmland ecosystem is an artificial ecosystem, and human activities have had a non-negligible impact on the C cycle of the farmland ecosystem. Different fertilization measures can increase farmland C storage at different rates. Changes in fertilization measures and tillage measures can change the habitat of soil microorganisms by affecting the soil physicochemical properties, thereby affecting the C cycle process they participate in. For instance, some studies showed that organic agricultural systems generally show high biodiversity and soil microbial biomass, and have a strong ability to decompose and transform SOC (Schjønning et al. 2002). Therefore, the development of optimal agricultural practices that enhance SOC sequestration via mediating and altering soil properties for different regional agricultural systems in China is a potential goal for future C sequestration studies.

In this research, we tried to address the research questions of i) quantifying the relative influence of these soil properties to microbial community variations under

long-term fertilization and ii) analyzing the main microbial drivers that promote carbon storage at aggregate scale and iii) comparing the contribution of microorganisms to carbon storage in different sized aggregates. To achieve the objective, we used a 27-year long term fertilization experiment, including 4 fertilization treatments, CK: no fertilization management; NP: inorganic fertilizers application alone; NPS: inorganic fertilization plus the incorporation of maize straw; NPM: inorganic fertilization plus the incorporation of composted cow manure, and present a comprehensive analysis of soil microbial community trait, soil physicochemical properties, and SOC content, and their relationship under long-term fertilization regime. Finally, we got several interesting conclusions.

1.1 Analysis of the contribution of microorganisms to C storage under straw addition

Microorganisms play an important role in the fate of organic substrates in soils through their contribution to SOC formation and decomposition processes. Different microbial groups prefer different C sources, and the dominant microbial groups in soils may alter C storage and cycling (Six et al. 2006). For instance, fungal-dominated communities contribute to soil C sequestration through increasing their biomass, which are thought to have slower rates of residues decomposition relative to bacterial residues (Koechli et al. 2019). Therefore, an increase in soil fungal-to-bacterial abundance is thought to induce greater soil C accumulation (Smith 2012). Waldrop and Firestone (2004) reported that gram-positive bacteria are thought to preferentially use older and more microbially-processed SOM as a C source, whereas Gram-negative bacteria favor fresh plant residues (Bai et al. 2016). Moreover, some specific autotrophic microorganisms need to absorb and utilize carbon dioxide or inorganic C in order to meet their own needs for carbonization. Xanthomonas has been confirmed to be able to synthesize complex organic C from simple organic C such as glucose, and Gaiellaceae can utilize inorganic C in soil to synthesize various organic compounds, which are of great significance to the SOC accumulation (Albuquerque and Costa 2014). Therefore, we can alter the microbial community structure by improving the relative abundance of specific microbial groups in the future, thereby enhance the formation of SOC and nutrient transformation.

As the functional unit of soil, soil aggregates act as the micro-habitat for the microbes and a stable pool of SOC. In common, soil aggregates are classified into < 250 mm micro-aggregates and > 0.25 mm macro-aggregates (Jastrow 1996; Six et al. 2004). About 90% of soil bacteria is found to associate with macro-aggregates, and nearly 70% live within micro-aggregates (Ranjard et al. 2000). It was reported that macro-aggregates contain relatively fresh C inputs and prefer labile C inside, which is sensitive to environmental disturbance (Six et al. 2000; Tisdall & Oades 1982). Conversely, the relatively stable C was accumulated in micro-aggregates with many micropores. Due to inaccessibility, microorganisms and their enzymes cannot access and decompose the C inside the micro-aggregates (Denef et al. 2007; Six et al. 2000). While free organic C located outside of

aggregates is susceptible to be decomposed by microorganisms. Therefore, microbial changes may reflect differences of C distribution at aggregate scale. Current research on relationship between microorganisms and C at aggregate scale has revealed variable results, and the high variability may be due to soil-specific properties, i.e., the differences in soil C and nutrient content, or to the differences in methods for detecting microbial community properties (Smith et al. 2014).

The amendment of straw and livestock to soil is thought as an effective measure to increase SOC content, create soil structure and improve soil fertility. The exogenous organic matter applied into soil provides substrate for microorganisms. As the metabolic products of microorganisms, the residue C is stored in different soil fractions or attached on small particles during the processes of organic transformation and aggregate formation (Guggenberger et al. 1995; Six et al. 2004). Thus, exogenous organic matter input could alter the distribution of SOC in aggregates. Therefore, exploring the relationship between microorganisms and C in aggregates is of great significance for the C sequestration efficiency of crop residue returning to the field.

In our research, straw application can change C accumulation in independent aggregates, which is closely related to the microbial community biomass and structure derived from differently sized aggregates (Chapter 4). SOC content increased in independent aggregates under straw addition. > 5 mm aggregates were more capable of improving unstable C sequestration and C derived from straw (C_{straw}), compared to other smaller sized aggregates. In addition, fungi and G– bacteria were more important to increase C accumulation in > 2 mm aggregates and G+ bacteria dominated in < 2 mm aggregates under straw addition, compared with other indicators. To maintain sustainable SOC cycling including C availability and storage many different microbial species are necessary. Overall, the activities of microorganisms in independent aggregates contributed to inner C accumulation under straw application, which was closely associated with aggregate sizes under straw application. Consequently, it is necessary to take the aggregate sizes into consideration when increasing C accumulation by straw returning in future.

1.2 Analysis of the impacts of microbial characteristics on C sequestration

Soil microorganisms directly or indirectly participate in many soil ecological processes, such as the formation of humus, the degradation of litter, and the transport and circulation of nutrients. Therefore, soil microorganisms are considered to be an important part of soil ecosystem. In previous studies, microbial biomass has been confirmed to reflect the active fraction of SOC. Wang et al. (2018) showed that dissolved organic C content in soil was significantly correlated with microbial biomass C. Cotrufo et al. (2013) reported that microbial biomolecules such as proteins and carbohydrates were thought to greatly contribute to stable SOM in soil. However, soil ecological processes cannot perform these functions just by the abundance of microorganisms, they can only play their role under the combined interaction of microbial abundance, vigor and diversity.

In recent years, soil enzymes, produced by soil microorganisms, are reported to regulate the overall processing of SOC through degrading different molecules or depolymerizing macromolecular substrates (Mooshammer et al. 2014). Several studies revealed that the alterations in microbial activity could cause priming effects due to the addition of substrate, which might simulate the turnover of natural organic matter in soil (Mooshammer et al. 2014; Romaní et al. 2006). Therefore, the microbial activity is considered to be closely related to SOC stability.

Additionally, soil microbial diversity has become a hot spot in farmland ecosystem research and is discussed to be an important indicator for evaluating soil quality. Numerous studies have used molecular biology methods to identify the variation of community diversity to reveal the contribution of soil microorganisms in the conservation of soil ecological functions. Inevitably, there are also several studies that disagree with this statement, which prefer functional redundancy in soil ecology (Rousk et al. 2009). Functional redundancy believes that the loss of one species in an ecosystem can be replaced by another species, and the reduction of biodiversity has no major impact on ecosystem function. Schimel (1995) thought that functional redundancy was more obvious in ecological processes controlled by a large abundance of microorganisms than by a small abundance. Therefore, further studies on the relationship between microbial properties and functional redundancy are extremely necessary.

Regarding food demands, the application of inorganic or organic fertilizers in agricultural systems is necessary to increase crop productivity in the world. A large number of research results have confirmed that the combined application of organic and inorganic fertilizers is a relatively scientific fertilization method at present. Additionally, long-term experience in Europe provides important information that organic agriculture has altered the microbial community structure, which plays a key role in soil C cycling, and has greatly improved soil fertility (Mäder et al. 2007). Consequently, investigating soil microbial processes under agricultural measures such as fertilization and exogenous organic materials returning to the field can provide a theoretical basis for revealing the C cycle and transformation in soil.

In our study, the application of inorganic fertilization was observed to decrease all of the microbial groups biomasses, while organic amendments significantly reduced this negative effect of inorganic fertilizer on microbial biomass (Chapter 3). It showed that microbial variables were significantly correlated with SOC content in > 0.25 mm aggregates, not in < 0.25 mm aggregates. And the alteration of microorganism community (i.e., fungal and AM biomass and F/B ratio) and enzyme activities (BXYL and LAP), rather than microbial diversity, contributed greatly to C in macro-aggregates (> 0.25 mm), which explained 21% and 2% on C, respectively. Compared to inorganic fertilization, combined application of organic and inorganic fertilizer increased the microbial contribution to C storages.

Overall, the contribution of microbial processes to C accumulation not only depends on the aggregate size, but also on the variety of the microbial properties and their interrelationships under different fertilization regimes. Our study

indicated that organic matter addition could contribute to higher C storage by boosting fungal community and enzyme activity rather than by changing microbial community diversity in macro-aggregates. Consequently, it is necessary to study the microbial properties and dynamics in agricultural ecosystems under different agricultural practices to explore soil C turnover.

1.3 The influence of soil properties on soil microorganism community

As an early sensitive indicator for evaluating agricultural practices, soil microorganisms can reflect the changes in soil physical, chemical and biological properties after disturbance more timely and sensitively than other indicators. It has been confirmed that changes in agricultural practices or land-use patterns in agriculture can significantly affect the microbial community environment in soil and the stabilization mechanism of SOC (Fanin et al. 2015; Song et al. 2021). This is because farmland practices can directly or indirectly change soil properties, such as the physical properties of soil aggregates, and then alter the habitat of soil microbes, which in turn affect the C cycling processes.

It is widely proven that, changes in fertilization practices are accompanied by dramatic changes in soil microbial species, abundance and richness (Wang et al. 2020). Compared with inorganic fertilizers, the organic fertilization or the combination of organic and inorganic fertilizers in most researches significantly increased the activity and abundance of microorganisms, as well as microbial biomass C, which contributed greatly to C sequestration. However, studies on Shajiang black soil showed inconsistent results, with no significant increase in microbial activity and slow organic matter formation under organic fertilization (Li et al. 2019). As a typical low- and medium-yield arable land, Shajiang black soil has poor physical properties, i.e., high bulk density, low total porosity, and poor soil aeration, resulting in low microbial activity. Since then, the exogenous organic matter cannot be well utilized and transformed by microorganisms after entering the soil, which is one of the main reasons for the barrenness of Shajiang black soil. Therefore, the influence of soil physicochemical properties on microorganisms will be directly and rapidly reflected in the SOC turnover (Zhang et al. 2020). Future research should focus on altering the microbial properties through combining agricultural practices and soil physicochemical properties to enhance the formation of soil organic matter.

In our research, we found that there were strong relationships among soil physicochemical properties and microbial community across different fertilization (Chapter 2). Microbial biomass had close relationships with both soil chemical and physical properties, and soil chemical properties explained a larger proportion of variation of microbial biomass than physical ones. These physical properties, i.e., GMD, MWD were more strongly correlated with PLFA under application of organic amendments. The same result with variation of bacterial community composition. Differently, fungal community composition was more greatly correlated with soil chemical properties under long-term fertilization. Overall, soil

chemical properties captured a greater proportion of the variations in soil microbial biomass and community composition under different fertilization regimes, comparing with soil physical properties. In addition, the application of organic amendments could possibly increase the decisive impact of aggregate stability on microbial community. This indicated that it is possible to evaluate or control directly soil microbial community via the regulation of main driving factors and agricultural management in future.

2. Future prospects and recommendation for long-term fertilization regime in Loess Plateau and China

This dissertation provided new insights on the effects of different fertilization regimes on soil microbial community characteristics and C sequestration by a long-term field experiment in Loess Plateau of China. Our study indicated that the application of organic amendments can improve microbial biomass and alter microbial community structure through affecting the soil physicochemical properties, which is useful for preserving soil biodiversity in the intensive farmland ecosystem in Loess Plateau of China. From the results presented, it was obvious that the application of organic amendments increased the aggregate stability and its impact on the microbial community. However, aggregate stability is closely related to the soil pore structure in bulk soil. In order to fully understand the impact of soil physical properties on the microbial environment, both pore structure and aggregate stability should be considered here. X-ray microtomography is a very promising technique for investigating soil pore characteristics. In addition, further work could be focused on quantitatively regulating the micro-ecological environment through organic amendment to better meet soil fertility needs.

In this research, we investigated the interrelations of SOC with biological drivers and their relative importance at aggregate scale. Organic matter addition could contribute to higher C storage by boosting fungal community and enzyme activity rather than by changing microbial community diversity. In addition, the activities of microorganisms inside aggregates contributed to inner C accumulation under straw application. Due to the differences in soil properties in different regions of China, the dominant species of microorganisms and their contribution to C accumulation may vary greatly. Therefore, it is necessary to adapt to local conditions. Further research is needed to reduce the negative impact of inorganic fertilizers on soil flora by using OM application and the specific microbes naturally occurring in soils while maximizing the soil C stock in the agroecosystems.

In the light of our study, linking fertilization management to microbial community regulation is very important for soil C sequestration in the agroecosystems. Organic amendments can increase C stock through biomass input, and also lead to C loss through increasing the rate of decomposition of organic

matter. The balance between C input and output determines the C sequestration situation under these practices. As the key medium, microbial community is in charge of the net balance. In the future, the sequestration and loss of soil C will be better quantified by regulating the microbial characteristics and making it serve organic amendment. It may be an optimized strategy for soil C sequestration and reply to the debate about the sustainability of organic amendments with respect to soil C sequestration in the agroecosystems. As a major source of atmospheric CO_2, increasing soil C sequestration in agroecosystems will benefit climate change adaptation and mitigation. Therefore, further research is needed to understand the microbial process and mechanisms of SOC sequestration induced by different agricultural practices.

Based on the results in this thesis, the following points are recommended to promote C sequestration more effectively through agricultural practices:

1) Assess the changes of soil physical properties under agricultural fertilization, especially of non-destructive testing of soil pore structure, to identify the key factors affecting the soil microbial environment. Then these key factors can be used as one of the basis for determining the optimum combination of fertilization method and fertilizer amount.

2) Consider the effect of soil aggregate sizes on dominant microbial community variation in soil. The difference in aggregate distribution under agricultural practices directly induces huge variation in the distribution and abundance of dominant microorganisms in soil, which directly reflected in the SOC turnover.

3) Screen for indigenous microorganisms with high C sequestration capabilities in soils and use them in the production of specific microbial fertilizers. Identify functional genes and major microbial contributors involved in the metabolic process of marker substrates using biotechnological means, such as DNA-SIP (DNA- stable isotope probing) methods and high-throughput sequencing technologies. Different from traditional methods, this technique is not only suitable for the identification of functional genes of culturable microorganisms, but also applicable to non-culturable microorganisms.

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