DISSERTATION

FEEDING THE SOIL TO FEED THE PLANET: SOIL HEALTH OUTCOMES FROM NOVEL AMENDMENTS TO RESIDUE MANAGEMENT

Submitted by

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In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Summer 2021

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ABSTRACT

FEEDING THE SOIL TO FEED THE PLANET: SOIL HEALTH OUTCOMES FROM NOVEL AMENDMENTS TO RESIDUE MANAGEMENT

Healthy soils are the foundation for the continued capacity of agricultural lands to supply essential ecosystem services while also meeting demands for food, fuel and fiber. From academia to policymakers and other key stakeholders, attention towards soil health continues to rise due to global environmental challenges such as climate change and food security that can be potentially mitigated through the sustainable and innovative management of soils. Specifically, the application of organic inputs including composts and animal manures can help enhance water holding capacity, organic matter accumulation and crop production. However, the heterogeneous nature of soils and diversity of production systems precludes a single 'silver bullet' solution to optimize soil health. In addition, outstanding questions persist on the differences in spatiotemporal effects of different organic inputs and their application frequency as well as the linkages between different soil health properties.

This dissertation examines soil health under two different organic input management regimes including a novel soil amendment derived from cheese manufacturing as well as corn residue management in semi-arid agroecosystems. Both the novel soil amendment and corn residue management approaches were established with the goal of conserving soil water in these water limited systems. The novel soil amendment approach involved the one-time, direct application of a byproduct from cheese production known as lactobionate (LB) to soils through an agronomic

trial irrigatedwith wheat and corn. I found that LB applied to soils increased the water retention capacity as well as the microbial biomass content of soils in the 5-15 cm soil zone under the wheat trial. I also found a non-statistically significant 14% increase in corn yield for LB-amended plots. However, I did not observe any difference in wheat yield and some soil properties (soil pH, soil carbon (C), soil nitrogen (N), and soil ammonium concentration for both trials) with lactobionate addition. My observations suggest the potential for lactobionate to modify soil water content, microbial biomass, nitrate, and yield but outcomes varied by crop trial and amendment rates. This implies that while recycling industrial food processing waste for use as a soil amendment may have benefits for key soil properties, the timing, mode and application rate need to be optimized for maximal effects on soil properties.

Due to the effect of LB on soil health observed in the field trials, I conducted an 84-day laboratory incubation experiment to understand specific mechanisms of how LB influences soil organic matter (SOM) decomposition and accumulation via different SOM fractions. I collected soils from the field and split them to add ^{13}C lactobionate to some soils and water only to other soils. I found that about 53% of added lactobionate was respired over 84 days, and observed a positive priming effect after 14 days. In response to LB addition, the total C content of the water extractable organic matter (WEOM) fraction increased by 100% at the initial stage of the incubation but declined exponentially and quicker than other SOM fractions. In addition, the total C content of the light-fraction particulate organic matter (LF-POM) fraction also declined, while both the sand-sized POM and mineral-associated organic (MAOM) C fractions strongly increased relative to unamended control. My results suggest that while lactobionate can help improve soil water retention, it also presents an avenue to building more persistent C through its impacts on the internal cycling of SOM fractions and more importantly on the mineral-associated organic matter

fraction considered more relevant to SOC long-term persistence and relative resilience to disturbance.

The corn residue management study included a four-treatment combination of residue management (residue retained versus residue harvested) and tillage (no-tillage versus conventional tillage) implemented in the field consistently for 6 years, in contrast to the one-time application of lactobionate. My results showed that the most significant differences across soil properties measured were more apparent at the 0-10 cm zone and were mainly driven by residue retention with minor tillage effects. Regardless of tillage mode employed, retaining residues in the 0-10 cm soil layer led to higher soil water content, soil C, aggregate stability, available phosphate, soil macrofauna and fungal abundance and diversity. Furthermore, residue retention was the main driver of macrofauna and microbial community composition; however, an interaction between tillage and residue management suggested that the effect of tillage on microbial communities was most pronounced when residues were retained. I also found significant covariation between soil physicochemical, macrofauna and microbial datasets, indicating a strong association between different soil properties and cascading effects of management on multiple soil properties.

Overall, my findings suggest the impact of both novel amendment and corn residue inputs on soil health varied with application strategy, as the corn residues applied consistently for 6 years had a stronger effect on soil health in the top layer of soils (0 – 15 cm) as compared to lactobionate which was applied one-time. Certain soil properties also responded more quickly to management as compared to others. In addition, while organic inputs are usually applied to target a specific soil health property, other soil health elements can also be affected in a similar magnitude and direction due to latent linkages between different soil properties.

ACKNOWLEDGEMENTS

This dissertation would not have been possible without the guidance, support and assistance of many amazing people in my professional and personal life. I want to begin by expressing my deepest gratitude to my PhD advisor, Dr. Matthew Wallenstein for taking a 'risk' on me by accepting me into his research group and for helping me to thrive, grow and succeed during the course of my studies. Matt's incredible mentorship and assistance has forever left an indelible mark on my life. I am also very grateful to my co-advisor Dr. Steven Fonte for his support and guidance in my academic journey. Steve's guidance has helped me mature in my academic voyage. To my advisory committee, Dr. Schipanski, Dr. Trivedi and Dr. Conant, I am also thankful for giving me important feedback and advice on all my research projects.

My sincere gratitude also goes out to all my collaborators whose valuable support have made this work a reality and include Dr. Cynthia Kallenbach at McGill University (I am so fortunate to call her my academic 'mom' and an incredible mentor!); Dr. Richard Merrill and Dr. Ranjeeta Wadwhani (both at Leprino Foods and Ornua Inc respectively); Dr. Francisco Calderon, Dr. Merle Vigil and Joel Schneekloth (all trio at the USDA Central Great Plains Station, Akron, CO); Dr. Jason Corwin; Dr. Will Reeves and Dr. Boris Kondratieff (both at CSU Entomology).

Furthermore, my special gratitude goes to Aaron Lynch and Dr Erika Foster. Aaron, it was great working with you and thanks for your dedication to working with me through thick and thin. Erika, I am grateful for the countless fun trips you gave me to Akron and for the intellectual conversations. I also want to extend my gratitude to everyone that worked with me and assisted me both in the lab and field including Dr. Megan Machmuller, Kris Otto, Siwook Hwang, Priscilla Matos, Katherin MezaMeza, Christopher Toy and the undergraduate interns at the USDA-ARS

Central Great Plains, Akron, CO for their immense support in field sampling, macrofauna sorting and laboratory analyses. I also want to say a big thank you to Dan Reuss, Guy Beresford, Michelle Haddix and the EcoCore Analytical Facilities at the Natural Resources Ecology Lab (NREL-CSU) for the technical support provided to me during my doctorate journey. I also want to add the lab group members of the Fonte and Schipanski agroecology labs for the valuable feedback during lab meetings. The administrative staff at the Soil and Crop Sciences Department at CSU are not to left out of this gratitude as Jeannie Roberts, Kierra Jewell and former staff Karen Allison for their support as well. Let also include the amazing folks running the stackoverflow.com site for helping me answer thorny stats and R code issues.

I am also grateful for financial support and assistance gotten from various sources including Leprino Foods Inc, Annie's Sustainable Agriculture Scholarship, CSU VPR Fellowship, the Colorado Water Center and the USDA-NIFA grants.

Finally, I want to express my gratitude to my siblings, friends and well-wishers who have supported me mentally and emotionally for these 4 incredible years including Bola and Bimpe Olayemi, Amy and Kaya Matthews, Wale Odukomaiya and the entire Odukomaiya family, Tosin Olanipekun, Nora Flynn, Tobi Oke, Mark and Heather Hallett, Prof A.K. Akintokun and her family, Dr. Ed Hall, Ben Patella and his family, Ryan Boudreau, Tomiwa Bifarin and Bruce William.

DEDICATION

to my amazing parents,

Raphael and Florence Olayemi,

Thank you for your irrefragable love and immaculate care.

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CHAPTER 1: INTRODUCTION

Soils are an important but greatly undervalued non-renewable natural resource. The continued supply of food, fiber and fuel for an ever-increasing global population is dependent on the productivity or 'health status' of soils. Healthy soils are conceptualized as functional living ecosystems with the capacity to not only sustain humans but also plants and animals (USDA, 2020). This implies that healthy soils must play a balancing act in providing the necessities of life required to support all macro and microorganisms alongside providing ecosystem and cultural services.

However, a challenge in understanding soil health is the contextual nature of this topic as inherent soil properties differ from one location to another and from one purpose to another (Norris et al., 2020; Janzen et al., 2021). For example, soils termed healthy may differ in attributes between semi-arid Colorado and rain-fed Illinois and soil health requirements for growing corn may differ from soils where C storage is the primary goal. Due to this context-based knowledge, soil health should be evaluated through predicted outcomes reflective of land use, soil stewardship and management practices (Karlen, et al., 2019).

Historically, soils have been managed with the primary goal of meeting food demand through continuous crop production (Larsen, 2006). Conventional management approaches include the application of synthetic agrochemicals, the tilling of soils prior to planting and often the removal of crop residues post-harvest (Jian et al., 2020). These practices, together with crop genetic improvements, have all led to increasing crop yields and economic returns over several decades. Despite these gains, these practices also led to unintended consequences for soils and the environment. Soil health decline, erosion, groundwater pollution and the loss of vital biodiversity

needed for multiple soil functions are some of the challenges arising from conventional management (Montgomery, 2007). Recent estimates suggest a global economic loss of up to $8 billion per year due to soil erosion (Sartori et al., 2019). These unforeseen outcomes have therefore led to a new wave of scientific and policy interests in alternative but sustainable management approaches that can promote and maintain soil health.

One such approach is the innovative application of organic inputs to soils (Lavelle et al., 2001; Wu et al., 2017). This approach can be re-imagined as 'feeding' the soil to build soil health and is akin to feeding humans to maintain health and socio-economic productivity. The concept of feeding soils in a similar fashion to feeding people is a powerful one considering that healthy soils are conceptualized as functional ecosystems teeming with life. Just as low-quality foods can lead to poor health, stunted growth, sickness and even death in humans, conventional inputs to soils including synthetic agrochemicals can lead to unintended consequences for soils as described earlier.

Soil health outcomes can be maintained by feeding the soils with abundant and high-quality inputs, which in turn support a diverse soil microbiome and active food web. This is reflected in meta-analyses that have described soil health benefits including increased water holding capacity, greater soil organic matter (SOM) and higher biodiversity derived from the application of composts, manures, crop residues and other organic amendments (Diacono and Montemurro, 2011). Further exploration of alternative sources of high-quality organic inputs is needed to refine context-dependent best practices, as soil health properties vary across spatial and temporal scales. It is also important that we take a cursory look at soil health properties that are often interlinked and can respond differently to organic inputs at different scales.

Thus, this dissertation seeks to unravel soil health outcomes under different organic input management ranging from a novel soil amendment derived from cheese manufacturing to improved corn residue management in semi-arid agroecosystems. The location of my study area is near Akron, Colorado, and serves as a model for semi-arid agroecosystems with soil water content being a major limiting factor to soil and crop productivity. Semi-arid areas such as the High Plains of eastern Colorado have been predicted to get drier as the climate intensifies and water sources become scarcer thereby affecting irrigated cropping systems. Hence, the first chapter of this dissertation is aimed at studying at field scale how soil-water retention and other soil health properties including soil C and microbial biomass are impacted under a novel soil amendment approach. This approach involves the one-time, field application of an organic by-product of cheese manufacturing known as lactobionate.

Lactobionate is a low molecular weight sugar acid derivative of whey that is stabilized by cations. Whey is produced in large quantities during cheese manufacturing and the disposal of this waste represents a significant challenge for the dairy industry. In a previous laboratory experiment, different forms of lactobionate (potassium lactobionate, calcium lactobionate and ammonium lactobionate) were applied to soils with contrasting C contents obtained from Colorado and California (Kallenbach et al., 2019).

Lactobionate-amended soils regardless of initial C content and origin retained 37% more soil water and had 70 times greater microbial biomass than unamended soils within 2 months of the experiment (Kallenbach et al., 2019). Of the three forms of lactobionate, potassium lactobionate was the most effective, increasing soil water content between 100-600% (Kallenbach et al., 2019). While lactobionate showed great potential as a soil amendment in our laboratory experiment, it might not exhibit the same effect under field conditions. High variability in the field with respect

to climate and other associated parameters, coupled with the unknown potential interactions with plants and other uncontrollable factors are reasons why lactobionate and other soil amendments may not show the same success in the field as compared to controlled laboratory observations. Hence, the objectives of this chapter are: (1) to validate laboratory observations at the field-level in two different cropping system, and (2) to assess lactobionate impacts on soil water retention, crop nutrient availability, soil C and crop production across different application levels to achieve the optimal impacts of lactobionate.

At a fundamental level, the second chapter of the dissertation also builds upon the previous laboratory findings on lactobionate to understand how lactobionate affects SOM dynamics. SOM is a key soil health property that provides essential ecosystem services including nutrient cycling and retention, biodiversity proliferation, and erosion control (Wall, 2012). In semi-arid systems such as my study area, building SOM is vital but highly challenging due to low C inputs and high erosive nature of soils in the area. As the climate intensifies, low levels of SOM increases the potential for soil and crop failure particularly under water-limited systems (Ko et al., 2012). More importantly, C is stored and lost from SOM fractions including the particulate organic matter (POM) fraction, the dissolved or water-extractable organic matter (DOM/WEOM) fraction and the mineral-associated organic matter (MAOM) fraction. These fractions differ in their C protection and storage mechanisms and the extent to which soil management, using labile inputs such as lactobionate, affects these fractions remains unresolved. The objective of this chapter is to study the persistence of lactobionate in soils and to examine C dynamics and changes within SOM fractions over an 84-day incubation experiment by using isotopically enriched ^{13}C-lactobionate.

The third chapter of this dissertation is focused on the impact of residue management and tillage on soil biological health properties with a special focus on linkages between biological and

physicochemical soil properties. Consistently feeding soils with crop residues to boost soil health represents a departure from the conventional approach of removing crop residues from the field for feed or bioenergy purposes, exposing soils to wind and water erosion as well as nutrient depletion. When combined with conventional tillage, the removal of crop residues after harvest can have mid-to-long-term deleterious effects on soil biodiversity and other soil properties particularly in semi-arid agroecosystems such as my study location. For instance, the machinery used during conventional tillage can cause direct mortality to earthworms – organisms considered as 'ecosystem engineers' that directly and indirectly influence soil health.

Alternative practices such as residue retention and no-till have gained considerable momentum in recent times especially due to the observed benefits on soil health including the accumulation of SOM, increased water infiltration and retention and improved soil aggregation, mostly restricted to the soil surface zone (West and Post 2002; Page et al., 2020). However, we still have a limited understanding of how these practices influence whole soil biological communities simultaneously. Furthermore, it remains unclear to what extent management-induced shifts in soil biological communities and physicochemical properties are linked across spatial scales. Based on these knowledge gaps, this chapter's objective is to distinguish the specific effects of residue management (residue retention vs. residue removal) combined with tillage (conventional vs. no-till) on soil macrofauna and microbial communities alongside a suite of other soil physical and chemical properties 6 years after establishing the management practices.

In three main chapters, this dissertation seeks to answer the following questions:

1. What are the soil health benefits derived from the one-time, field application of lactobionate?

2. What is the fate and persistence of lactobionate in soils and how are SOM fractions responding to lactobionate application with respect to C loss and stabilization?
3. Are there any linkages between soil biological and physicochemical health properties derived from the consistent field retention of corn residues and are these differences magnified when residues are left on the soil surface (no-till) versus incorporated into soils (conventional tillage)?

To resolve these questions, a combination of field experiments (mainly at the USDA Central Great Plains Region, Akron, CO) and laboratory experiments are employed that are discussed in details in the next chapters. A conclusion chapter is also presented to summarize key findings and the contributions to knowledge that this work has made.

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CHAPTER 2: FROM FACTORY TO FIELD: EFFECTS OF A NOVEL SOIL AMENDMENT DERIVED FROM CHEESE PRODUCTION ON WHEAT AND CORN PRODUCTION[1]

Introduction

Productive soils are the foundation of a sustainable and secure global food supply. Human activity has degraded nearly 40% of the world's soils (Oldeman, 1994) through intensive tilling, erosion, mining and industrial activities, and excessive chemical inputs. This has led to a decline in many indicators of soil health, including nitrogen (N) retention and use efficiency, carbon (C) sequestration, and water infiltration and retention (Gugino et al., 2009). Water limitations and nutrient supply remain the major limiting factors to crop productivity globally (Tillman et al., 2002). As a result, modern day agriculture often depends significantly on the continuous use of freshwater irrigation and synthetic agrochemicals for optimal crop production. Such reliance on high water and chemical inputs contributes to the depletion of limited water resources—particularly in arid and semi-arid locations (Morison et al., 2007), eutrophication, and ground water pollution (Cassman, 1999).

To feed a growing global human population and achieve optimal crop productivity in the face of increasing climate variability, we need to rapidly regenerate soil health in a sustainable manner by targeting the key indicators of soil functional capacity relevant to the challenges of a particular cropping system.

[1]Olayemi, O. P., Kallenbach, C. M., Schneekloth, J. P., Calderón, F. J., Vigil, M. F., & Wallenstein, M. D. (2020). From Factory to Field: Effects of a Novel Soil Amendment Derived From Cheese Production on Wheat and Corn Production. *Frontiers in Sustainable Food Systems*, *3*, 127.

While there is no universal definition for soil health, it broadly acknowledges the functional capacity of soils to sustain plant productivity, maintain water and air quality, and support human well-being and other essential ecosystem services (Doran and Parkin, 1994; Doran, 2002; Kibblewhite et al., 2008).

Healthy soils have also been described as active living entities (Kibblewhite et al., 2008; Lal, 2016) and thus imply that biological presence and activity are key to soil functions. Management approaches that can support soil water retention, increase soil C stocks, sustain microbial activity, and improve the timing of nutrient supply are especially needed in regions such as the U.S. Great Plains where water limitation, and subsequently soil C and N, are often the pivotal attributes impacting soil functioning and crop productivity (Ko et al., 2012; Robertson et al., 2017). These soil properties are related to each other and connected to the overall functional capacity of soils. For instance, soil water retention has been linked with soil C (Lal, 2014) while recent reports have shown that soil microbial biomass and their byproducts form a significant source of stable soil C (Kallenbach et al., 2016). One common approach for both increasing soil water retention and soil C and N is through the application of organic amendments to soils. For example, in a global meta-analysis carried out by Eden et al., (2017), the addition of organic wastes to soils improved plant available water on a long term basis and also conferred benefits on other soil properties.

Most studies on the recycling of organic wastes in agriculture have focused on composts and of manure from livestock production (Peterson et al., 2007; Hargreaves et al., 2008; Vasilica et al., 2009; Annabi et al., 2011). The application of these organic wastes to soils have been shown to improve crop yield (Luo et al., 2018), enhance microbial biomass and activity (Kallenbach and Grandy, 2011), support soil fertility (Chaparro et al., 2012), and sustain long term soil health long term (Xie et al., 2014). While it is well documented that compost and other organic inputs confer

positive effects on soils, there are many barriers to widespread implementation. Variation in composition and physicochemical properties of different soil amendments has been shown to modulate their effects on soils, thereby generating uncertainty in their efficacy and sustainability (Fereidooni et al., 2013; Malik et al., 2013, Ninh et al., 2015). The economic, labor and public health costs (including pathogen transmission and unpleasant odors) associated with compost and manure-based organic amendments also limits their accessibility to growers. Thus, there is an urgent need to develop alternative, sustainable approaches that can rapidly regenerate soil functional capacity, including water storage and nutrient retention, by considering other sources of single stream wastes such as food processing products.

A promising approach to enhance soil services and crop productivity is by the conversion of food byproducts and waste into soil amendments. In developed nations such as the United States, roughly 40% (52 million tons) of the food produced annually is not eaten, with most of the waste disposed in landfills (ReFED, 2016; Gunders and Bloom, 2017). As a result, $218 billion worth of labor and resources invested in agricultural production is wasted annually (ReFED, 2016). For developing countries, post-harvest food losses total $4 billion per year, contributing to chronic poverty and hunger (FAO, 2013). With 868 million malnourished people facing starvation daily, this wasted food is a missed opportunity (Bond et al., 2013). Food wastes occur at every level of the food supply chain, from field to fork. Food waste is not only an economic, but an environmental and moral issue as well. Foods diverted to landfills contribute directly to climate change via the emission of methane (CH_4), a significant greenhouse gas 25 times more potent than carbon dioxide (CO_2) (USEPA, 2019). Significant attention has been focused on food waste management at the retail and post-consumer level, reflecting the USEPA's food recovery hierarchy strategy (USEPA, 2016). However, industrial food processing and manufacturing byproducts represent 14% of total

food waste generated (CEC, 2017). Food waste and food production byproducts diverted from landfills and incinerators to agricultural fields can also provide a rich source of organic nutrients and C that may facilitate microbial-mediated nutrient and C cycling, a foundation to biological agroecosystem management (Drinkwater et al., 2017). These food processing/manufacturing byproducts present a meaningful opportunity to put waste to work if they can be converted to useful organic soil amendments.

The production of mozzarella cheese produces large amounts of whey byproduct. For every kilogram of cheese produced, 9 kg of whey is generated (Robbins et al., 1996; Prazeres et al., 2012). In 2013, global whey production was estimated at 180 million tonnes (Dairy Processing Handbook, 2014). Whey primarily consists of water (93 - 94%), lactose (4.5 – 6 %), proteins (0.6 - 1.1 %), minerals (0.8 - 1.1 %) and fats (0.06 %) (Guimarães et al., 2010; Prazeres et al., 2012; Carvalho et al., 2013). Lactose constitutes up to 90 % of whey organic load content and contributes to whey's high biodegradability index, biological oxygen demand and chemical oxygen demand that burdens municipal sewage treatment systems (Berruga, et al., 1997; Janczukowicz et al., 2008; Yadav et al., 2015). As a result, direct disposal of this waste without pre-treatment has been widely banned (Yadav et al., 2015). Whey and its lactose derivatives are highly underutilized (Affertsholt, 2007). Thus, there is a need to find alternative applications for lactose (Gänzle et al., 2008; Alonso et al., 2013).

A derivative of lactose called lactobionate contains unique properties that we previously showed improved soil water retention, soil microbial biomass, and soil C content (Kallenbach et al., 2019). Lactobionate is produced from the enzymatic oxidation of lactose and consists of gluconic acid, galactose and metal ions (Potassium, Calcium). Antioxidant, chelating and emulsifying properties of lactobionate have made it useful in the pharmaceutical, cosmetic and infant formula industries

(Green et al., 2009; Wu et al., 2009; Gutiérrez et al., 2012). In a prior laboratory trial, we applied different forms of lactobionate (potassium lactobionate, calcium lactobionate, ammonium lactobionate) to soils of contrasting C contents from semi-arid locations in the USA (Colorado and California) (Kallenbach et al., 2019). All forms of lactobionate increased soil water retention as well as soil C and microbial biomass compared to the control. Potassium lactobionate showed the greatest and most dramatic effects on all of the soil properties measured. Soils amended with potassium lactobionate improved their water holding capacity by 100 - 600 % compared to unamended soils. Lactobionate amended soils also exhibited a persistent increase (87 %) in soil organic C two months after the amendment was applied (Kallenbach et al., 2019).

While lactobionate showed great potential as a soil amendment in our laboratory experiment, it might not exhibit the same effect under field conditions. High variability in the field with respect to climate and other associated parameters, coupled with the unknown potential interactions with plants and other uncontrollable factors are reasons why lactobionate and other soil amendments may not show the same success in the field as compared to controlled laboratory observations. Hence, we conducted agronomic trials on winter wheat and corn to assess the impact of potassium lactobionate on soil properties relevant to agronomic challenges in dryland agriculture. We focused on soil water retention since in dryland agriculture, increasing soil moisture is likely to have one of the most significant impacts on crop yields (Ko et al., 2012; Kallenbach et al., 2019). We also examined changes in soil C, mineral nitrogen, and microbial biomass, typically at low concentrations in this region and thus further limiting both water retention and nutrient supply. The objectives of the agronomic trials were (1) to validate laboratory observations at the field-level in two different cropping system, and (2) to assess lactobionate impacts on soil water

retention, crop nutrient availability, and soil C across different application levels to achieve the optimal impacts of lactobionate.

Materials and methods

Field layout and soil sample collection

A field experiment was conducted at the USDA-ARS Central Great Plains Research Station located in Akron, CO (40.15 °N, 103.15 °W, 4540 feet elevation). The climate is semiarid, with an annual rainfall of 420.624 mm (average normal from 1981 to 2010) (usclimatedata.com, accessed 2018). The soil type is classified as a silty loam Weld series (Calderon et al., 2015) with a total C of 1.0 %, total nitrogen (N) of 0.1 % and pH of 5.7. Soils used for this trial were under high crop residue retention with no tillage management for both wheat and corn trials respectively.

Potassium lactobionate (referred to as lactobionate for the remainder of this paper) was used in this study and supplied in liquid formulation by Leprino Foods Company (Denver, CO). This formulation had a 65 % moisture and 35 % solids (lactobionate) content (Fig. 2.1). Lactobionate consists of 36.6 % C, 4.8 % potassium (K), pH of 6.7, and has no other nutrients. A complete randomized block design was used in this study, consisting of 5 levels of lactobionate treatments replicated 5 times for the wheat trial (0, 159, 318, 655 and 1871 liters/hectare) and corn trial (0, 187, 374, 561, and 748 liters/hectare). Plots were 15.34 m long by 4.57 m wide for winter wheat and 24.38 m by 6.09 m wide for corn. The application rates were selected based on the economic feasibility of lactobionate supply from the manufacturer. The lactobionate treatments were applied using two approaches: for the wheat trial, it was directly applied to the soil surface as liquid formulation via spraying with the aid of a tractor mounted sprayer; and for the corn trial, it was banded into the soil near the zone of planting to a depth of 10 cm. The different rates and routes

of application were selected to examine their differential influence on soil properties and crop productivity with no intentions on comparing routes of application but to examine the most economically feasible and efficient application rates. In September of 2017, winter wheat (*Triticum aestivum*) variety Snowmass 2.0 was planted across the plots three days after lactobionate applications. In May, 2018, a separate field trial was set up by banding lactobionate together with corn seeds (*Zea Mays*) variety DeKalb 45-65 RIB. Since 1994, all plots have been managed using no-till dryland practices, common to the region for enhancing soil moisture retention. Soils were fertilized at typical rates for this region with urea ammonium nitrate (UAN) at a rate of 67.25 kg N/hectare and with diammonium phosphate at 22.41 kg P/hectare.

Prior to lactobionate application, soils were collected from each plot at two depths (0-5 cm, 5-15 cm) with a 2.5 cm-diameter hand-held soil corer. For the winter wheat trial, 5 soil cores were collected randomly in each plot and composited. For the corn trial, 5 soil cores were collected randomly between lactobionate-banded corn rows and composited. Soils were placed in ziplock bags on ice and transported at field-moisture level. The same procedure was completed 4 weeks after lactobionate application for the corn trial and at 5 weeks for the wheat trial due to unusually intense rainfall episodes immediately after lactobionate application. Soil samples were stored in a 4 ºC refrigerator upon arrival. The samples were then sieved to 2 mm and all analyses were conducted within a week of sample collection.

Soil Analyses

Soil moisture and pH

Gravimetric soil moisture was estimated on a 10 g subsample by drying in a 105 °C oven for 24 h, and the difference between the soil weight pre-drying and post drying was used for soil dry weight

correction. We also collected wheat volumetric moisture data directly from each plot using a soil moisture sensor probe (integrated across 12-cm depth) (Stevens Hydraprobe, Portland OR). Five volumetric moisture data points were collected per plot on the 21st of October and 22nd of November 2017 for the winter wheat trial. For the corn trial, volumetric moisture data was collected on the 11th of May and 8th of June, 2019. Soil pH was determined in 1:5 soil:water mixture using an Orion EA 9110 m (Thermo Scientific, Beverly, MA, USA). There was no treatment effect on soil pH across soil depths for the two field trials conducted (Table 1 and 2).

Soil C and N

We determined total soil C and N on oven-dry, pulverized soil samples analyzed on a LECO True-Spec CN analyzer (Leco Corp., St. Joseph, MI, USA). The output was estimated as a percentage. Soil nitrate (NO_3^-)-N and ammonium (NH_4^+)-N was determined by extraction using 25 ml of 2 M KCl, filtered using a Whatmann Filter paper no. 42 and estimated on the Alpkem Flow Solution IV Automated wet chemistry system (O.I. Analytical, College Station TX, USA).

Microbial Biomass C and N

To determine microbial biomass C and N, the chloroform fumigation extraction method (as described by Vance et al., (1987) was conducted on 10 g of soils stored in the 4 ºC refrigerator. Two ml of alcohol-free chloroform was added to a subset of soils and then extracted using 40 ml of 0.5 M K_2SO_4. Total organic C and total N was then measured with a TOC-V-TN analyzer (Shimadzu Corp., Kyoto, Japan). We calculated microbial biomass C and N by subtracting the unfumigated extracts from the fumigated extracts, using a conversion efficiency factor (k) of 0.45.

Wheat and corn yield and protein content

Wheat and corn yields were measured in July and November 2018 respectively. Both wheat and corn were harvested with a field-plot combine harvester with one pass through the center of each plot and yield quantified by plot. To determine grain protein content, N was first estimated using the same method as in soil total C and N on wheat and corn grain sub samples collected in each plot at harvest. Protein content was calculated from total grain N using a factor of 5.68 for corn (Sriperm et al., 2011) and 5.7 for wheat (AOAC, 1984)

Statistical Analyses

To examine the effects of lactobionate application on soil moisture, soil mineral N, soil microbial biomass, wheat and corn yield, we fitted a general linear model with treatment as the fixed effect, and ran a one-way analysis of variance (ANOVA), followed by pairwise comparisons of control to each treatment using the Dunnett's test in the estimated marginal means (emmeans) package in R. The level of significance (p) was set at 0.1 due to the minimal number of replicates and also the variability of field trials. The datasets were evaluated for outliers, normality and equal variance assumptions using the diagnostics function in R. Identified outliers were removed from the dataset and not used in the final analyses. All figures were created using the ggplot2 package in R (Wickham, 2009). All analyses were conducted in R version 3.5 (R Development Core Team, Vienna, Austria, 2017).

Results

Wheat trial

Soil Inorganic N

Four weeks after lactobionate was broadcasted for the winter wheat trial, we observed no significant difference in soil nitrate between the control treatment (0 L ha^{-1}) and the intermediate treatment (159 L ha^{-1}) at both soil depths (0-5 cm, 5-15 cm). However, we did observe a decreasing trend and significant reduction in soil nitrate concentration at higher lactobionate application rates at both soil depths examined ($p = 0.03$, Fig. 2.2, Fig. 2.3). Soil ammonium did not differ significantly between the treatments in the surface soil (0-5 cm), but in the deeper soil (5-15 cm), a significant increase ($p = 0.0371$) was observed in the 318 L ha^{-1} treatment as compared with the control (Appendix I: Fig. S1.1, Fig. S1.2). Surface soil had greater soil nitrate and ammonium concentration across all treatment as compared to the deeper soil.

Soil C and N

Total soil C content in the surface soil ranged from 1.06 - 1.2 % for the surface soil and from 0.67 - 0.8 % for the lower depth across all treatments (Table 2.1). There were no significant differences between control and treatment plots in total C content ($p = 0.22$) across soil depths. Total soil N in the surface soil ranged from 0.10 - 0.12 % and 0.07 - 0.08 % in the deeper soil across treatments. Similar to C, no significant differences in total N were observed between control and treatments across soil depth ($p = 0.15$). However, total soil C and N were higher in the surface soil as compared to deeper soil.

Soil moisture

Mean gravimetric moisture content ranged from 0.12 g g^{-1} - 0.15 g g^{-1} across treatments and depths while volumetric moisture content ranged from 10 - 27 % (Fig. 2.4, Fig. 2.5, Appendix I: Fig. S1.3). The only significant treatment effect in the surface soil was between control and the 655 L ha-1 treatment ($p = 0.027$). We also observed higher gravimetric moisture content for treatments 159, 318 and 655 L ha^{-1} compared to control at the deeper soil depth ($p = 0.036$). Volumetric moisture content was measured at 4 and 8 weeks after lactobionate application with the aid of a moisture sensor probe. Higher volumetric moisture content was found in the higher application treatments (318, 655 and 1871 L ha^{-1}) as compared with the control when measured in October 2017, but by November 2017, a drastic fall in moisture content occurred across all treatments and there were no clear differences at that point (Appendix I: Fig. S1.3, Fig. S1.4).

Soil microbial biomass C and N

No differences were observed in microbial biomass C and N among treatments in the surface soil ($p = 0.21$) (Table 2.1). The mean microbial biomass C and N in the surface soil ranged from 19-23 μg g^{-1} dry soil and 0.9-1.65 μg g^{-1} dry soil respectively and across treatments. In the deeper soil depth examined, we found higher microbial biomass C for the 318 L ha^{-1} and 1871 L ha^{-1} treatments ($p = 0.095$, $p = 0.06$) as compared with the control (Table 2.1), while there was also no observable difference in microbial biomass N. However, the surface soil had higher microbial biomass C and N as compared with the deeper soil.

Wheat yield and grain C and N content

Wheat yield ranged between 5224 – 5497 kg ha^{-1} across treatments (Fig. 2.6). There was no significant difference in wheat yield between control plots and lactobionate-amended plots. There was also no observable difference in grain protein content between control and treatment plots ($p > 0.1$) (Appendix I: Fig. S1.5).

Corn trial

Soil inorganic N

Lactobionate application did not alter soil nitrate relative to unamended soils for both surface and deeper soil ($p > 0.1$) (Table 2.3). Surface soil nitrate concentration across all treatments was higher than deeper soil. Average soil nitrate concentration ranged from 31 to 39 μg g^{-1} dry soil for surface soil and from 11 to 16 μg g^{-1} dry soil for deeper soil across all treatments. Average soil ammonium concentration in the surface soil ranged from 11 to 47 μg g^{-1} drysoil and from 3 to 9 μg g^{-1} dry soil for the lower depths across all treatments (Appendix I: Fig. S1.6, Fig. S1.7). Lactobionate applied at 374 L ha^{-1} significantly decreased surface soil ammonium ($p = 0.052$). There was no effect of lactobionate application on the deeper soil ammonium concentration.

Soil C and N

Lactobionate application did not influence soil C and N at either soil depth ($p > 0.1$). Topsoil C (0.67 - 0.75 %) was however higher than subsoil C (0.5 - 0.55 %) across all treatments and a similar trend observed for soil N (Table 2.2).

Soil moisture

Average gravimetric soil moisture ranged from 0.09 to 0.10 g g^{-1} in the surface soil and 0.13 to 0.15 g g^{-1} in the deeper soil across treatments (Table 2.3). No significant differences were observed in the gravimetric moisture content of lactobionate-amended soils and control soils at both soil depths sampled ($p > 0.1$). However, soil moisture tended to be slightly higher in the deeper soil with the 561 and 748 L ha^{-1} treatments than the control treatment. Likewise, no significant differences were observed in the volumetric moisture content of the treatment and control plots (data not shown).

Soil microbial biomass C and N

Average microbial biomass C at both soil depths sampled ranged from 73 to 125 μg g^{-1} dry soil, and there was no observable significant difference ($p > 0.1$) between treatments at both soil depths (Table 2.3). Microbial biomass N decreased in the 187 L ha^{-1} treatment ($p = 0.061$) as compared to control and also showed a decreasing trend, although this was not statistically significant in the surface soil. However, in the deeper soil, an increase in microbial biomass N was observed in the 561 L ha^{-1} treatment as compared to the control ($p = 0.081$).

Corn yield and grain C and N content

Corn yield ranged from 3565 to 4080 kg ha^{-1} across treatments (Fig. 2.7). Lactobionate application did not have a significant effect on corn yield ($p > 0.1$). However, a consistent increase in yield with increasing application rate was observed. Corn grain protein content did not increase or decrease with lactobionate application ($p > 0.1$) (Appendix I: Fig. S1.8).

Discussion

Agronomic practices

While it is common practice to apply fertilizers and soil amendments directly to soil by spraying or broadcasting, recent studies have shown that a more efficient way of amendment application is subsurface banding. Subsurface banding of fertilizers has been shown to reduce ammonia volatilization of fertilizers (Bouwmeester et al., 1985), increase crop yield (Stevens et al., 2007), increase plant nitrogen use efficiency (Malhi et al., 2001; Sommer et al., 2004) and reduce nutrient leaching and runoff (Lamba et al., 2013; Watts et al., 2011). In our field experiments, the application of lactobionate through two different modes (broadcasting and banding) on wheat and corn respectively was not done to compare and contrast the more efficient method but to assess the impact of lactobionate under different systems to better elucidate if these impacts can be broadly applied across systems or are unique to a particular system. The key effects seen with broadcast application in wheat were an increase in soil moisture at the deeper soil depth, an increase in microbial biomass and a decrease in soil nitrate after four weeks of application. However, substantial rainfall events immediately after lactobionate broadcast application may have resulted in much of the lactobionate to leach through the soil profile or lost through overland water flow. This, along with evidence for the benefits of banding amendments described above, led us to apply lactobionate through banding in the corn trial. We believed that concentrating the byproduct at the zone of planting will prevent it from being easily leached or washed off while also maximizing its effect on the key indicators of interest.

Overall, with banding in the corn trial, no major effects were seen on the soil properties we measured. This may be a result of the fact that soils were not collected at the exact zone of banding

(since this was where the corn was seeded) but between corn rows. Moreover, the differences in the lactobionate effects between the two trials may have been co-founded by season, as the corn lactobionate application was conducted in the summer as compared to the fall for the winter wheat trial. However, a key observation in the corn trial was the increasing trend in corn yield with lactobionate application rate. Other studies have also shown that subsurface banding can improve crop yield as shown in a study involving subsurface banding of poultry litter that increased the yield of cotton by 22 % (Tewolde et al., 2015) and the banding of N fertilizer that increased sugarbeet yield (Stevens et al., 2007). While this is not an attempt to compare the most efficient mode of application considering the differences in crop, planting season and year, we suspect that the different application modes contribute to the varying degree of influence of lactobionate we observed on soil and crop properties between corn and wheat.

Soil moisture

Given the chemical properties of lactobionate, we anticipated that it could be effective at increasing water retention in dryland cropping systems and thus be a potential amendment for mitigating plant drought stress. The potential for lactobionate to increase soil moisture could be controlled by four key mechanisms: cations (potassium, K^+) that can improve soil aggregation by binding to negatively charged soil particles; hydroxyl groups (OH) present in lactobionate which directly improve water absorption; lactobionate-mediated increases in soil organic C (SOC) leading to increased soil aggregation and water sorption; and lactobionate-stimulated increase in microbial biomass resulting in higher SOC retention and biogenic aggregation via microbial polymeric exudation (Kallenbach et al., 2019).

We examined the effect of lactobionate application on soil moisture using two metrics (gravimetric and volumetric) at two soil depths and observed different effects in the dryland wheat and corn trials. For the wheat field soils, lactobionate applied at even the lowest rate (159 L ha^{-1}) had a positive effect on gravimetric soil moisture in the subsoil while a high application rate (655 L ha^{-1}) led to increased soil moisture in the surface soils. No effects were observed at 1871 L ha^{-1} and this may be a result of overloading the soils with organic material to the point where it was clogging soil pores, limiting infiltration and hydraulic conductivity (Lehrsch and Robbins, 1994). The positive effect of lactobionate on wheat soil moisture in the subsoil might be the result of multiple rainfall episodes that occurred after its application in the wheat field trial, potentially transporting lactobionate deeper into the soil profile.

Adding materials rich in organic C such as sucrose and sawdust have also been found to increase soil moisture levels (Blumenthal et al., 2003, Averett et al., 2004). In a previous study, cheese whey applied directly to soils via furrow irrigation increased soil aggregate stability and infiltration, thus reducing erosion and enhancing water retention (Lehrsch et al., 2008). Furthermore, the volumetric moisture content of the wheat field soils was greatest under the highest lactobionate application rate as compared to the control for October 2017. However, by November, no differences were observed for volumetric moisture content of the treatments. For corn field soils, no clear differences in either gravimetric or volumetric soil moisture were seen at either soil depth. These differences in effects between the wheat field and corn field soils may be explained by the timing and mode of lactobionate application in each field, along with inherent differences in crop physiology and water uptake. Lactobionate was sprayed across the wheat field soils during the fall season while lactobionate was banded into the soil at the exact zone of planting for the corn field soils in the summer. As the soil samples were not collected from the exact

banding zone but adjacent to it (in between corn rows), this may be a contributing factor to the lack of differences between control and treatment rates in the corn trial. Also, the differences seen by month (October vs November) may imply that a one-time application may not be enough to see desired changes in soil moisture retention.

Soil nitrogen

In dryland systems, following water, the next most often limiting factor for crop productivity is plant available nutrients, especially N. The global use of chemical fertilizers addresses N deficiencies, but has also contributed to greenhouse gas emissions and eutrophication. The addition of high C organic materials including lactobionate to soils could potentially alter microbial-mediated N cycling, primarily by stimulating microbial immobilization and subsequent turnover. We measured the impact of lactobionate application on soil nitrate and ammonium, to understand potential effects on N mineralization and crop available N. While we observed a significant decline in soil nitrate at intermediate to high levels of lactobionate applied at both soil depths for wheat field soils, we did not see this trend in the corn field soils.

The decline in soil nitrate may be a result of the high level of bioavailable C present in lactobionate, which might have stimulated a temporal microbial immobilization of N. A similar trend has also been observed in field and laboratory studies of relatively high C organic materials (sucrose, wheat straw, vinasses and sawdust) on soil N dynamics (Blumenthal et al., 2003; Ghani et al., 2005; Baruah et al., 2013; Moran-Salazar et al., 2016; Averett et al., 2004). This effect could be either beneficial or deleterious to soils and crop productivity depending on timing and frequency of application. If microbes feeding on C-rich lactobionate assimilate soil N into their biomass, when they eventually turnover, N is released for plant uptake. Also, the slight increase in soil nitrate seen

in the 159 L ha^{-1} treatment may indicate that this rate was stimulating microbial activity just enough to increase N mineralization without inducing N limitations. However, at higher rates, microbial biomass may cross a threshold, moving from C to N limitations. Plant nitrate uptake could also be another reason for the decline in soil nitrate as plants are often better competitors for nitrate compared to microbes. Thus, the combination of plant mineral N uptake coupled with increased microbial biomass due to lactobionate application could have led to this temporal decline in soil nitrate. Lactobionate application did not significantly impact the level of soil ammonium in either the wheat or corn trials at both soil depths examined. This may be due to the higher concentration of nitrate supplied via fertilizer application. The application of C-rich organic materials including lactobionate to soils has previously been suggested to help reduce nitrate leaching and ammonia volatilization by sequestering soil mineral N in microbial biomass (Manevski et al., 2016; Cao et al., 2018).

Soil microbial biomass

Biological management of agricultural nutrient cycling can reduce nutrient losses and support microbial mineralization of organic N (Drinkwater et al., 2017). Soil organic amendments are a key tool to enhance soil nutrient cycling since they provide a readily available source of energy for the microbial community. Supporting high levels of microbial activity may also lead to SOM formation and soil aggregate stability which in turn can increase soil water retention (Murphy, 2015; Kallenbach et al., 2016). The challenge however in using soil amendments is balancing soil organic inputs with the C and N requirements of both the microbial community and crop needs.

In this field experiment, we determined how soil microbes respond to lactobionate application by measuring their biomass C and N. The increase we observed in microbial biomass C at the lower

soil depth in wheat parallels what we observed for soil moisture and is not unexpected, as lactobionate contains monosaccharides fueling microbial metabolism. Our results are consistent with other studies that have observed an increase in microbial biomass C and N in soils treated with dairy effluents (Degens et al., 2000; Sparling et al., 2001; Sarathchandra et al., 2006). This increase in microbial biomass could decrease soil organic C mineralization by selecting for microbes with greater ability to degrade lactose and its derivatives rather than SOM (Degens et al., 2000).

The observable changes in microbial biomass C within four weeks of amendment application is not surprising as this fraction is known to have a short turnover time and with high sensitivity to environmental changes and management relative to other soil C fractions (Joergensen and Emmerling, 2006; Kallenbach and Grandy, 2011). Additions of labile C materials including sucrose and lactobionate to soils have temporarily increased microbial biomass and activity (Török et al., 2000; Eschen et al., 2006; Kallenbach et al., 2019). In contrast, the addition of C sources such as wheat straw and sawdust containing structurally complex molecules requiring enzymatic degradation are likely to have less of an impact on elevating microbial biomass (Dalenberg and Jager, 1981; Magill and Aber, 2000). Thus, the impact of a soil amendment on the soil microbial biomass will depend primarily on how labile or recalcitrant its C source is. While a clear change in biomass C was observed in the winter wheat field experiment, there was no observable effect on the total soil C. This could be as a result of the lower biomass input rates relative to the total soil C fraction and changes may have occurred in only certain SOC fractions. For instance, increases in fractions with relatively faster turnover times, such as the particulate organic C and the mineral associated organic C fractions would not be detectable from our total SOC measurements.

Lactobionate impact on wheat and corn

Ultimately, for a soil amendment to be adopted, its effects on soil health must translate into increased crop yield and/or quality, or reduced input costs. Thus, we assessed the effect of lactobionate on yield and protein content. For the wheat trial, lactobionate had no significant effect on yield. Wheat and corn grain protein content was also not significantly affected by lactobionate application. While corn yield was not significantly affected by lactobionate, an increase in yield with increasing lactobionate application rate was observed (up to 14% at the highest application rate), suggesting a dose-dependent benefit on corn yield. The differences between treatment effects on corn and wheat grain yields could be a result of multiple environmental and physiological factors associated with the crops, along with the different application modes. The length (8 months) and growing season (winter, spring, summer) of winter wheat as compared to that of corn (5 months, summer, fall) could partly account for the difference in lactobionate effects on yields of the two crops. Winter wheat is likely to experience greater nutrient and water limitation as compared to corn, with just a single application of the amendment (Chen et al., 2016; Manevski et al., 2016). The timing of lactobionate application could have also played a role, as lactobionate applied during the summer (for the corn trial) could have stimulated greater microbial activity which in turn could affect N cycling dynamics as compared to fall application at lower temperatures.

The similarity in wheat and corn grain protein between lactobionate-amended plots and control could have been as a result of the one-time application of lactobionate at the early stage of crop growth, when effects on soil mineral N might have been short-lived. Despite the reduction in soil nitrate after 4 weeks of lactobionate application for the winter wheat trial, this did not result in any deleterious effect on the wheat grain yield and protein content. Furthermore, the application of

soil amendments such as compost, manure, straw, biochar and other materials rich in organic C has shown multiple inconsistencies on crop yields especially in the short term due to differences in crop, climate and location (Christian et al., 1999; Malhi and Lemke, 2007; Coulter and Nafziger, 2008).

Conclusion

We explored the potential benefits of lactobionate, a byproduct of cheese manufacturing, on soil properties and crop productivity in wheat and corn dryland systems. Our findings suggest that lactobionate has the potential to be an effective soil amendment based on the benefits we observed in our study, but it depended on the agronomic system under evaluation. Lactobionate caused a temporary increase in gravimetric soil water content and decrease in soil nitrate in the wheat trial but showed no effect in the corn trial. This suggests that timing, mode, and frequency of application needs to be further optimized for maximal soil benefits of lactobionate. The limited statistical power relative to plot variability in this trial constrained our ability to conclusively determine effects of lactobionate on crop yields. Like many soil amendments and management approaches, effects on soil properties may accumulate with repeated treatments and effects on crop yield or quality can take several years to manifest. This is a grand challenge for the adoption of soil amendments, as farmers often make purchasing decisions based on short-term returns on investment and discount long-term benefits. On the other hand, technologies like lactobionate also benefit society by diverting food waste from landfills to the farm, where they can potentially decrease the environmental impact of agriculture and move towards a regenerative, circular economy. Given the potential benefits to farmers and global sustainability, more long term-field trials encompassing different crops and field sites are required to conclusively determine the potential benefits of lactobionate as a soil amendment.

Table 2.1

Effect of lactobionate application rates on microbial biomass C and N, soil pH, soil C and N across soil depths (0-5, 5-15cm) for the wheat field trial. Data are means (n=5) with standard error in parenthesis.

Treatment ($L\ ha^{-1}$)	Soil depth (cm)	Nitrate-N ($\mu g\ g^{-1}$)	MB C ($\mu g\ g^{-1}$)	MB N ($\mu g\ g^{-1}$)	GMC ($g\ g^{-1}$)
0	0-5 cm	33.97 (4.91)a	101.27 (25.18)b	19.21 (2.84)c	0.10 (0.009)d
187	0-5 cm	33.36 (3.78)a	124.03 (27.22)b	7.76 (3.83)m	0.10 (0.008)d
374	0-5 cm	30.55 (8.25)a	55.01 (44.45)b	12.19 (2.75)c	0.09 (0.011)d
561	0-5 cm	35.19 (5.48)a	105.06 (24.64)b	11.36 (1.31)c	0.10 (0.010)d
748	0-5 cm	38.50 (0.63)a	130.74 (12.19)b	16.05 (1.09)c	0.10 (0.011)d
0	5-15 cm	13.83 (2.29)e	89.61 (14.23)f	7.18 (0.13)g	0.13 (0.009)h
187	5-15 cm	13.34 (1.41)e	100.55 (24.21)f	10.22 (1.71)g	0.14 (0.005)h
374	5-15 cm	11.44 (2.95)e	94.09 (18.19)f	7.05 (0.92)g	0.14 (0.007)h
561	5-15 cm	14.57 (2.60)e	73.61 (5.91)f	10.30 (0.71)g	0.15 (0.005)i
748	5-15 cm	16.19 (2.99)e	122.7 (53.33)f	10.45 (2.06)g	0.15 (0.002)h

Values represent means and standard error while letters represent pairwise comparison of each treatment and control as analyzed using the Dunnett's test in the emmeans package in R. Similar letters by column represents no significant difference ($p > 0.1$).

Table 2.2

Effect of lactobionate application rates on soil pH, soil C and N across soil depths (0-5, 5-15cm) for the corn field trial. Data are means (n=5) with standard error in parenthesis.

Treatment (L ha^{-1})	**Soil depth (cm)**	**pH**	**Soil C (%)**	**Soil N (%)**
0	0-5 cm	6.26 (0.09)a	0.72 (0.09)e	0.083 (0.014)d
187	0-5 cm	6.38 (0.10)a	0.67 (0.05)e	0.076 (0.006)d
374	0-5 cm	6.3 (0.07)a	0.71 (0.09)e	0.080 (0.009)d
561	0-5 cm	6.3 (0.12)a	0.74 (0.09)e	0.086 (0.012)d
748	0-5 cm	6.32 (0.10)a	0.75 (0.10)e	0.086 (0.001)d
0	5-15 cm	6.92 (0.14)c	0.50 (0.05)b	0.059 (0.006)f
187	5-15 cm	7.22 (0.31)c	0.51 (0.05)b	0.054 (0.007)f
374	5-15 cm	6.94 (0.06)c	0.51 (0.06)b	0.059 (0.007)f
561	5-15 cm	7.02 (0.25)c	0.53 (0.04)b	0.062 (0.006)f
748	5-15 cm	6.86 (0.08)c	0.55 (0.04)b	0.058 (0.011)f

Values represent means and standard error while letters represent pairwise comparison of each treatment and control as analyzed using the Dunnett's test in the emmeans package in R. Similar letters by column represents no significant difference ($p > 0.1$).

Table 2.3

Effect of lactobionate application rates on microbial biomass C and N, soil pH, soil C and N across soil depths (0-5, 5-15cm) for the corn field trial. Data are means (n=5) with standard error in parenthesis.

Treatment ($L\ ha^{-1}$)	Soil depth (cm)	Nitrate-N ($\mu g\ g^{-1}$)	MB C ($\mu g\ g^{-1}$)	MB N ($\mu g\ g^{-1}$)	GMC ($g\ g^{-1}$)
0	0-5 cm	33.97 (4.91)a	101.27 (25.18)b	19.21 (2.84)c	0.10 (0.009)d
187	0-5 cm	33.36 (3.78)a	124.03 (27.22)b	7.76 (3.83)m	0.10 (0.008)d
374	0-5 cm	30.55 (8.25)a	55.01 (44.45)b	12.19 (2.75)c	0.09 (0.011)d
561	0-5 cm	35.19 (5.48)a	105.06 (24.64)b	11.36 (1.31)c	0.10 (0.010)d
748	0-5 cm	38.50 (0.63)a	130.74 (12.19)b	16.05 (1.09)c	0.10 (0.011)d
0	5-15 cm	13.83 (2.29e	89.61 (14.23)f	7.18 (0.13)g	0.13 (0.009)h
187	5-15 cm	13.34 (1.41)e	100.55 (24.21)f	10.22 (1.71)g	0.14 (0.005)h
374	5-15 cm	11.44 (2.95)e	94.09 (18.19)f	7.05 (0.92)g	0.14 (0.007)h
561	5-15 cm	14.57 (2.60)e	73.61 (5.91)f	10.30 (0.71)g	0.15 (0.005)i
748	5-15 cm	16.19 (2.99)e	122.7 (53.33)f	10.45 (2.06)g	0.15 (0.002)h

Values represent means and standard error while letters represent pairwise comparison of each treatment and control as analyzed using the Dunnett's test in the emmeans package in R. Similar letters by column represents no significant difference ($p > 0.1$).

Figure 2.1. Production of lactobionate in a cheesefactory (Shutterstock.com/Giuseppe Parisi).

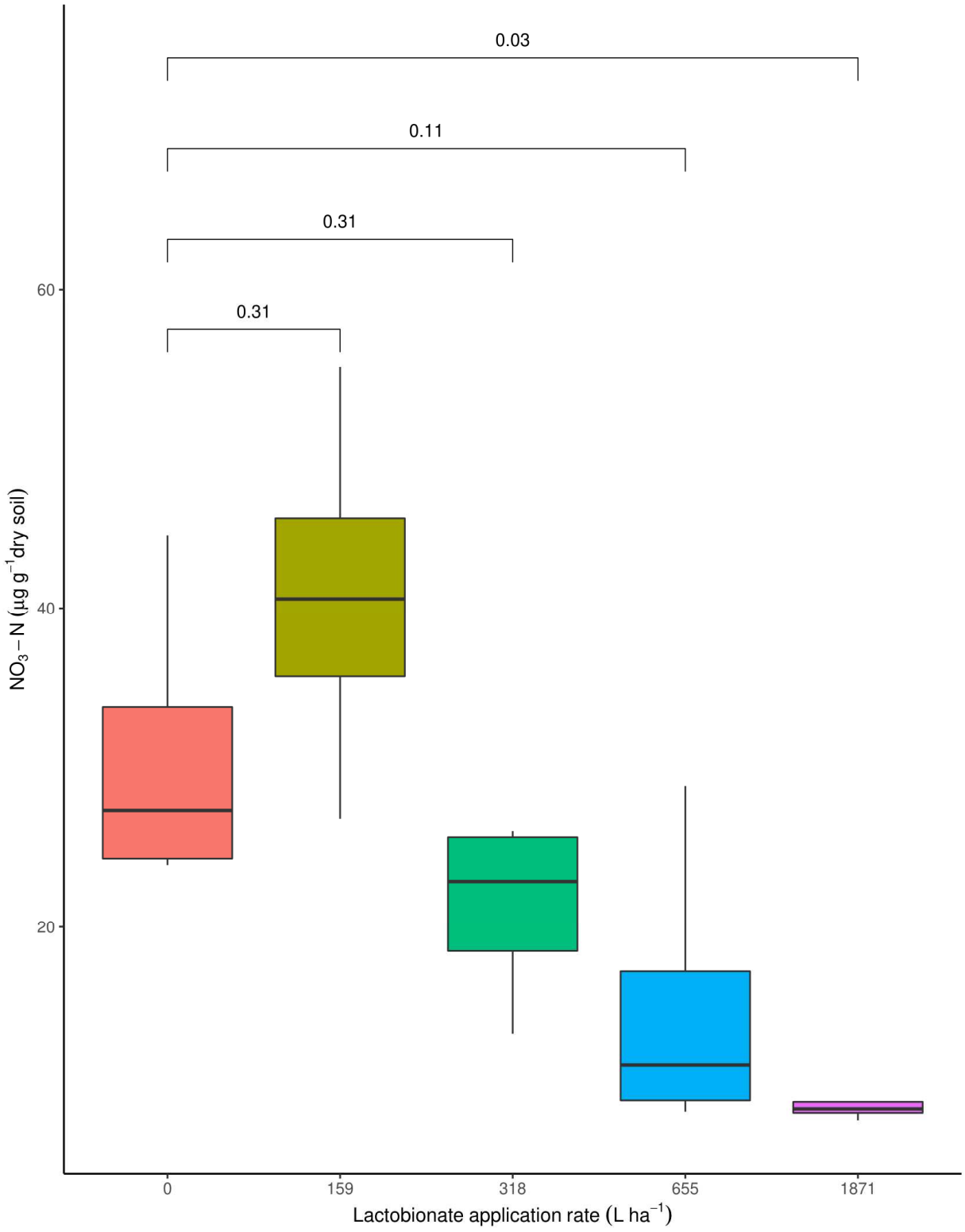

Figure 2.2. Effect of lactobionate application on soil nitrate-N for wheat trial (0-5 cm). Horizontal lines and p-values above each boxplot is the pairwise comparison of each treatment and control.

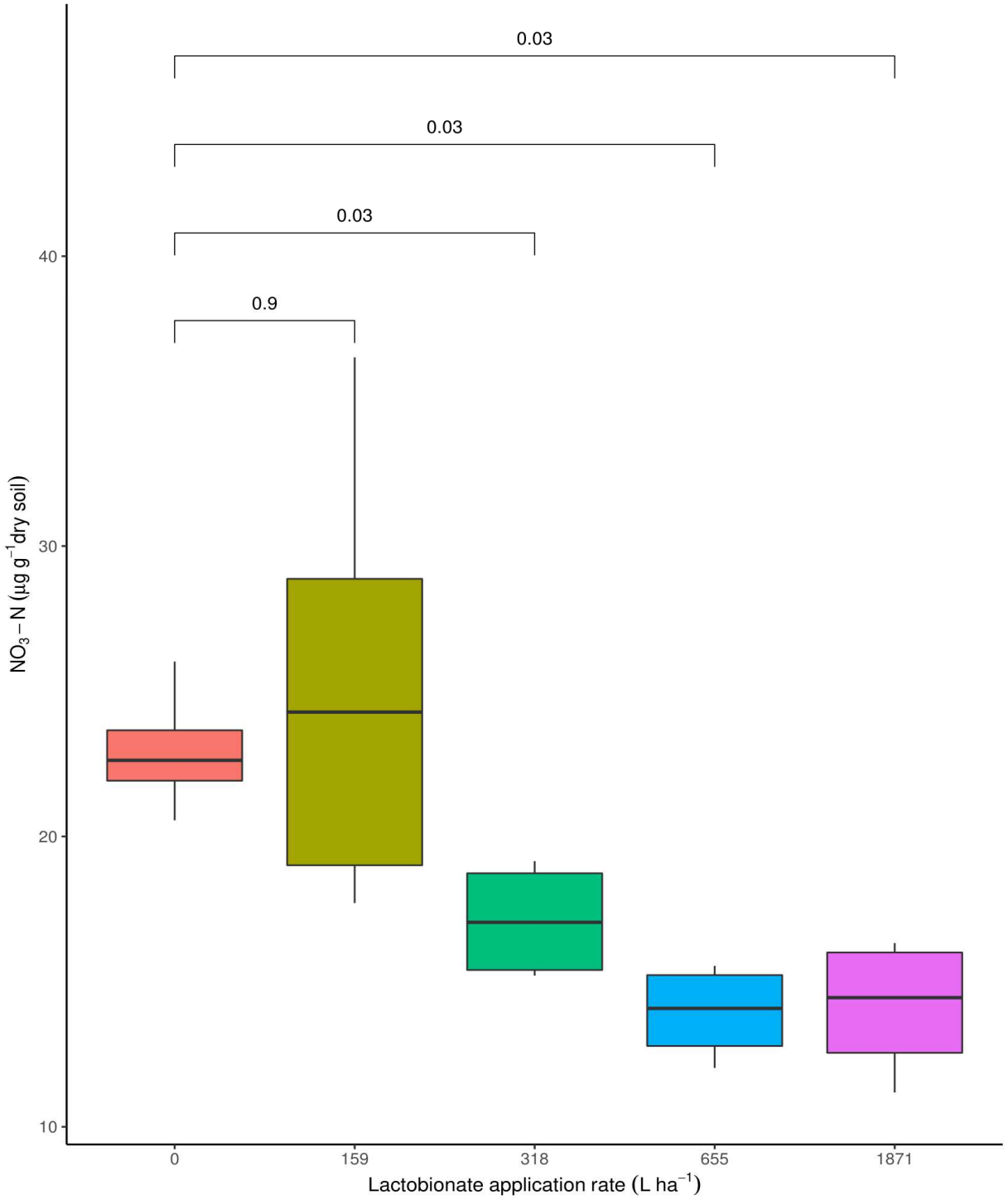

Figure 2.3. Effect of lactobionate application on soil nitrate-N for wheat trial (5-15 cm). Horizontal lines and p-values above each boxplot is the pairwise comparison of each treatment and control.

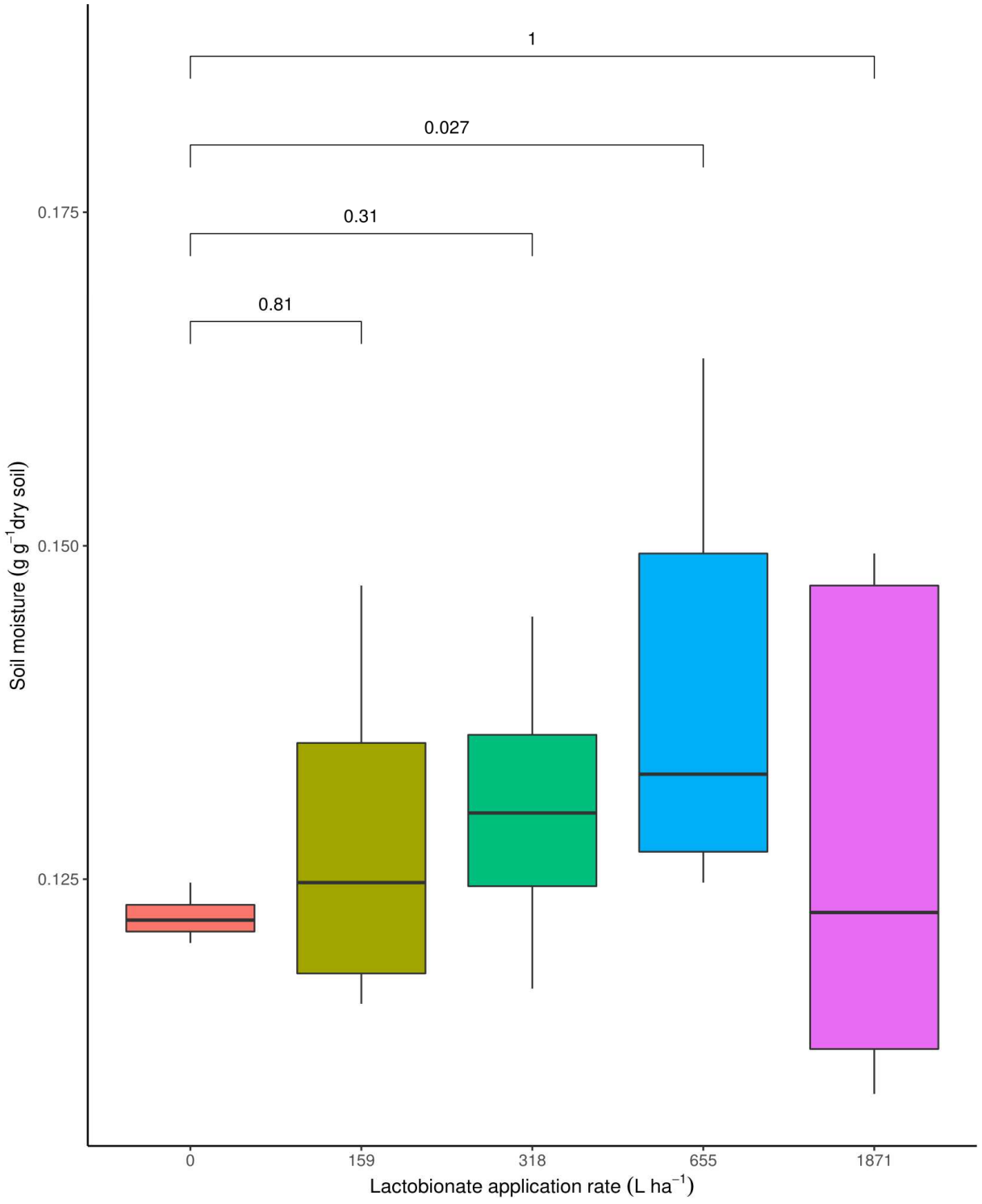

Figure 2.4. Effect of lactobionate application on gravimetric soil moisture for wheat trial (0-5 cm). Horizontal lines and p-values above each boxplot is the pairwise comparison of each treatment and control.

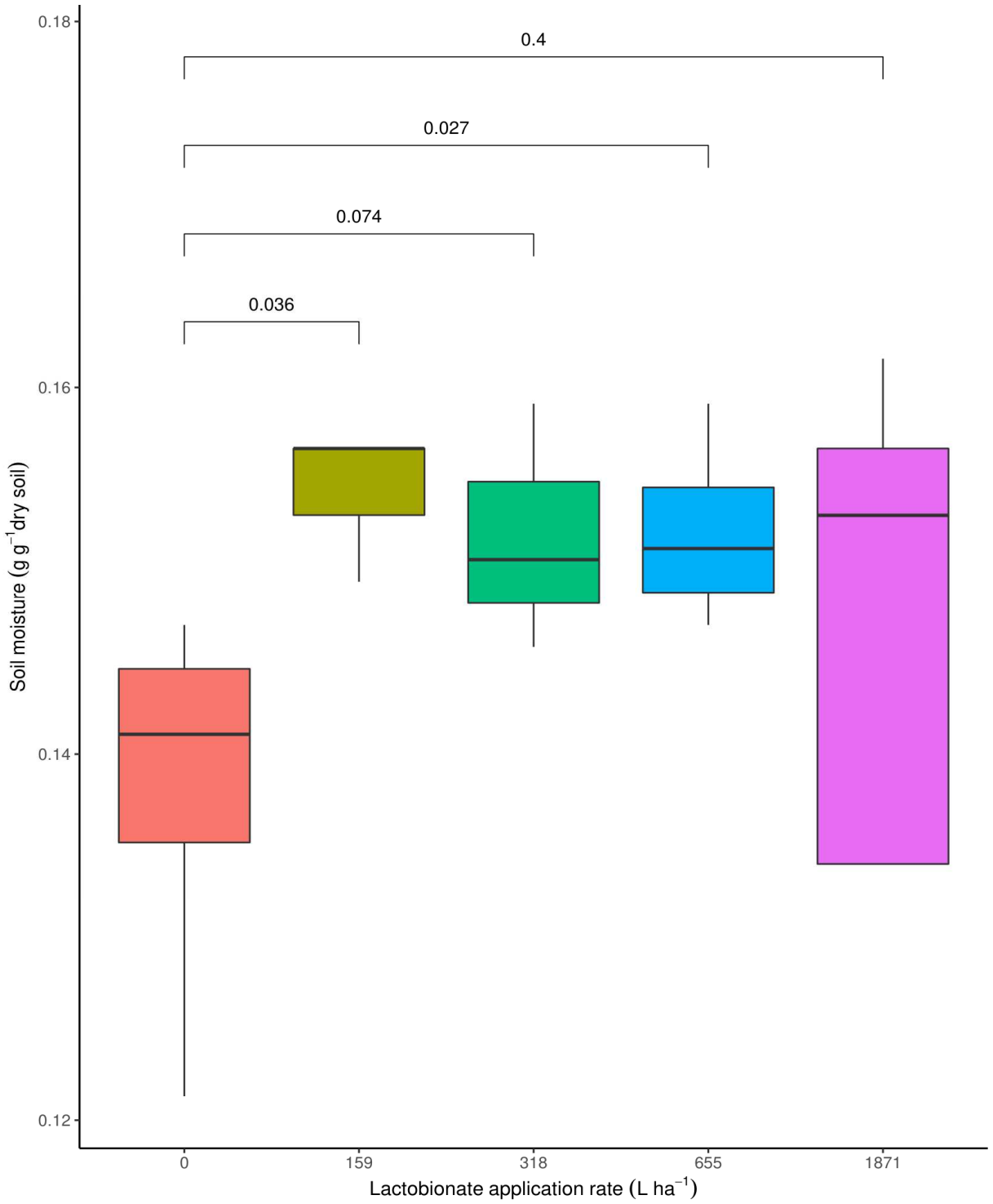

Figure 2.5. Effect of lactobionate application on gravimetric soil moisture for wheat trial (5-15 cm). Horizontal lines and p-values above each boxplot is the pairwise comparison of each treatment and control.

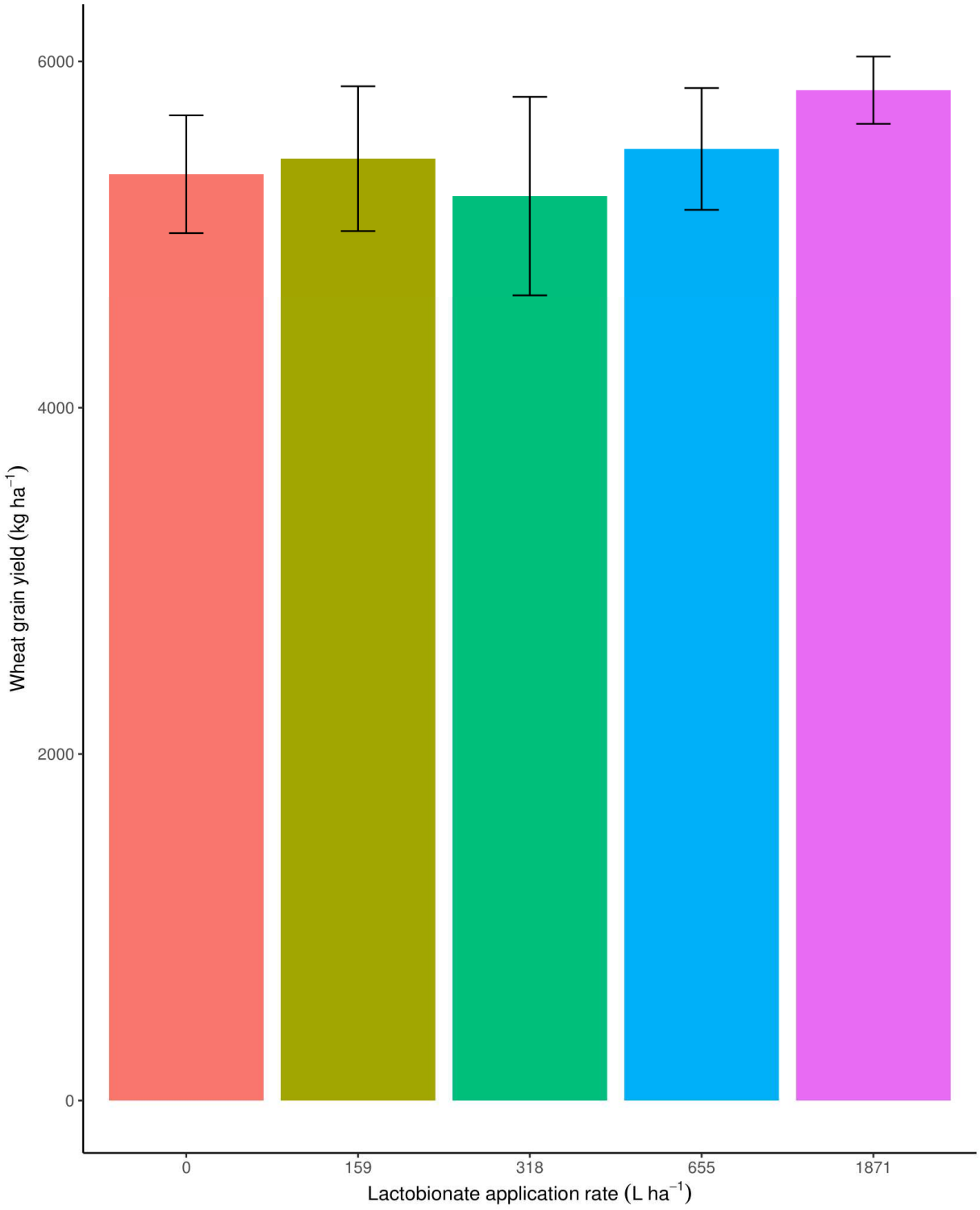

Figure 2.6. Effect of lactobionate application on wheat grain yield.

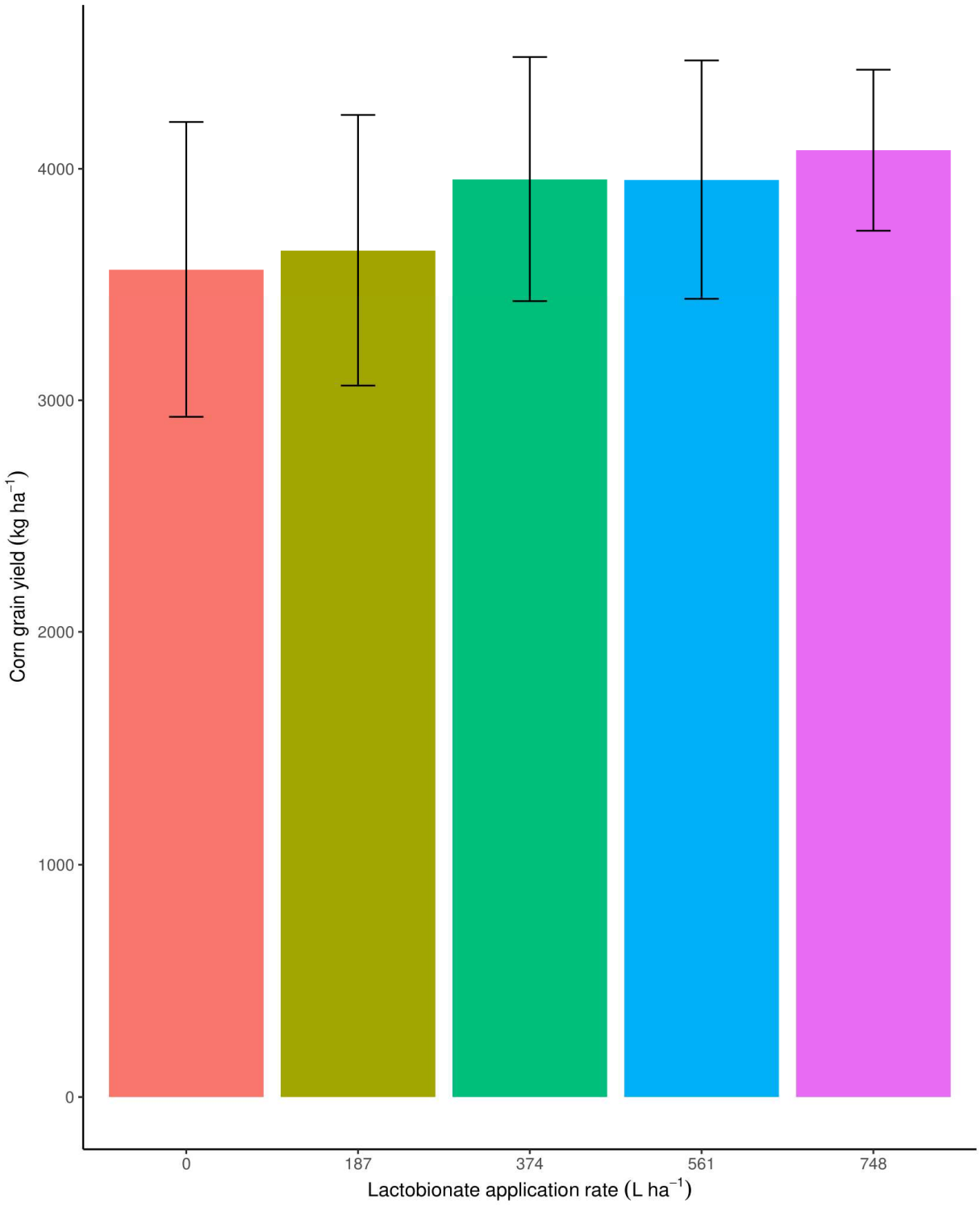

Figure 2.7. Effect of lactobionate application on corn grain yield.

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CHAPTER 3: DISTRIBUTION OF SOIL ORGANIC MATTER FRACTIONS ARE ALTERED WITH SOIL PRIMING.

Introduction

As the global climate crisis intensifies, managing soils to accumulate soil organic matter (SOM) is gaining widespread interest and investment as one potential climate and soil health solution. Increasing root biomass, especially living roots in managed soils is one approach that is receiving attention due to recent evidence that low-molecular weight bioavailable root compounds are more effective at building the C that contributes to long-term persistent SOM (Sokol et al., 2019; Villarino et al., 2021). At the same time, root exudates have been shown to stimulate SOM decomposition in a process known as priming (Kuzyakov et al., 2010; Dijkstra et al., 2013). As alternatives to more traditional organic amendments such as composts and crop residues, novel C-based soil amendments that exhibit properties similar to root exudates in terms of their solubility and low molecular weight are thus being considered (Olayemi et al., 2020). Central to understanding the net SOM balance and ultimately the impact on long-term soil C storage of such amendments, we need clarity surrounding how priming may influence distinct SOM fractions differently (Villarino et al., 2021). Not all SOM is functionally the same− some fractions of SOM may be relatively more important for aggregation, while other fractions may be more critical for supporting an active soil biological community. Still, much of our current understanding of SOM priming is limited to impacts on total soil C net balances. Unfortunately, this limits our understanding of which fractions of SOM are susceptible to loss and which fractions might instead be transformed within the soil, potentially altering the functional role of SOM.

The delineation of SOM into particulate organic matter (POM) and mineral-associated organic matter (MAOM) fractions has been a useful framework to improve our understanding of SOM and management recommendations for increasing soil C storage (Lavallee et al., 2020). Carbon associated with POM is likely to accumulate rapidly following crop harvest and residue inputs and respond to management changes, with a mean residence time on annual to decadal scales. This fraction consists of both the dominant light fraction, non-occluded POM containing partly decomposing plant materials and the heavy fraction− sand-sized POM containing more decomposed plant and microbial residues that retains C via physical protection from microbial access through occlusion in macroaggregates (Haddix et al., 2020; Mosier et al., 2021). The MAOM fraction stabilizes C onto clay and silt minerals via strong organo-mineral bonds that provide greater protection from microbial access and decomposition compared to POM, contributing to its relatively longer turnover time (Kogel-Knabner et al., 2008). Due to its slow turnover (on millennial scales), the MAOM fraction is well suited to more efficient long-term C storage as compared to POM, though some evidence suggests that MAOM can be desorbed, released back into solution under changing environment conditions, e.g anoxia, or following labile root inputs (Keiluweit et al., 2015). Understanding SOM dynamics through the lens of these fractions is vital to predicting management influences on long-term C accumulation.

As C is stored in these fractions, it can simultaneously be lost via mineralization and priming. When exogenous C amendments are added to soils, microbial activity is often stimulated via rapid microbial anabolism of the added material (De Nobili et al., 2001, Stenström et al., 2001). This subsequent increase in microbial biomass may result in two simultaneous, non-mutually exclusive consequence to SOM dynamics: 1) increased production of microbial compounds that are preferentially sorbed and retained in the MAOM fraction (Cotrufo et al., 2013), or 2) elevated soil

microbial biomass that enhances mineralization of existing SOM through priming (Kuyzakov, 2008, Bladgoskaya and Kuyzakov, 2010). While priming should decrease extant (or native) C stocks, the response may vary among SOM fractions. In some instances, net increases in soil C with priming have been observed (Blagodatskaya and Kuzyakov, 2011). It may be that these observed increases in soil C with priming occur due to the cascading and interacting effects of stimulated decomposition transforming the distribution of SOM from ephemeral to the more persistent fractions. Because POM is relatively unprotected, it is reasonable to expect that elevated microbial biomass will accelerate POM depolymerization, causing a priming of POM. In this process, POM becomes biologically altered, increasing its potential to be occluded, and thus protected, in aggregates (Lehmann and Kleber, 2015). Additionally, priming-induced POM depolymerization should increase the production of soluble C or dissolved organic matter (DOM) that can also be directly sorbed into the MAOM fraction or can be rapidly assimilated by microbes, which may enter the MAOM fraction following their biomass turnover. (Chantigny, 2003; van Hess et al., 2005; Kaiser and Kalbitz, 2012). As such, each of these fractions (POM, DOM, and MAOM) likely interact and influence one another to affect the outcome of C storage as described by soil continuum model (Lehmann and Kleber, 2015). Within this context, is it then possible that the potential priming of unprotected SOM fractions results in the formation of more persistent SOM fractions like MAOM?

To resolve this question, we conducted a laboratory incubation to examine SOM dynamics and interactions of different SOM fractions in response to soil amended with lactobionate. Lactobionate is a low molecular weight sugar acid derived from whey that is separated during cheese production. Large quantities of lactobionate are produced as a byproduct each year (180 million tons in 2013 alone), but current uses for this material fall short of the amount available,

leading to waste (Dairy Processing Handbook, 2014; Olayemi et al., 2020). Meanwhile, few options exist for increasing low molecular weight C-rich inputs to soils. In addition, few studies have examined the influence of low molecular weight organic amendments across different SOM fractions with respect to both C stabilization and priming. Our objective was to study the persistence and priming effects of lactobionate in soils by examining C changes within SOM fractions over an 84-day incubation experiment. We added isotopically enriched ^{13}C-lactobionate to soils from an agricultural field and used quantitative tracing to determine lactobionate contribution to soil CO_2 efflux and distinct SOM fractions. We hypothesized that due to the absence of C protection mechanisms, a lactobionate-induced priming effect will lead to C depletion in DOM and free-light POM fractions as compared to MAOM. We also predicted that lactobionate-amended soils would contain greater levels of C in their MAOM and interaggregate POM fractions as compared to unamended soils due to the potential lactobionate-induced activation of microbial activity and physical-chemical protection of C in these fractions via mineral sorption and occlusion in aggregates respectively.

Materials and Methods

We obtained field soils to a depth of 20 cm from the USDA-ARS Central Great Plains Research Station located in Akron, CO (40.15 °N, 103.15 °W, 4540 feet elevation). The climate is semiarid, with an average annual precipitation of 420.624 mm (usclimatedata.com, accessed 2020). The soil is classified as a silty loam mesic Aridic Argiustolls of the Weld series (Calderon et al., 2015) or Calcic Kastanozems (WRB, 2006) with an average total C of 1.0 %, total nitrogen (N) of 0.1 % and pH of 5.7. Soils used for this incubation experiment were collected from a wheat field during the active growing season under high crop residue retention with no tillage management since

1995. Soils were temporarily kept in ziplock bags on ice during collection and transport and then stored at field-moisture level in a 4 ºC refrigerator upon arrival at Colorado State University.

Incubation setup

To study the priming and C-stabilization effects of lactobionate in soils, we set up an 84-day laboratory experiment using a design that consisted of ^{13}C lactobionate-amended and unamended soils sampled destructively at four time points and replicated 5 times for a total of 40 incubation units. Additional 15 incubation units with no destructive sampling were used to capture soil CO_2 respiration dynamics and priming effects. These additional units consisted of ^{13}C lactobionate-amended soils, natural abundance ^{12}C lactobionate-amended soils, and unamended soils (control) replicated 5 times.

For all incubation units, soils were first sieved using a 2 mm-mesh sieve to homogenize samples and to remove large (> 2 mm) surface and belowground organic material. Thereafter, sieved soils were weighed into 55 specimen cups (66.3 g of dry soil per cup) and then placed in 1 L Mason jars and lids were fitted with Swagelok thread connectors (Swagelok, Denver, CO). The incubation units were then placed in a constant temperature room at 25 °C for 7 days to allow for stabilization of soil respiration. Uniformly labelled (1215 ‰) as well as natural abundance lactobionate (-25‰) was used in this study and supplied in liquid formulation by Leprino Foods Company (Denver, CO). This formulation had a 65 % moisture and 35 % solids (lactobionate) content. Lactobionate is a low molecular weight sugar acid (< 900 Daltons) that consists of 36.6 % C, 4.8 % potassium (K), pH of 6.7, and has no other nutrients.

We set up the lactobionate-amended incubation units by adding labeled and unlabeled liquid lactobionate at a rate of 0.00536 g C g^{-} dry soil (0.015 g g^{-} dry soil), increasing initial soil C

content by 0.54%. We chose this rate for two main reasons; to reflect potential field application rates of lactobionate and to ensure that the microbial community in our low C soils (1%) were not saturated with lactobionate C that can often cause a negative priming effect. Unamended control units received deionized water of the same quantity as the lactobionate treatments. Lactobionate was added to incubation units using a pipette and the soils were not mixed after lactobionate addition. All incubation units were kept in a dark constant temperature room at 25°C for 84 days. The incubation units were maintained at 60% water holding capacity for the duration of the incubation. To prevent CO_2 accumulation, the destructively sampled incubation units were unsealed every 3 days for 3 hours to allow for dissipation of the accumulated gas in the overhead space. Samples were destructively harvested at days 14, 28, 56 and 84 from the start of the incubation for further analyses discussed below.

Soil respiration and $\delta^{13}C$-CO_2

To capture soil CO_2 efflux and ^{13}C-CO_2 signature from both amended and control incubation units, 15 incubation units amended with ^{13}C lactobionate, ^{12}C lactobionate and deionized water (control) were tightly sealed and connected to a Picarro G2131-I Cavity Ring Down Spectrometer (CRDS; Santa Clara, California, USA). Prior to its use, the CRDS was calibrated according to the manufacturer's instruction. The CRDS was used to collect CO_2 concentration and the $\delta^{13}C$-CO_2 signature from the incubation jars within 10 minutes of connection. Soil respiration was measured every day for the first 15 days of the incubation (including two measurements on days 3 and 5 due to the rapid accumulation of CO_2 exceeding the 3% threshold) and every 2-3 days afterwards until the termination of the experiment on the 84th day. To obtain the CO_2 concentration at day 0, we measured headspace CO_2 concentration of each jar immediately after placing the specimen cups

in the Mason jars. After each CO_2 measurement, all 15 incubation units were flushed with reconstituted, moistened and decarbonated air from a tank.

Soil fractionation and C and nitrogen content

Prior to SOM fractionation, we attempted to measure microbial biomass in all incubation units by the chloroform fumigation extraction method but due to methodological issues, the data was considered unreliable and thus not included in our analysis. To determine changes in SOM fractions for each treatment over time, we employed a SOM fractionation scheme adapted from Haddix et al. (2020). By using a combination of size and density fractionation, four SOM fractions were sequentially obtained that include: water-extractable organic matter (WEOM) as a proxy for DOM, free-light POM (LF-POM), heavy POM (H-POM) and MAOM. We chose to separate POM into the light and heavy fraction because of their potential differences in their degree of decomposition, C:N, and aggregate protection (Christensen, 2001; Soong and Cotrufo, 2015). The fractionation process was carried out on 5.5-6.0 g of air-dried soil from each incubation unit and then oven-drying these samples overnight at 60°C.

WEOM was obtained by adding 35 ml of deionized water to the oven-dried soils that were then shaken for 15 minutes and centrifuged at 1069 gfc for 15 min with a subsequent decanting of the liquid supernatant as WEOM. The soil samples post-WEOM were re-suspended in 35 ml of sodium polytungstate (SPT) at a density of 1.85 g cm^{-3} and centrifuged at 1069 gfc for 30 min. The floating material (LF-POM) was then aspirated off and rinsed four times to remove any remaining SPT. Following LF-POM removal, the soil samples were dispersed by shaking for 18 h with glass beads and 0.5% sodium hexametaphosphate to break all aggregates (Haddix et al., 2020). This was followed by the rinsing of dispersed samples over a 53-μm sieve to separate the H-POM (> 53 μm) from MAOM (< 53 μm).

The WEOM extracts were freeze-dried and all other SOM fractions (LF-POM, HF-POM, MAOM) were dried at 60 °C prior to weighing and analysis of C, N, and $\delta^{13}C$ on an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS model: Optima; Micromass, Manchester, UK). The total fractions mass recovery was within ± 5% of the initial mass.

Statistical analyses

We determined the relative contribution of lactobionate-derived C to soil CO_2 efflux and SOM fractions (^{12}C) using the isotopic mixing model as shown in the equation below (Balesdent and Mariotti, 1996):

Equation 1:

$$f_{lactobionate} = \frac{(\delta t - \delta c)}{(\delta L - \delta c)}$$

where $f_{lactobionate}$ is the lactobionate-derived C contribution to SOM fraction and CO_2. The δ_t and δ_C are the $\delta^{13}C$ of the specific SOM fraction and CO_2 sample from the lactobionate (δ_t) and the control (δ_c) treatment, respectively. The δ_L is the $\delta^{13}C$ of the initial lactobionate used for the incubation experiment (1215‰).

The lactobionate-induced priming effect intensity was also computed as a percentage of the control cumulative soil CO_2 respiration by using the equation below modified from Zhang et al., (2017):

Equation 2:

$$\text{Priming intensity (\%)} = \left(\frac{F_{SOM} * Q_{treatment} - Q_{control}}{Q_{control}}\right) * 100$$

where F_{SOM} (native SOM-derived C) = 1 – $f_{lactobionate}$ and Q is the cumulative CO_2 respired from treatment ($Q_{treatment}$) or control ($Q_{control}$) in μg C-CO_2 g^{-1} soil day^{-1}.

To examine the effect of lactobionate on the C, N and $\delta^{13}C$ of the WEOM, LF-POM, H-POM and MAOM, we fitted a two-way repeated measures ANOVA (Type III) using both treatment and timepoints (time) as fixed effects followed by pairwise comparison of control versus treatment at each timepoint using the Dunnett's test under the *emmeans* package in R. The level of significance (p) was set at 0.05 for all analyses. The dataset was evaluated for outliers, normality and equal variance assumptions using the diagnostics function in R (Q-Q plots, Residuals vs fitted plots). All figures were created using the ggplot2 package in R (Wickham, 2009). All analyses were conducted in R version 3.5 (R Development Core Team, Vienna, Austria, 2017).

Results

Soil respiration

Soils amended with ^{13}C-labelled lactobionate had higher soil CO_2 cumulative respiration across the entire incubation period as compared with unamended soils ($p = 0.001$) (Fig. 3.1). The majority of total respired CO_2 in the lactobionate-amended soils was derived from the added lactobionate (Equation 1; Fig. 3.1; Appendix II: Fig. S2.1). Most of the lactobionate contributions to CO_2 occurred within the first 14 days and then continuously declined, shown by the decrease in the $\delta^{13}C$-CO_2 throughout the experiment. The flux pattern of CO_2 derived from native SOM (^{12}C) in the lactobionate-amended soils was similar to the control (unamended soils) for the first 14 days of the experiment but afterwards diverged from one another, with more CO_2 respired from the native SOM in lactobionate-amended soils as compared to unamended soils (Fig. 3.1).

Lactobionate persistence and priming intensity

At the end of the 84-day experiment, 52% of the added lactobionate was respired as CO_2 in lactobionate-amended soils (Appendix II: Fig. S2.2), indicating that almost half of the lactobionate

remained in the soil after 84 days. Similarly, 48% of added lactobionate was in the bulk ^{13}C soil after 84 days. Lactobionate-amended soils displayed a dynamic priming effect, shifting in direction and magnitude over the course of incubation. A negative priming effect (ranging from 0 to -40% relative to unamended soils) was observed for the first 14 days of the incubation (Fig. 3.2). The magnitude of negative priming peaked roughly on the 9th day of the experiment. This was followed by a switch to positive priming (0 to 40 %) in the lactobionate-amended soils for the remainder of the experiment. The intensity of positive priming increased steadily from day 14 to 67, and thereafter plateaued until the incubation was terminated on day 84 (Fig. 3.2).

Total SOM fractions and their responses to lactobionate addition

Heavy-particulate organic matter (H-POM)

Relative to unamended soils, lactobionate addition led to a maximum 40% increase in the total C content of the H-POM fraction as compared with unamended soils coupled (Fig. 3.3). The N content of the H-POM fraction was also higher in lactobionate-amended soils ($p = 0.004$) as compared with unamended soils and there was also a marginal interaction of treatment and time ($p = 0.06$) (Table 3.1). Further, the C:N of the total H-POM fraction for the lactobionate-amended soils declined over time, from 12.5 (day 14) to 8.19 (day 84) and this was lower than the unamended soils ($p < 0.01$) (Table 3.1). A steady decline in the $\delta^{13}C$ of the H-POM fraction was also seen across the incubation period (Table 3.2).

Light-fraction particulate organic matter (LF-POM)

Compared to the unamended soils, the lactobionate-amended soils decreased the total C content of the LF-POM fraction by a maximum of 25% (Fig. 3.3). Similar to C, the N content of LF-POM was lower in amended soils as compared to unamended soils and sampling time had no effect on

both treatment and control soils with respect to LF-POM N content (Table 3.1, $p > 0.05$). The C:N of the LF-POM also differed by treatment ($p < 0.001$) and time ($p = 0.07$), with the amended soils having a higher C:N ranging from 17.7 to 16.1 as compared to 15 for control soils from days 14 to 84 of the experiment (Table 3.1). A steady decline in the $\delta^{13}C$ of the LF-POM fraction was also seen in the amended soils across the incubation period, starting at -8.21 ‰ on day 14 and declining to -15.29 ‰ on day 84 (Table 3.2).

Mineral-associated organic matter (MAOM)

The total C content in the MAOM fraction increased by a maximum of 15% under amended soils relative to unamended soils and this increase was consistent for the entire incubation period (Fig. 3.3). A similar time and treatment effect was observed for the MAOM N content of the amended soils ($p = 0.04$), which also had a lower C:N ($p < 0.01$) (Table 3.1). Like the H-POM fractions, lactobionate-amended MAOM fraction was enriched in $\delta^{13}C$ but declined steadily from 48.6 ‰ to 20.0 ‰ during the incubation period (Table 3.2).

Water-extractable organic matter (WEOM)

Lactobionate increased the total C content of the WEOM fraction to a maximum of 100% relative to control soils but an exponential decline was observed with time (Fig. 3.3). The opposite trend was observed for the N content as unamended soils retained more N content as compared to the lactobionate-amended soils with a significant time by treatment interaction ($p = 0.01$, Table 3.1). Similar to the other examined SOM fractions, $\delta^{13}C$ of the WEOM fraction declined steadily from 92.9 ‰ to -3.06 ‰ (Table 3.2).

Effect of lactobionate on native (^{12}C) SOM fractions

Lactobionate additions changed the amount of SOM-derived C (^{12}C) across the SOM fractions, inducing increases, decreases or no change depending on the individual SOM fraction. The WEOM fraction had higher SOM-derived C in the lactobionate-amended soils as compared to unamended soils (Fig. 3.4). However, we saw a clear decline of WEOM SOM-derived C in the amended soils throughout the incubation period. SOM-derived C of LF-POM was significantly higher in unamended soils as compared to lactobionate-amended soils for the entire period of the incubation (Fig. 3.4). In contrast, both MAOM and H-POM fractions showed no significant differences in their SOM-derived C between lactobionate-amended and unamended soils but the lactobionate-amended soils trended higher for both fractions (Fig. 3.4).

Distribution of lactobionate-derived C in SOM fractions

The relative contribution of lactobionate-derived C to SOM fractions in lactobionate-amended soils varied across fractions and by time (Fig. 3.5). The MAOM and WEOM fractions contained more lactobionate-derived C as compared to the POM fractions for the entire duration of the experiment. However, by the end of the incubation, the MAOM fraction contained the most lactobionate-derived C as compared to all other fractions. Less than 1% of lactobionate C was added to both the LF-POM and H-POM during the course of the experiment (Fig. 3.5).

Discussion

To effectively utilize soil amendments to increase SOM content, we need to better elucidate the mechanistic underpinnings of C dynamics through SOM fractions. Our study was thus designed to understand how labile inputs such as lactobionate affect the persistence or loss of soil C and its impact on different SOM fractions, given the different C protection mechanisms of these fractions.

Soil respiration and lactobionate persistence

As expected, cumulative soil respiration was greater under lactobionate-amended soils as compared to control soils, likely caused by the high bioavailability of lactobionate stimulating microbial activity and respiration (Daufresne and Loreau, 2001; Blagodatsky et al., 2010). This is supported by findings from a previous laboratory experiment where lactobionate-amended soils had on average 70 times more microbial biomass as compared to unamended soils over a 2-month period (Kallenbach et al., 2019). Despite elevated respiration with lactobionate, especially during the first two weeks of the incubation, nearly half of the labile lactobionate persisted in soil after 84 days. Other studies similarly show labile C materials such as glucose and cellulose persisting in soils (Kiem and Kogel-Knabner, 2003; Schmidt et al., 2011; Bore et al., 2019).

Lactobionate priming effects

We observed negative priming in lactobionate-amended soils for the first 14 days of the incubation experiment (Fig. 3.2). This may be attributed to microbial stimulation and resource utilization switch induced by the addition of lactobionate, a C-rich material that helped alleviate microbial C-limitation in a low C soil such as the one used in the experiment (Zhang et al., 2017). Initial negative priming after substrate addition has also been observed in other studies that have used similar quantities of labile C inputs (Cheng et al., 2014; Zhang et al., 2017). A switch from microbial lactobionate C utilization to native soil C respiration and subsequent positive priming began to occur around the 17^{th} day of the experiment. It is worth noting that in our study, positive priming occurred even when there was still an abundant supply of lactobionate in the soil, including in the WEOM fraction. Thus, the switch from negative to positive priming may be less because of a change in substrate supply and more due to a shift in microbial communities. For example, dynamic priming effects have been explained by slow SOM-feeding K-strategy microbes

replacing fast-feeding r-strategy microbes (Fontaine et al., 2003; Blagodatskaya and Kuzyakov, 2008). It is also possible that the shift to positive priming in our experiment could be attributed to N-limitations induce by lactobionate. Lactobionate contains no N and after a relative short period, the microbes likely became N limited and may mine native soil N to meet their nutritional requirements, as demonstrated in a number of studies (Craine et al., 2007; Guenet et al., 2010; Kuzyakov, 2010; Fontaine et al., 2011).

We hypothesized that the more unprotected WEOM and LF-POM SOM fractions would be the most susceptible to priming. While we cannot directly identify the source SOM fraction that is contributing to the CO_2 induced from positive priming, we can infer this by considering how the native SOM fractions change with priming (Fig. 3.4). The LF-POM fraction was the only native SOM fraction that decreased with the lactobionate amendment. This fraction has limited C protection compared to the other SOM fractions and should thus be more susceptible to priming with the lactobionate addition. The lactobionate-stimulated microbial community may have also been responding to N-limitation caused by high C inputs of lactobionate, potentially explaining the lower N content and higher C:N of LF-POM in lactobionate amended soil. While we did not observe an overall decrease in native WEOM-C in response to lactobionate and relative to the unamended soils, the native WEOM steeply declined over time in the amended soils, suggesting that native WEOM was also contributing to SOM-derived CO_2. Furthermore, neither the native MAOM nor the H-POM fraction decreased in response to lactobionate. Hence, our first hypothesis was partly supported as we saw a clear decrease in native LF-POM in response to priming and the native WEOM fraction appeared to respond to lactobionate-induced positive priming by the initial buildup but then its gradual depletion.

Lactobionate effect on SOM fractions

While the magnitude and direction of priming is crucial to understand C loss, fewer studies have comprehensively examined labile input-induced priming effects alongside C storage in specific SOM fractions. Examining the response of specific SOM fractions under priming provides a mechanistic understanding of SOM dynamics as influenced by labile soil C amendments. We show that lactobionate decreases the amount of both total (^{13}C and ^{12}C) and native (^{12}C) LF-POM but generally caused an increase in the other SOM fractions, suggesting that lactobionate is accelerating the movement of newer, less protected C (LF-POM) into more protected fractions (H-POM and MAOM). Thus, while some C may be lost through priming, this appears to have stimulated native SOM transformations into more persistent fractions.

For instance, the relatively higher WEOM-C we observed with the lactobionate amendment (Fig. 3.4) may imply either an accelerated production or input rates to WEOM from stimulated POM decomposition, increased water-extractable microbial biomass, or from desorption of MAOM-C. Soluble labile materials such as lactobionate likely contributed directly to the initial increases we saw in total WEOM-C, however, native WEOM-C was also higher with lactobionate amendments. Even though WEOM-C concentrations were consistently higher with lactobionate compared to unamended soil, both native- and lactobionate-derived WEOM-C decreased over time (Fig. 3.5). Labile WEOM compounds are rapidly lost via microbial CO_2 respiration (Kuzyakov et al., 2000; van Hees et al., 2005), but decreases in WEOM may also occur as WEOM moves out of solution directly into MAOM or indirectly via microbial WEOM utilization and then microbial biomass sorption (Cotrufo et al., 2015). While we cannot be certain if WEOM loss over time was due to respiration or sorption, given that the lactobionate-derived C in WEOM-C declined simultaneously with increase in MAOM-C (Fig. 3.5), it is clear that this fraction declines over time with labile input addition.

The changes we observed in POM with lactobionate further suggest that priming of available SOM fractions is simultaneously inducing the movement of unprotected C to more protected SOM fractions. Lactobionate amendment decreased the LF-POM fraction more so than any other fraction we measured, with observed decreases in the C and N content of both the total and the native LF-POM fraction relative to unamended soils (Fig. 3.4, Table 3.1). However, LF-POM can also contribute to H-POM and MAOM formation by the gradual depolymerization of this fraction via microbial activity. While it is unclear where LF-POM was lost to (H-POM, MAOM or CO_2), lactobionate-induced decreases in native LF-POM were matched by consistent increases in the H-POM with lactobionate addition (Table 3.1). The POM C:N ratio is often used as a proxy for the degree of plant residue decomposition, decreasing as decomposition advances, where compared to LF-POM, H-POM tends to contain more decomposed plant residues and microbial decomposition products (Golchin et al., 1997). The decrease in the C:N of the H-POM by lactobionate addition may therefore suggest that its decomposition is higher with lactobionate, potentially increasing its occlusion between and within aggregates and thus protection from further microbial attack. The decreased H-POM C:N could also be attributed to higher and more rapid turnover of microbial biomass induced from lactobionate. Although studies have suggested that the majority of microbial necromass and byproducts following microbial turnover in soil ends up in MAOM fraction, a new study has shown that the H-POM fraction can also retain a significant portion of microbial residues (Angst et al., 2019). Lastly, another explanation for the greater H-POM C levels with lactobionate addition could be a result of biogenic aggregation via microbial polymeric exudation that leads to more POM being occluded in aggregates (Deng et al., 2015; Cosentino et al., 2006; Kallenbach et al., 2019) protecting POM from microbial access. But if this were the case, we might not expect the large C:N decreases we observed over time with the lactobionate amendments. Regardless of

the mechanism, our observations that lactobionate increases H-POM C suggests that our amendment is shifting the distribution of native C into the more persistent H-POM fraction, since no new POM can be created in our systems.

Similar to trends in the H-POM fraction, lactobionate-amended soils resulted in more native MAOM-C and N compared to unamended soils (Fig. 3.3). As compared to other fractions, the MAOM fraction also retained more lactobionate-derived C at the end of the experiment. The increase we observed in this fraction could be attributed to changes in three main sources of inputs to MAOM. First, the dominant constituents of MAOM are turnover residues and byproducts from microbial biomass and highly decomposed organic matter, both of which are relatively enriched in N compared to fresh and less decomposed organic matter. The lower MAOM C:N we observed in amended soils and relative to our POM fractions may thus be partly explained by the likely lactobionate-induced stimulation and turnover of microbial biomass that preferentially accumulate in MAOM. This explanation is supported by recent frameworks including the 'in vivo modification' pathway (sensu Liang et al., 2017) and the Microbial Efficiency-Matrix Stabilization (MEMS) model (Cotrufo et al., 2013) that have described MAOM formation as SOM passing through a microbial loop that increases its MAOM sorption potential. Similar to lactobionate, other low molecular weight compounds including glucose and root exudates have been shown to stabilize in soil through the mechanism described above (Bore et al., 2019; Sokol et al., 2019; Villarino et al., 2021). Secondly, the elevated lactobionate-derived MAOM-C may have also accumulated via direct sorption of non-microbial WEOM to mineral surfaces (Cotrufo et al., 2015; Haddix et al., 2020). However, ~25% of lactobionate was in the WEOM fraction after 84 d, suggesting that not all WEOM is directly sorbed, or at least is only temporarily sorbed, or that the ^{13}C-labeled WEOM fraction is being replenished from microbial biomass turnover.

Third, native MAOM-C increases could arise from enhanced POM decomposition. Our results support the idea that following labile C additions, enhanced decomposition or priming of LF-POM increases the feedstock of WEOM (whether derived directly from depolymerized POM or from increased microbial biomass from POM monomers) that could directly contribute to MAOM. The specific sources of C and N to explain the higher MAOM with lactobionate are likely a combination of all of the mechanisms described above. However, the decreases in WEOM and LF-POM and parallel increases in MAOM and H-POM contents and the shifts towards lower C:N ratio suggest that labile inputs induce transformations of existing C from easily accessible to more persistent C fractions. We contend that not only is the MAOM fraction not readily influenced by positive priming induced by lactobionate application but that it increases via a greater production of potential sources that contribute to MAOM. Given MAOM's strong organo-mineral bonding and the occlusion in microaggregates, funneling more C into MAOM in response to priming LF-POM could represent a potential unexplored pathway for enhancing SOM protection.

Conclusion

We unraveled the effects of labile inputs on SOM dynamics by tracking the fate of ^{13}C lactobionate into soil CO_2 and SOM through its distinctive fractions (WEOM, LF-POM, H-POM and MAOM). While we observed a positive priming effect after 14 d, about 48% of the initial lactobionate remained in the soil after 84 d. Importantly, while lactobionate resulted in a net priming effect it changed the fractions where C was stored, potentially increasing its long-term persistence as lactobionate led to more SOM in the more protected H-POM and MAOM fractions. In our study, we focused on a labile C addition under the rationale that this would more likely elevate microbial biomass growth and depolymerization rates with positive consequences to MAOM fraction. Our

results suggest that C-rich soil amendments such as lactobionate may facilitate increased decomposition at the same time as building more persistent SOM.

Table 3.1
Effect of lactobionate on total N content and C:N ratios of heavy-fraction particulate organic matter (H-POM); and light-fraction particulate organic matter (LF-POM). Data are means (n=5) with standard error in parenthesis.

Treatment	Time (days)	H-POM N (mg N g^{-1})	H-POM C:N	LF-POM N (mg N g^{-1})	LF-POM C:N	MAOM N (mg N g^{-1})	MAOM C:N	WEOM N (mg N g^{-1})
Control	14	0.07 (0.00)	12.3 (0.37)	0.13 (0.01)	15.5 (0.19)	0.79 (0.01)	7.85 (0.08)	0.07 (0.01)
	28	0.06 (0.01)	14.1 (2.05)	0.14 (0.01)	16.0 (0.17)	0.70 (0.04)	8.07 (0.21)	0.07 (0.01)
	56	0.07 (0.00)	16.8 (1.16)	0.15 (0.02)	15.3 (0.31)	0.73 (0.02)	8.37 (0.08)	0.05 (0.01)
	84	0.07 (0.00)	13.5 (0.87)	0.13 (0.01)	15.2 (0.20)	0.70 (0.03)	8.17 (0.12)	0.10 (0.00)
Lactobionate	14	0.07 (0.01)	12.5 (0.42)	0.09 (0.01)	17.7 (0.28)	0.81 (0.04)	7.59 (0.06)	0.02 (0.00)
	28	0.09 (0.01)	12.6 (0.79)	0.11 (0.01)	17.1 (0.38)	0.86 (0.03)	7.41 (0.15)	0.02 (0.00)
	56	0.17 (0.05)	8.8 (2.05)	0.10 (0.01)	17.9 (0.82)	1.17 (0.15)	6.24 (0.84)	0.03 (0.00)
	84	0.26 (0.09)	8.2 (3.45)	0.12 (0.01)	16.1 (0.66)	0.90 (0.11)	7.32 (0.76)	0.06 (0.00)
					p-values			
Treatment		**0.004**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.002**	**<0.001**
Time		**0.050**	0.508	0.665	0.079	**0.088**	0.666	**<0.001**
Treatment* Time		0.067	0.091	0.378	0.162	**0.044**	0.152	**0.015**

Table 3.2

Summary of the $\delta^{13}C$ of heavy-fraction particulate organic matter (H-POM); light-fraction particulate organic matter (LF-POM); mineral-associated organic matter (MAOM); and water-extractable organic matter (WEOM) in ^{13}C lactobionate-amended soils. Data are means (n=5) with standard error in parenthesis.

Treatment	Time (days)	H-POM $\delta^{13}C$ (‰)	LF-POM $\delta^{13}C$ (‰)	MAOM $\delta^{13}C$ (‰)	WEOM $\delta^{13}C$ (‰)
Control	14	-23.5 (0.12)	-21.7 (0.21)	-20.1 (0.08)	-23.9 (0.30)
	28	-23.6 (0.15)	-21.6 (0.23)	-20.3 (0.11)	-23.5 (0.36)
	56	-23.3 (0.19)	-21.8 (0.21)	-20.4 (0.07)	-23.1 (0.57)
	84	-22.9 (0.19)	-21.8 (0.07)	-20.4 (0.05)	-22.2 (0.38)
Lactobionate	14	-8.2 (1.13)	-9.0 (0.99)	48.6 (1.42)	92.90 (17.8)
	28	-11.3 (0.79)	-13.0 (0.51)	35.2 (1.32)	25.80 (2.24)
	56	-14.4 (0.53)	-14.9 (0.26)	25.8 (0.70)	-1.04 (1.78)
	84	-15.9 (0.44)	-15.2 (0.30)	20.0 (0.69)	-3.06 (1.34)
			p-values		
Treatment		**<0.001**	**<0.001**	**<0.001**	**<0.001**
Time		**<0.001**	**<0.001**	**<0.001**	**<0.001**
Treatment*Time		**<0.001**	**<0.001**	**<0.001**	**<0.001**

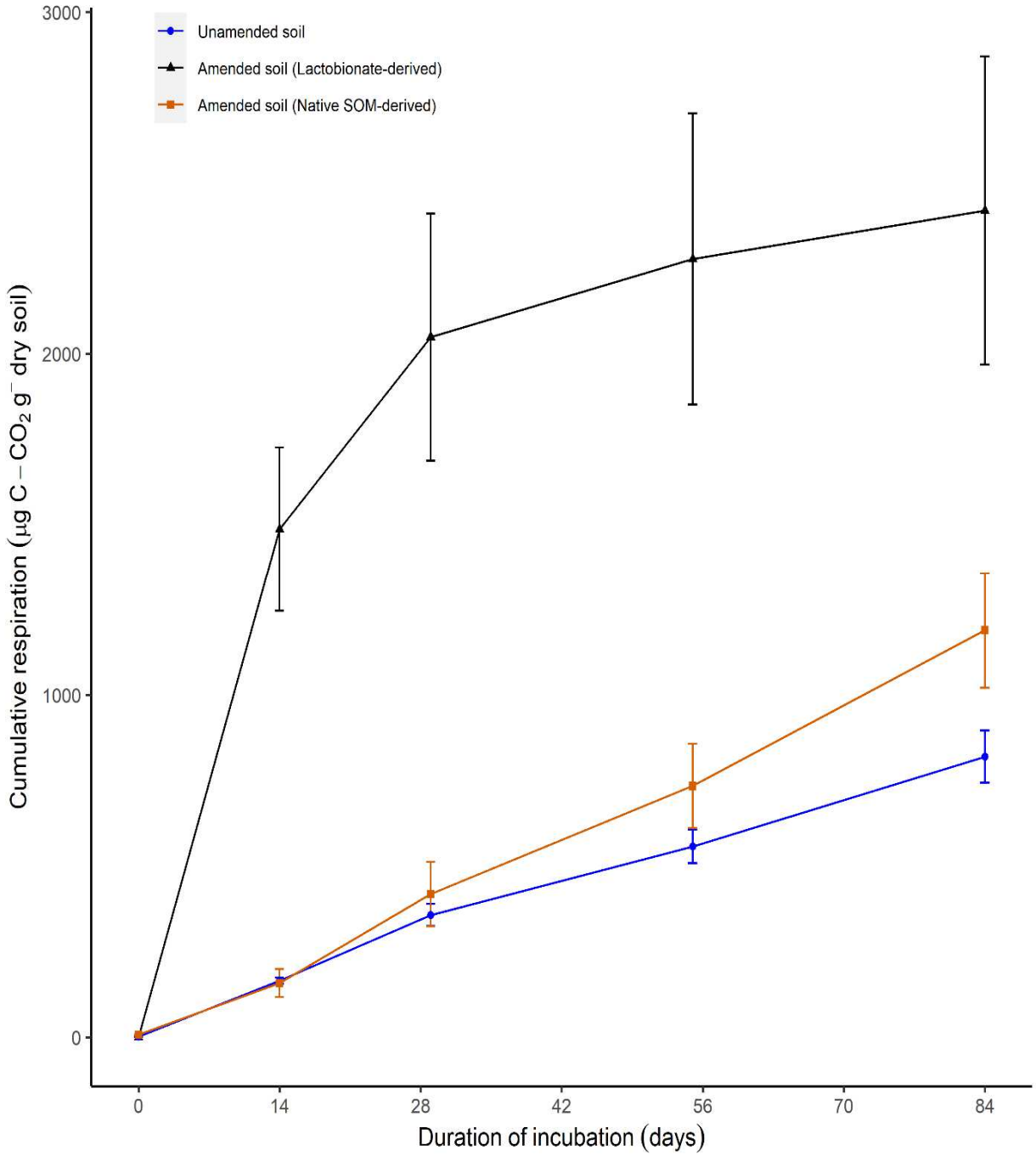

Figure 3.1. Mean cumulative respiration of lactobionate-amended (treatment) and unamended soils (control) during an 84-day incubation (n = 5, error bars are standard error of the mean).

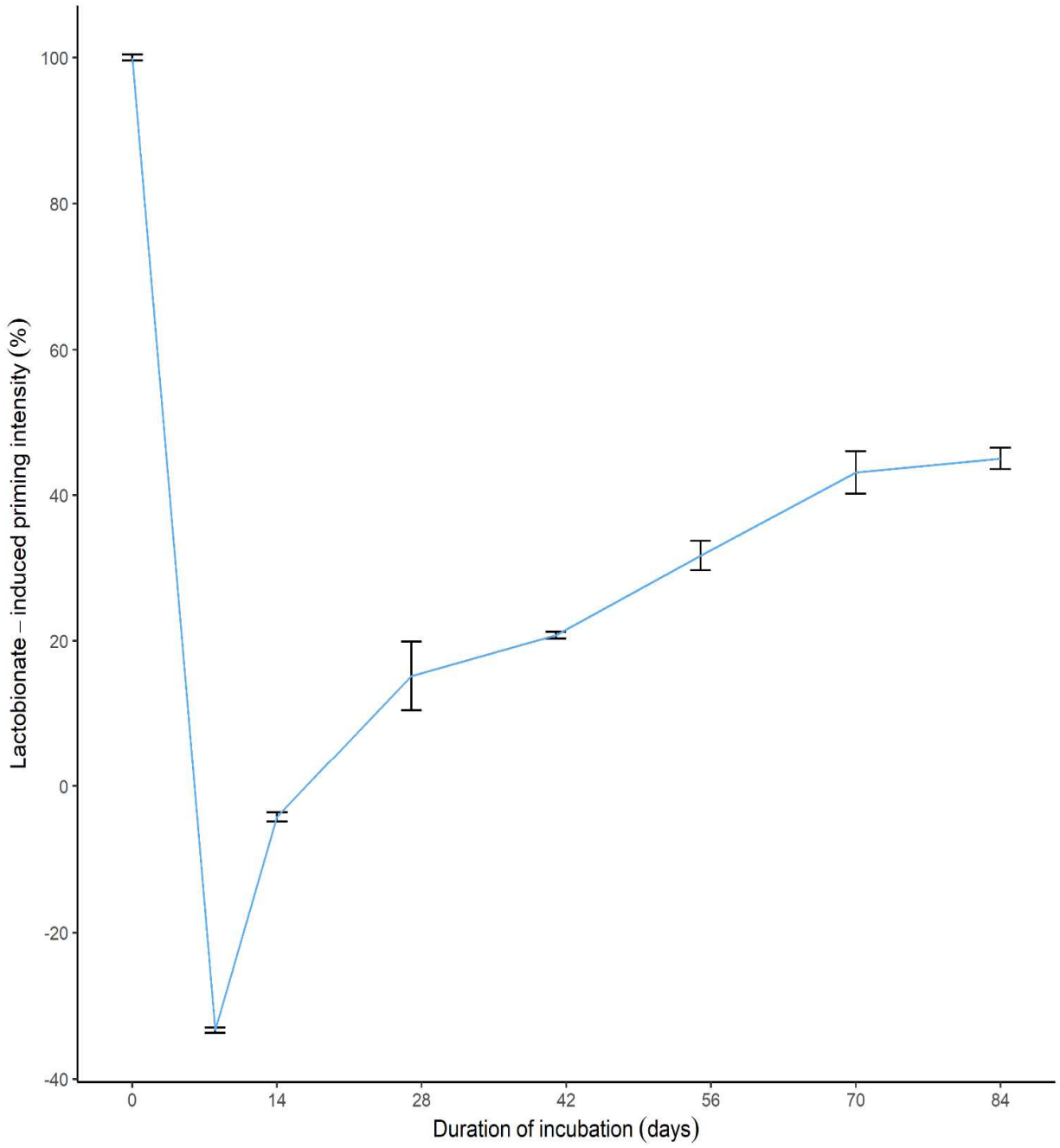

Figure 3.2. Mean priming effect intensity of lactobionate-amended soils relative to unamended soils (n = 5, error bars are standard error of the mean).

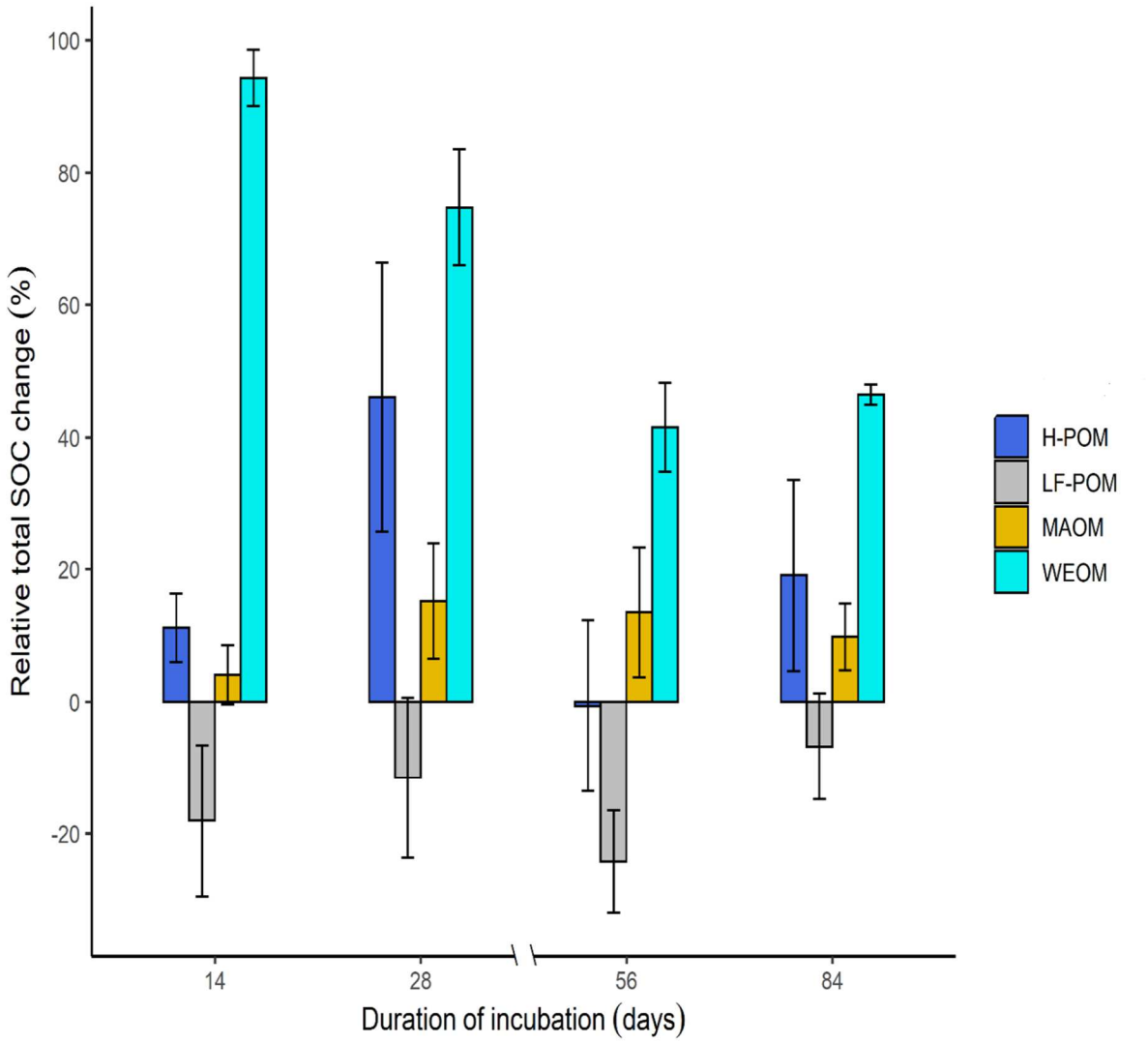

Figure 3.3. Comparing changes in mean ^{12}C and ^{13}C soil organic carbon in lactobionate-amended (Lacto) and unamended soils (Control) for A: mineral-associated organic matter (MAOM); and B; water-extractable organic matter (WEOM) (n = 5, error bars are standard error of the mean).

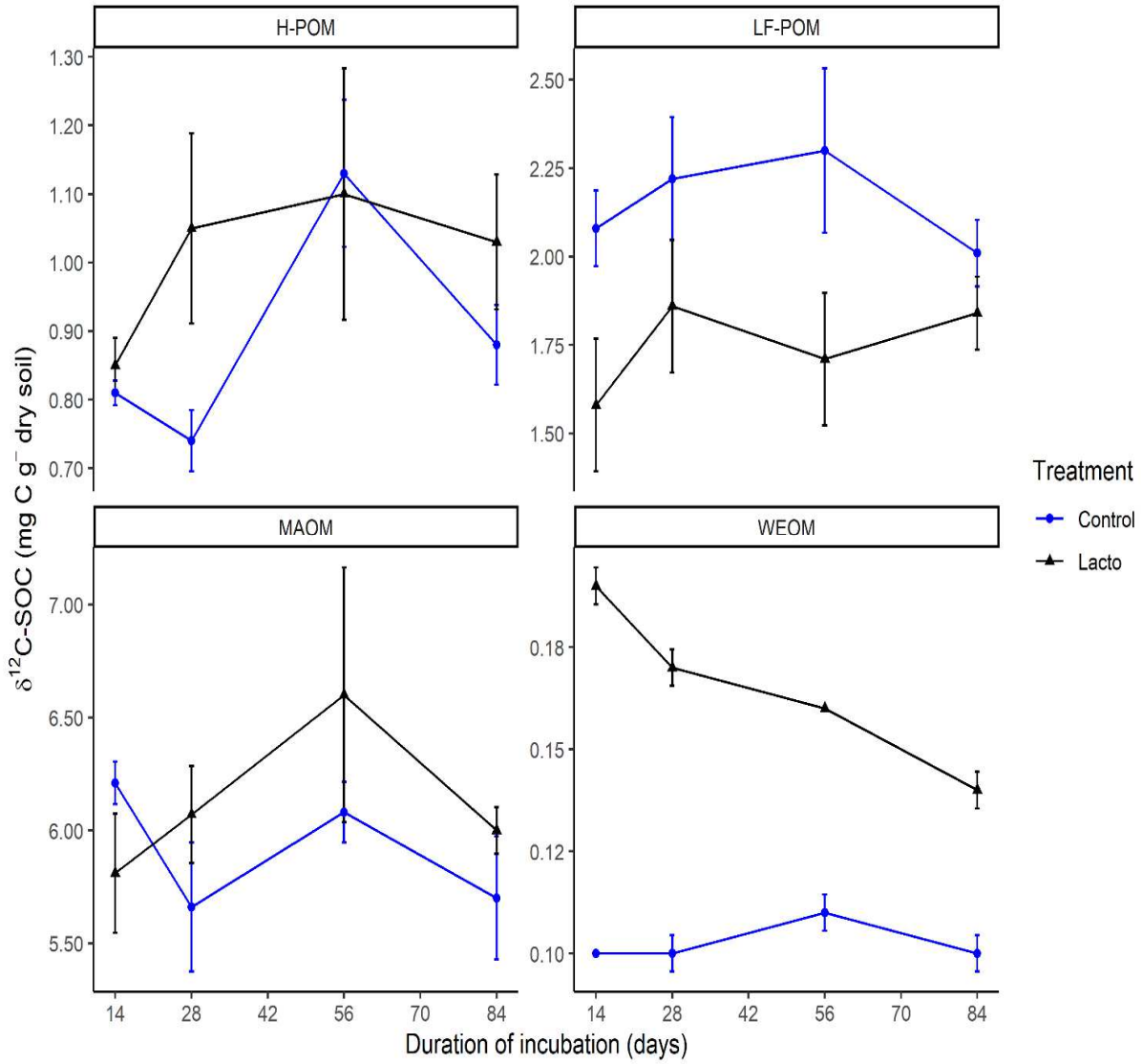

Figure 3.4. Comparing changes in mean ^{12}C soil organic carbon (native SOC) in lactobionate-amended (Lacto) and unamended soils (Control) for heavy-fraction particulate organic matter (H-POM); light-fraction particulate organic matter (LF-POM); mineral-associated organic matter (MAOM); and water-extractable organic matter (WEOM) (n = 5, error bars are standard error of the mean).

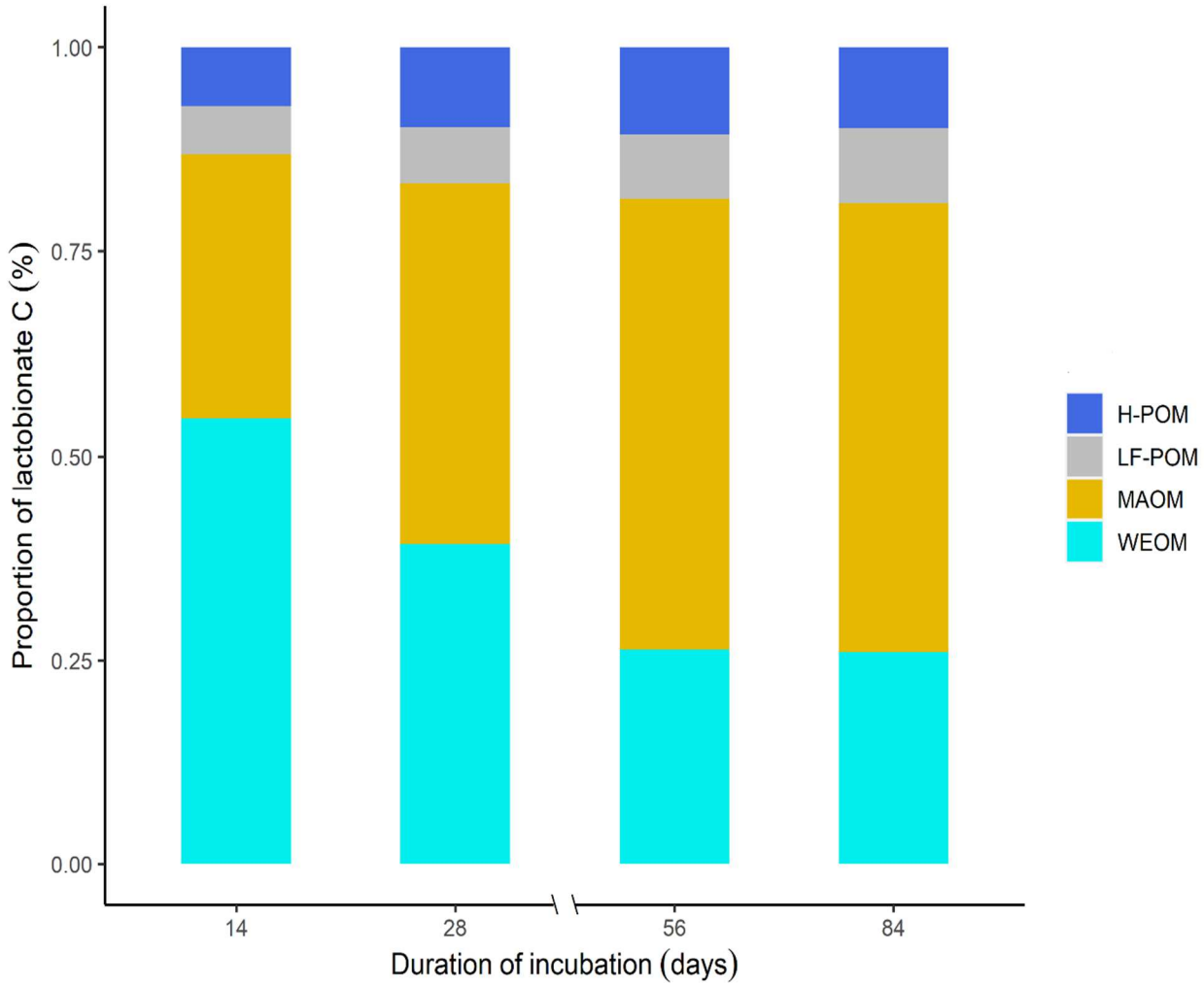

Figure 3.5. Relative contribution of lactobionate derived-C to heavy-fraction particulate organic matter (H-POM); light-fraction particulate organic matter (LF-POM); mineral-associated organic matter (MAOM); and water-extractable organic matter (WEOM) fractions (n = 5).

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CHAPTER 4: RESIDUE MANAGEMENT AND TILLAGE SHAPE SOIL MACROFAUNA AND MICROBIAL COMMUNITIES IN SIMILAR WAYS IN A SEMI-ARID AGROECOSYSTEM OF COLORADO.

Introduction

Healthy soils must not only offer the physical and chemical attributes necessary to support plant growth and a range of key soil functions, but also provide energy resources and suitable habitat for a diverse array of soil organisms (Kibblewhite et al., 2008; Bardgett and Van Der Putten 2014). Soil biota can in turn influence the productivity of soils through their roles in soil organic matter (SOM) and nutrient cycling and regulation of soil structure (Lavelle et al., 2006; Fierer et al., 2020). A variety of soil organisms contribute to the regulation of key soil functions and ecosystem services across multiple spatial and temporal scales, leading to their recognition as drivers and bio-indicators of soil health (Rousseau et al., 2013; Schloter et al., 2018). Hence, understanding soil management strategies that support soil organisms and their beneficial activities in soils has become critical to efforts to enhance the resilience and overall sustainability of agroecosystems.

Soil biological communities are exceptionally diverse, both taxonomically and in the functions they carry out. Soil macrofauna (i.e., invertebrates larger than 2 mm in size) can substantially modify the soil environment due to their larger size and high activity (Lavelle et al., 2006). Earthworms, termites and ants, in particular, are known as 'ecosystem engineers' that can modify soil structure through their bioturbation and tunneling activities (Lavelle, 1997). Other prominent groups of soil macrofauna include beetles (*Coleoptera*), spiders (*Araneae*), centipedes (Chilopoda), and flies (*Diptera*), with numerous larval and/or adult stages operating in soils. The

ecosystem services that these soil-dwelling macroarthropods provide, including biological control of arthropod pests and weeds (through predation and parasitism) and the regulation of SOM dynamics, have made conservation of these organisms of high importance in agroecosystems (Pretorius et al., 2018). At the other end of the size spectrum exists a diverse array of soil microorganisms, consisting mainly of bacteria and fungi. These organisms also play vital roles in ecosystem functioning through regulation of nutrient cycling, decomposition of organic matter, pest and disease control, and contributions to soil structure (van der Heijden et al., 2008; Bissett et al., 2011; Delgado-Baquerizo et al., 2016a). While numerous studies have considered these disparate groups in isolation, it remains unclear to what extent soil microbial communities and macrofauna co-occur and/or shift in similar ways in response to key management drivers, and how these groups may be interacting with each other to influence a wide range of soil processes.

Efforts to manage diverse biological communities must recognize that these organisms do not operate in isolation, but rather are intimately linked across multiple spatial scales. For example, ecosystem engineers such as earthworms and ants can have profound effects on soil microbial habitats through their casting and tunneling activities, and associated impacts on soil aggregation, aeration and water movement (Brown, 2000; Jouquet et al. 2013). At the same time, macrofauna graze on soil microbes, either intentionally or inadvertently, and in the process selectively reduce or enhance the presence and activity of different microbial taxa (Crowther et al., 2012; Bray et al., 2019). At the same time, microbes within the gut of detritivore macrofauna can aid in the digestion of complex organic materials such as cellulose through a mutualistic microbe-macrofauna association (Drake and Horn, 2007; Douglas, 2015). Thus, linkages between soil macrofauna and microbial communities appear to be quite pervasive and are likely important for regulating

multiple soil biological functions; however, these linkages and the role of management remain poorly understood.

Tillage and residue harvest are common management practices that are used to facilitate planting, irrigation, nutrient availability, weed control and provide additional farm income, but these practices can also lead to soil degradation and impact soil biodiversity and functioning (Zarea, 2010). Under long-term conventional tillage, soil organisms ranging from large-sized earthworms to microscopic bacteria are often reduced in abundance and diversity (Briones and Schmidt, 2017; Tsiafouli et al., 2015). The homogenizing effect of conventional tillage on soils can lead to a reduction of available niches for soil organisms, with larger organisms potentially impacted to a greater extent than smaller ones (Postma-Blaauw et al., 2010). Furthermore, soft-bodied organisms such as earthworms and fungal hyphae are likely to experience higher mortality due to the direct impact of tillage implements as compared to hard-bodied organisms such as beetles. In addition, tillage and residue management can also elicit inconsistent responses from microbial groups and thus different microbial groups show variation in their response to tillage (Schmidt et al., 2019; Marshall and Lynch, 2020).

Alternative management approaches including no- or reduced-tillage and residue retention can improve soil physical and chemical properties and lead to the accumulation of SOM, increased water infiltration and retention and improved soil aggregation (West and Post 2002; Page et al., 2020). While the benefits of no-till and residue retention have generated considerable interest in these management practices, their influence on whole soil biological communities remains poorly understood. Studies examining no-till and its influence on soil organisms have focused largely on individual taxa (Abail and Whalen, 2018; Schmidt et al., 2019; Li et al., 2021) or a limited number of taxa (Degrune et al., 2017; Jiang et al., 2018), and we thus lack a holistic understanding of the

different responses from multiple soil organisms under no-till and residue retention management. This knowledge is important for our ability to optimize management that can maintain and promote robust soil communities associated with soil health.

Our study aimed to quantify the effects of tillage (conventional vs. no-till) and residue management (residue retention vs. residue removal) on soil macrofauna and microbial communities as well as a suite of key soil physical and chemical properties. Previous work in this same field experiment documented strong effects of tillage and residue management on soil macrofauna communities just two seasons after trial establishment (Melman et al., 2019). In this study, we sought to elucidate longer-term impacts on soil biological communities and to understand how changes in soil macrofauna are associated with soil microbial communities. Specifically, we hypothesized that:

1. Despite their functional and physiological differences and the spatial scales at which they operate, bacterial, fungi and macrofauna increase in abundance and diversity under residue retention due to greater resource (C) availability.
2. Conventional tillage reduces the abundance and diversity of most soil organisms, but disproportionately affects large organisms more than smaller ones (i.e., earthworms > beetles > fungi > bacteria on the basis of their body size differences).
3. Soil macrofauna communities are strongly associated with the abundance and diversity of smaller-sized organisms including fungi and bacteria, due to similar responses to changes in habitat and resource availability as well as the ecosystem engineering effects of soil macrofauna, earthworms in particular.

Materials and methods

Site description and experimental layout

We conducted this study at the USDA-ARS Central Great Plains field station near Akron, Colorado (40°09′09" N, 103°08′09" W). This region experiences a semi-arid climate with mean annual rainfall of 420 mm and mean monthly temperatures ranging from 23 °C in the summer to −5 °C in the winter. Soils at the field site are classified as Weld silt loam (fine, smectitic, mesic Aridic Argiustolls; Nielsen et al., 2015).

In April 2014, we established a field trial to study the effects of tillage and residue management on soil properties, water dynamics and corn (*Zea mays*) productivity. Tillage and residue treatments were applied in a full factorial, randomized complete block design, resulting in four treatments: no-till with residue retention (NT-R); no-till with residue harvest (NT-RH); conventional tillage with residue retention (CT-R); and conventional tillage with residue harvest (CT-RH). Each of the four treatments was randomly assigned to plots (18.2 m wide x 24.2 m long) within four replicate blocks. Tillage was conducted each spring using a tandem disc to a depth of 20 cm, with a single pass for CT-RH and up to three passes for CT-R (to fully incorporate crop residues). For the entire duration of the trial, the no-till plots were left undisturbed (other than planting). All residues were removed shortly after corn harvest in the residue harvest treatments, while all residues were left in place in the NT-R and CT-R for the entire trial duration. Overhead sprinklers were used to irrigate the corn and supplement natural precipitation each growing season. Fertilizer was applied at corn planting in May of each year and consisted of 112 kg N ha^{-1}, 45 kg P_2O_5 ha^{-1}, as well as zinc and sulfur at recommended rates. Additional fertigation was conducted

from mid-June through July each year with the addition of roughly 34 kg N ha^{-1} in irrigation water. Fertilizer N was mainly applied as liquid urea ammonium nitrate (UAN), but also some liquid ammonium phosphate (10–34-0) was included at planting.

Soil sampling

We conducted soil sampling in August of 2019, as this time of the year is thought to be optimal for soil biological activity following 3 months of warm and moist soil conditions with an actively growing crop and full canopy cover. Based on the sampling approach of Melman et al., (2019), conducted within these same plots, we selected two sampling points at opposite ends of each treatment plot (≥ 5m from the plot edge) for assessment of all soil parameters at two soil depths (0-10 and 10-20 cm). This approach resulted in a total of 64 samples (4 treatments x 4 replicates x 2 sampling points x 2 depths), each analyzed separately for all soil parameters described below.

Soil macrofauna

Macrofauna communities were obtained using a modified Tropical Soil Biology and Fertility (TSBF) method (Anderson and Ingram, 1993). At both sampling points in each treatment plot, a soil monolith (25×25 cm) was excavated to a depth of 20 cm and split into two layers (0-10 cm and 10-20 cm). All excavated material (soil and surface residues) was hand-sorted to collect visible macro-invertebrates (> 2 mm). Collected individuals were stored in 70 % ethanol and returned to the lab for identification. Specimens were generally classified to the level of species and tallied. Earthworms were identified to species level following the key of Gates and Reynolds (2017). Earthworms were also weighed to assess their fresh biomass (including soil in their intestinal tract). The different soil taxa obtained and abundance of each were used to calculate richness (total number of observed taxonomic groups), and the Shannon Diversity Index for alpha diversity.

Soil DNA extraction and sequencing

To examine the effects of management on soil bacterial and fungal communities, we extracted DNA from soils and utilized 16S and ITS amplicon sequencing to assess bacterial and fungal community structure, respectively. A total of three soil sub-samples were collected immediately adjacent to each monolith using a 2.5 cm-diameter hand-held soil corer that was cleaned with 90% ethanol between samples. The soil cores were separated into two depths (0-10 cm and 10-20 cm), composited by depth for each sampling point, and placed in sealed plastic bags on ice for transport to the lab. Soil samples were stored in a 4 ºC refrigerator for 3 days and processed. The samples were sieved to 2 mm and sieve was cleaned with ethanol between samples. Plant roots and other debris were removed using forceps that were also wiped cleaned with ethanol between samples. Sieved soils were stored in a -20 ºC freezer until analysis. We extracted soil DNA from 0.25 g of sieved soil samples using the DNeasy PowerSoil Pro Kit (Qiagen Inc., Valencia, CA , USA) following the manufacturer's instructions and the resulting DNA samples was stored at −80°C until further use. The DNA quality and quantity were determined by using a NanoDrop Spectrophotometer (Thermo Scientific, Wilmington, DE). Isolated DNA concentrations ranged from 5.3 – 56.8 ng/ml and all isolated DNA had an absorbance ratio (A260/A280) between 1.8 and 2.0. Sequencing libraries were constructed by the amplification of the V4 region of the 16S rRNA gene using primers 515F and 806R (Bates et al., 2011) and the amplification of the specific primers of the ITS 2 region (ITS1f/ITS2 primers) for fungi (White et al., 1990; Gardes and Bruns, 1993). To generate the DNA libraries for subsequent sequencing, we amplified each sample in duplicate in 25 µl PCR mixtures using a one-step PCR reaction with individually barcoded Illumina adapters ligated to the relevant primers. The PCR thermal cycler program was set as

follows: (i) 94°C for 5 min; (ii) 35 cycles, with 1 cycle consisting of 94°C for 45 s, 60 s at 50°C, and 72°C for 90 s; and (iii) a final extension step of 72°C at 10 min. All sample libraries were pooled, cleaned, and normalized using the ThermoFisher Scientific SequalPrep normalization plate kit. Cleaned and normalized amplicons were pooled, and sequenced on an Illumina MiSeq using v2 250-cycle paired-end kits at the Colorado State University Genomics Sequencing Center.

Microbiome data processing

The resulting FASTQ files were processed using an in-house laboratory pipeline primarily based on the USEARCH v.11 protocol (Edgar, 2016). Briefly, Illumina adapters were removed using cutadapt software (Martin, 2011) and demultiplexed using the python script 'prep_fastq_for_uparse_paired.py'. Reads were merged using the USEARCH 'merge_pairs' function with a minimum overlap of 16 bases and filtered for a maximum expected error of 1.0 base per amplicon using 'fastq_filter'. Merged and filtered sequences were dereplicated using 'fastq_uniques' and denoised via UNOISE3 (Edgar, 2016) creating the representative set of amplicon sequence variants (ASVs). Merged and filtered sequences were mapped to the representative set to obtain ASV counts per sample. Taxonomic classification of the representative set was obtained against the SILVA v13.2 database (Quast et al., 2013) for 16S reads and UNITE v 8.2 database (Nilsson et al., 2019) for fungal ITS using 'sintax' with a 0.8 bootstrap cutoff.

Soil physical properties

To assess bulk density, we first gently scraped away soils on the vertical sections of the walls of the soil monolith that may have be compacted during the excavation process. We then inserted a metal ring (7.5 cm diameter and 7.5 cm length) horizontally into the cleaned vertical wall of the monolith to soil depths of 1-8.5 cm and 11-18.5 cm. The soil collected within this ring was gently

placed into ziplock bags, transported on ice to Colorado State University, and then stored in a refrigerator at 4°C upon arrival. The moist soil was then weighed and a sub-sample (~20 g) was collected and dried at 105 °C to for determination of gravimetric moisture content (GMC). The remainder of the field moist soil was passed through an 8 mm sieve by gently breaking soil clods along the natural planes of weakness, and then air-dried in the lab.

We measured aggregate stability using a method adapted from Elliott (1986). A sub-sample (40 g) of the 8 mm sieved, air-dried soil was spread on a 2 mm sieve and submerged in deionized water for 5 min to allow for slaking. The soil was then gently submerged in water repeatedly for a total of 50 cycles over a 2 min period. The aggregate fraction remaining on top of the sieve was rinsed into a pre-weighed aluminum pan, oven-dried at 60 °C and weighed. This procedure was repeated on the soil passing through a 250 μm and a 53 μm mesh sieve. As a result, we obtained four aggregate size fractions: large macroaggregates (> 2 mm), small macroaggregates (250-2000 μm), microaggregates (53-250 μm) and silt + clay (< 53 μm). Soil aggregate stability was estimated using the mean weight diameter (MWD) according to van Bavel (1950).

Soil chemical properties

After the removal of macrofauna from the monoliths, a representative subsample of soil (~1.0 kg) was collected from each depth. In the lab, all subsamples were then homogenized, air-dried, passed through a 2 mm sieve and analyzed for a suite of soil chemical properties. Electrical conductivity (EC) and pH were measured in a 1:1 soil to water mixture. Permanganate oxidizable C (POXC) was assessed with a 0.2M $KMnO_4$ reacting solution according to Weil et al., (2003). Available P was determined with a sodium bicarbonate ($NaHCO_3$) extracting solution as described by Olsen and Sommers (1982). Total C and N were estimated by dry combustion on a LECO True-Spec CN analyzer (Leco Corp., St. Joseph, MI, USA).

Statistical analyses

We applied a general linear mixed effect model to examine the effects of tillage and residue management as well as the tillage by residue interaction on soil C and N, MWD, pH, EC, bulk density, GMC, POXC, earthworm biomass, earthworm and macroarthropod abundance, fungal and bacterial alpha diversity indices, with treatments considered as fixed effects and blocks and plots considered as random effects. These analyses were conducted separately for each depth (0-10 cm, 10-20 cm) using plot level data (i.e., average of the two sub-samples in each plot; n = 16) for all comparisons. The dataset was evaluated for normality and equal variance assumptions using the diagnostics function in R (Q-Q plots, residuals vs fitted plots) and log transformations were applied as needed. All univariate analyses were conducted in R using the *nlme* and *emmeans* packages (R Development Core Team, Vienna, Austria, 2017).

Permutational analysis of variance (PERMANOVA) based on Bray-Curtis distance metrics with 999 permutations were conducted to understand the effect of tillage, residue management and their interaction on soil microbial and macrofauna communities. Cumulative sum normalization and log transformation were done for data normalization for both microbial and macrofauna communities prior to PERMANOVA. To minimize the inflation of rare microbial ASVs in the community analysis, samples with less than 1,000 sequences and taxa with less than 0.01 percent relative abundance across all samples were removed (Zakrzewski et al., 2017). Analyses of macrofauna community structure was conducted at the order level (phylum for earthworms) to simplify interpretation and reduce the number of zeros that is common when considering species level data. Microbial ASVs were rarified to a depth of 11000 sequences prior to alpha diversity estimation that was evaluated by calculating the Shannon index. Additionally, we conducted indicator species analysis and linear discriminant analysis of effect sizes (LEfSE) to identify indicator macrofauna

and microbial taxa (at phyla level) for each treatment. Differences in relative abundance of microbial communities (at phylum level) between treatments were also evaluated using Kruskal-Wallis tests and p-values were adjusted for the false discovery rate (FDR). The online Calypso web tool as well as the *indicspecies, vegan* and *phyloseq* packages in R were used for all community analyses, while visualizations were generated using the *ggplot* package in R (McMurdie and Holmes, 2013; Oksanen et al., 2018; Zakrzewski et al., 2017).

Finally, co-inertia analyses were utilized to examine multivariate relationships and the overall similarity in data structure considering the following four normalized data sets: 1) soil physicochemical properties, 2) macrofauna communities, 3)16S bacterial communities and 4) ITS fungal communities. Co-inertia analyses examines the co-variance structure between paired datasets and does not use distance matrices as in PERMANOVA (Dolédec and Chessel, 1994; Dray et al., 2003). These analyses were conducted using the *coin()* function in the *ade4* package in R (R Development Core Team, 2018).

Results

Effect of tillage and residue management on soil physicochemical properties

The effect of tillage and residue management largely depended on soil depth. At the 0-10 cm depth (topsoil), residue retention (NT-R and CT-R treatments) significantly increased soil C and N, soil moisture content (GMC), POXC, aggregate stability (MWD), and available soil P ($p < 0.05$; Table 4.1) as compared to residue harvest (NT-RH, CT-RH). At the same time, tillage (CT-R and CT-RH) significantly increased available P and EC, while also decreasing pH and bulk density as compared to no-till treatments (NT-R and NT-RH, Table 4.1). Interestingly, residue management and tillage had largely independent effects for the vast majority of soil properties. However, there

was a marginally significant interaction ($p = 0.090$) between residue management and tillage with respect to available soil P, where residue retention increased available P considerably more when combined with conventional tillage (Table 4.1). At the 10-20 cm depth, both tillage and residue retention had little effect on soil properties except for bulk density that increased with tillage and residue retention. In addition, available soil P was highest in NT-R treatment, with a significant tillage by residue interaction for both available P and EC at this depth (Appendix III: Table S3.1). In all, residue management had the most significant effects on measured soil properties but mainly in the topsoil with minimal treatment effects observed in the deeper soil layer.

Effect of tillage and residue management on soil macrofauna structure

A total of 642 individuals were collected from the plots across all treatments and depths. Earthworms (*Annelida*) were the most abundant species (58% of total), followed by the beetles (*Coleoptera,*15% of total). Other species collected include spiders (*Aranaea*, 3.9%), ants (*Hymenoptera*; 4.2%), flies (*Diptera*, 3.4%), centipedes (*Chilopoda,* 7.9%*), Lepidoptera* (0.5%) and *Hemiptera* (3.0 %) (Appendix III: Table S3.2). The dominant earthworm species collected were *Apporectodea trapezoides* (73 %) and *Lumbricus rubellus* (22 %). Earthworms and macroarthropods were generally more abundant in topsoil (0-10 cm; Table 2) than in the 10-20 cm depth (Appendix III: Table S3.3), with more than twice as many earthworms and seven times as many arthropods in the 0-10 cm layer across all treatments ($p < 0.05$). Earthworm biomass and abundance were higher under residue retention as compared to residue harvest treatments at both soil depths examined ($p < 0.01$; Table 4.2 and Appendix III: Table S3.2). However, there was a significant interaction with tillage at the 10-20 cm depth ($p = 0.03$) such that earthworms were nearly an order of magnitude more abundant under no till, residue retention (NT-R) relative to the other treatments (Appendix III: Table S3.3). Residue retention plots had on average a four times

higher abundance of earthworms in the topsoil as compared to residue harvest plots (Table 4.2). No-till with residue retention (NT-R) had the highest average earthworm count (242 ind m^{-2}) and biomass (20.6 g m^{-2}) in the 0-10 cm layer followed by CT-R, while the residue harvest plots (CT-RH, NT-RH) had the lowest earthworm count and biomass across both depths (Table 4.2). Macroarthropod abundance followed a similar pattern, with residue retention plots having significantly higher counts of macroarthropods (average of 163 ind m^{-2}) as compared to residue harvest plots (average of 38 ind m^{-2}) in the 0-10 cm layer (Table 4.2). Residue retention plots also showed significantly higher Shannon diversity as compared to residue harvest plots in the topsoil (Fig. 4.1a). Tillage had no significant impacts, nor were there any tillage by residue interactions, for any of the macrofauna variables considered (Table 4.2).

Exploration of overall soil macrofauna community differences for the 0-10 cm layer using NMDS showed that community structure clearly differed by treatment (Fig. 4.2a). Residue management was the main driver of soil macrofauna communities (PERMANOVA = 0.003, R^2 = 0.125). Indicator species analysis demonstrated that *Chilopoda* and *Aranaea* were significantly associated with NT-R treatment in the surface soils, while *Coleoptera* presence was indicative of CT-R (Table 4.3). In the subsoil, *Coleoptera* and *Annelida* were the major groups present, but low abundances of most macrofauna taxa precluded multivariate or indicator species analyses.

Tillage and residue management impacts on soil bacterial communities

After quality filtering and merging, 16S sequencing of the V-4 region produced a total of 739,528 reads and 13,969 unique bacterial OTUs in the topsoil and 933,434 total reads and 14,312 unique OTUs in the subsoil. On average, the dominant phyla across treatments in the topsoil include Proteobacteria (34.3%), Acidobacteria (19.9%), Actinobacteria (11.6%) and Bacteriodetes (10.9%) (Appendix III: Fig. S3.1). In the topsoil zone, more bacterial phyla responded to residue

management as compared to tillage. Phyla belonging to Proteobacteria, Bacteriodetes, Acidobacteria, Spirochaetae, Fibrobacteres, Planctomycetes, Latescibacteria, Chlamydiae, Microgenomates and Candidate division TM6 demonstrated significantly higher relative abundances in residue retention treatments (NT-R, CT-R) as compared to residue harvest treatments ($p < 0.05$; Appendix III: Table S3.4). Alternatively, members of the bacterial phylum Firmicutes were significantly enriched in the residue harvest treatments (NT-RH, CT-RH) in the topsoil (Appendix III: Table S3.4). In the subsoil, no clear treatment effect was observed on enrichment patterns among the identified bacterial phyla (data not presented). Shannon diversity index of samples showed significant variation across management practices and depths. In the topsoil, conventional tillage significantly increased Shannon ($p <0.01$; Fig 4.1a) but at the 10-20 cm layer, the Shannon index was significantly higher under residue retention management ($p < 0.05$; Appendix III: Table S3.5).

When examining changes in the structure of soil bacterial communities in the topsoil using the Bray-Curtis dissimilarity matrix, there were significant impacts of residue and tillage (PERMANOVA $P < 0.05$) as well as a tillage:residue interaction ($P = 0.035$) that shaped the bacterial community structure (Fig 4.2b, Appendix III: Table S3.6). However, these communities appeared to cluster more by residue management than tillage. No significant management effects were observed for bacterial community structure in the 10-20 cm layer (data not presented). LEfSE analysis indicated that Candidate division TM6 and Fibrobacteres were indicative of NT-R while Spirochaetae, Chlamydiae and Latescibaceria were indicative of CT-R (Table 4.3).

Tillage and residue management impacts on soil fungal communities

A total of 742,648 reads and 1,371 unique fungal ASVs in the topsoil and 636,407 reads and 1,371 unique fungal ASVs in the subsoil were considered for analysis. The dominant phyla identified

across all samples were Ascomycota (64.4%), Basidiomycota (25.3%), Mortierellomycota (6.23%), Blastocladiomycota (4.09%), Glomeromycota (0.85%) and Rozellomycota (0.68%) (Appendix III: Fig. S3.2). The relative abundance of Ascomyota and Rozellomycota were highest under NTR while Basidiomycota was highest under CTR in the surface layer (Appendix III). In addition, Mortierellomycota, Blastocladiomycota and Glomeromycota were enriched under residue harvest treatments (Appendix III: Table S3.4). In the topsoil, residue retention significantly increased Shannon diversity ($p < 0.01$; Fig. 4.1c) but at the 10-20 cm layer, there were no significant treatment effects on Shannon diversity (Appendix III: Table S3.5).

Similar to bacterial communities, there were significant tillage (PERMANOVA P = 0.001) and residue effects (PERMANOVA P = 0.002), as well as tillage by residue interaction for fungal community structure in the topsoil layer (PERMANOVA P = 0.013; Fig. 4.2c, Appendix III: Table S3.7). NT-R appeared to cluster distinct fungal communities as compared to other treatments (Fig. 4.2c). LEfSE analysis showed that Rozellomycota was indicative of NT-R, while Basidiomycota was indicative of CT-R (Table 4.3). In the subsoil, there were no clear treatment effects on fungal community composition.

Co-inertia analysis of relationships between soil physicochemical, macrofauna and microbial datasets

Co-inertia analyses of the topsoil was used to examine similarities in data structure between the normalized soil physicochemical properties, macrofauna, and microbial datasets (at the phylum level). Significant covariation was observed between all paired datasets, as is summarized in Figure 3. While details for all of the paired datasets are not provided here (see Appendix III: Fig. S3.3), these findings were exemplified by significant covariation between soil macrofauna and soil bacterial communities ($p = 0.003$; RV 36.5%), where the macrofauna taxa *Annelida*, *Chilopoda*

and *Aranaea* were positively associated with Bacteriodetes, Fibrobacteres, Verrucomicrobia, and negatively associated with Firmicutes and Actinobacteria (Fig. 4.4a). We also note significant covariation between soil macrofauna and soil fungal communities ($p = 0.015$; RV 23.5%), such that *Coleoptera* were positively associated with the fungal phyla Ascomycota and Basidiomycota (Fig. 4.4b; $p = 0.015$; RV 23.5%).

Discussion

Residue retention enhances soil physical and chemical properties

Six years after trial establishment, residue management was the most significant driver of soil physical and chemical properties, particularly in the surface layer (0-10 cm). Soil C and N, moisture content (GMC), aggregate stability (MWD), POXC, available P, pH and EC in the surface layer were all positively enhanced by the presence of residues, either on the surface (NT-R) or incorporated (CT-R; Table 4.1). These findings add to an extensive body of evidence that has demonstrated the beneficial effects of residue retention on a suite of soil physical and chemical properties (Liu et al., 2014). The return of residues to the soil surface under reduced tillage practices provides a natural cover for soils, thereby reducing erosion from raindrops or blowing winds (Nielsen et al., 2005; Lampurlanés and Cantero-Martínez, 2006). This protection of the soil surface and increased C input associated with residues not only helps maintain soil structure at the surface, but also supports SOC stabilization, aggregation, and improved water dynamics (Blanco-Canqui and Lal, 2009). These ideas are supported by previous findings from this same experiment, where residue retention resulted in higher levels of soil water infiltration, volumetric water content and maize yield (Schneekloth et al., 2020). Beyond the effects of residue management, tillage influenced several soil physicochemical properties, including bulk density, pH, and EC, but overall the effects of tillage were not as strong as those of residue management.

Residue retention enhances soil macrofauna diversity and community structure

Our study showed that regardless of soil depth, residue retention (NT-R and CT-R treatments) supported the highest earthworm and macroarthropod abundance and Shannon index as compared to the residue removal treatments (Table 4.2, Fig. 4.1). In a previous study conducted within this same trial 2.5 years after the establishment, Melman et al., (2019) reported a strong interaction between tillage and residue management, such that earthworm abundance was more than five times higher in NT-R, than for all other treatments, with no treatment effects on macroarthropod abundance. In the current study (6 years after trial establishment), we observed a significant effect of residue management on both earthworms and macroarthropod abundance. However, the interactive effect between residue management and tillage on earthworms largely disappeared due to a relative increase of earthworms in the CT-R treatment and a decrease under NT-R. This suggests that under a constant supply of crop residue inputs, earthworm populations may be able to recover from the deleterious effects of tillage over time. Similarly, the number of macroarthropods seen in the NT-R and CT-R treatments supports this explanation for soil dwelling insects as well, although macroarthropods are generally thought to be less susceptible to tillage than earthworms due to their smaller size and hard-body morphology (Wardle, 1995; Postma-Blaauw et al., 2010). In addition to nutritional resources provided by crop residues, we suspect that residues indirectly support the growth and maintenance of macrofauna communities by regulating the soil physicochemical environment related to soil moisture, porosity and temperature, especially when left on the surface under no-till (Hendrix et al., 1986; Mulumba and Lal, 2008).

Residue retention also significantly influenced the structure and composition of soil macrofauna communities as seen in the ordination plot for the topsoil (Fig. 4.2). The separation in the ordination plot was more strongly associated with residue management than tillage. These findings

are consistent with previous studies showing the positive effect of residue retention on soil macrofauna communities, particularly when combined with no-till management (Brévault et al., 2007, Jiang et al., 2018). The Shannon index demonstrates the crucial role that residue retention plays in supporting the diversity of soil macrofauna communities in semi-arid agroecosystems (Brévault et al., 2007; Melman et al., 2019).

Indicator species analyses was conducted to understand if some macrofauna taxa are indicative of particular treatments. We found that *Chilopoda* and *Aranaea* were strongly associated with the NT-R treatment, while *Coleoptera* was more associated with CT-R. *Aranaea, Chilopoda* and some members of the *Coleoptera* are generalist predators of soil and leaf dwelling invertebrates and play important ecological roles in soil food webs and may prevent economically important pest outbreaks (Lundgren and Fergen, 2011; Thorbek and Bilde, 2004). The presence of crop residues on soil surface in addition to the undisturbed environment provided by no-till has been shown to significantly increase the abundance of these predators (Wardle, 1995; Thorbek and Bilde, 2004). Previous studies have supported our findings that no-till increases *Aranaea* presence (Rivers et al., 2016), while others have found conventional tillage to enhance *Coleoptera* abundance, especially when residues are retained (Shearin et al., 2014). Given that residues serve as the energy base of soil food webs, the presence of these predators in high abundance under residue retention treatments may indicate ample prey resources alongside more structurally complex and stable food-webs (Rousseau et al., 2013).

Management impacts on soil bacterial community structure and diversity

Similar to macrofauna communities, residue retention was the main driver of bacterial community structure in the surface soil layer (Fig. 4.2). However, the interaction between residue management and tillage indicated that tillage effects were somewhat more pronounced when residues were retained. Regardless, we note that more bacterial phyla were enriched under residue retention as compared to when residues were removed. The presence and decomposition of residues in soils has been directly linked to the enrichment of these phyla (Bernard et al., 2012; Gong et al., 2018). The selective enrichment and/or depletion of different bacterial phyla under residue retention can also be linked with the life history strategy and traits possessed by each bacterial phylum, specifically with regards to where they fall on the oligtrophy-copiotrophy continuum (Fierer et al., 2007). Several classes of Proteobacteria and Bacteriodetes are generally reported to be copiotrophic, rapidly proliferating in systems in high C content such as in residue retention systems (McHugh and Schwartz, 2015; Hao et al., 2019). Additionally, several bacterial phyla enriched under residue retention are known to possess unique organic matter degrading capabilities. For instance, members of the Bacteriodetes and Latescibacteria phyla have been demonstrated to possess complex C degrading capabilities (Farag et al. 2017; Kraut-Cohen et al., 2020). On the other hand, the dominance of Firmicutes under residue harvest treatment supports the oligotrophic nature of this phylum particularly in semi-arid locations such as our study site (Bastida et al., 2015). Regardless of the life history trait at work, it was clear from our study that the presence of residues stimulated more bacterial phyla with minimal tillage influence.

Most studies have found that conventional tillage tends to decrease bacterial alpha diversity due to the homogenization of the soil microhabitats (González-Chávez et al., 2010), but others have shown positive to no effect of conventional tillage on bacterial alpha diversity (Pastorelli et al.,

2013; Srour et al., 2020). Our study demonstrated that conventional tillage increased Shannon alpha diversity (Fig. 4.1). Possible reasons for these contrasting findings include the resilience of soil bacterial communities that ensures their rapid recovery following disturbance, the frequency of tillage, differences in soil types, sampling depths, geographic and climatic conditions as well as soil use history (Allison and Martiny, 2008; Wagg et al., 2018; Wang et al., 2020). Another possible reason for the greater effect of tillage on bacterial alpha diversity, compared to residue management, could be associated with the intermediate disturbance hypothesis that posits that disturbances that are neither too frequent nor too rare lead to greater species richness due to conditions that allows the coexistence of competitive species and disturbance□tolerant species (Mackey and Currie, 2001). This was also observed in the work of Lienhard et al., (2014) and Degrune et al., (2017), where infrequent tillage was found to increase both bacterial and fungal alpha diversity. Despite the notable effects of tillage on bacterial alpha diversity, our results show that residue retention had a greater influence on overall bacterial community composition as compared to tillage.

Management impacts on soil fungal diversity and community structure

Similar to patterns seen in macrofauna and bacterial communities, residue retention was a major driver of fungal community structure, but a significant residue by tillage interaction indicated that the effect of tillage was only expressed in the presence of residues (Fig. 4.2c). Of the five fungal phyla identified in our study, Ascomycota, Basidiomycota and Rozellomycota were significantly enriched in residue retention treatments in the topsoil. Ascomycota are ubiquitous across arable soils and are easily influenced by crop residue presence and it has been reported that they can easily degrade cellulose and lignocellulose in residues (Ma et al., 2013; Su et al., 2020). Basidiomycota have also been reported to thrive in dry and cooler climates such as the Central

Great Plains and they also possess a unique lignin and cellulose degrading enzyme complex (Treseder et al., 2014). The functional role of Rozellomycota in soils is yet to be fully elucidated, but they have been shown to adapt to extreme environments and can proliferate under abundant nutritional resources such as crop residues (Tedersoo et al., 2017).

In contrast to patterns seen for bacterial alpha diversity of the surface layer, and similar to findings for macrofauna, residue retention treatments increased fungal alpha diversity (Fig. 4.2c). We suspect that differences in management effects on fungal versus bacterial alpha diversity reflect the differences in physiology of fungi and bacteria, as the smaller size of bacteria affords them resilience and quicker recovery (Babin et al., 2019). Conventional tillage has been shown to reduce fungal abundance and richness via the destruction of fungal hyphae network that takes a longer time for recovery (Verbruggen et al., 2010; Hartmann et al., 2015). The contrasting effects of conventional tillage and residue management on bacterial and fungal alpha diversity has also led to questions regarding the validity of alpha diversity indices as metrics of functional differences in these systems (Schmidt et al., 2019). This is also in light of the high functional redundancy observed in soil microbial communities. We argue that microbial beta diversity patterns may provide more relevant information with respect to management effects. Knowledge of the microbial taxa enriched or suppressed under different management and associated beta-diversity patterns provides a foundation to infer both plausible microbial responses to management and microbial functions that influence soil functioning that are not generally captured by alpha diversity metrics.

Relationships between soil physicochemical properties, macrofauna and microbial communities

Along with the direct effects of management (particularly residue retention) on soil microbial communities, we suspect that management-induced changes to soil C, pH, and macrofauna

communities were also likely to have direct effects on soil bacterial and fungal community structure. While numerous studies have demonstrated that soil pH and soil C are strong drivers of microbial community structure at different spatiotemporal scales, few studies have noted the linkages between soil macrofauna and soil microbial community composition (Aira et al., 2011; Delgado□Baquerizo et al., 2016b, Bray et al., 2019). Despite limited knowledge on the role of earthworms and macrofauna in microbial community assembly, macrofauna have been proposed to influence the soil microbiome via three main routes: stimulation via resource accessibility, dispersal and grazing (Bray and Wickings, 2019). In this study, microbial access to the energy from residues may be facilitated by soil macrofauna through their bioturbating and litter-fragmenting activities. Additionally, habitat modification via earthworm burrowing, mucus production and the excretion of earthworm casts and macroarthropod fecal pellets can shape microbial community dynamics (Winsome, 2005; Jouquet et al., 2013). Furthermore, the high number of predatory macrofauna under the residue retention treatments could be indirectly influencing soil microbes via their predation of fungal and bacterial feeders such as Collembola, mites and nematodes (Pollierer et al., 2010). While we were unable to directly evaluate these mechanisms, previous studies have found that grazing (microbivory) and grazing intensity can influence the activity, structure, and diversity of soil microbial communities (Crowther et al., 2012; Trap et al., 2016).

In exploring covariation between the soil physicochemical, microbial and macrofauna datasets in our study, we found significant relationships between all dataset pairs (Fig. 4.3). For example, significant covariation between the macrofauna and bacterial datasets were exemplified by a positive association of *Annelida* (earthworms), *Araneae* (spiders) and *Chilopoda* (centipedes) with the bacterial phyla Fibrobacteres, Verrucomicrobia, Bacteriodetes and a negative association with

Firmicutes (Fig. 4.4a). Prior studies have shown that endogeic earthworm presence (including the dominant *A. trapezoides* in our study) to be positively related to the enrichment of Bacteriodetes, Proteobacteria, and Verrucomicrobia phyla (Gong et al., 2018; Medina-Sauza et al., 2019) and a decrease in Firmicutes (De Menezes et al., 2018). Importantly, earthworms appear to only be strongly associated with relatively few bacterial phyla that are copiotrophic and possess unique C degrading enzyme capabilities, indicating that these bacterial phyla may be stimulated by labile C in casts or mucus produced by earthworms (Bernard et al., 2012; De Menezes et al., 2018; Schlatter et al., 2019). Furthermore, these copiotrophic bacteria have been shown to thrive under aerobic conditions made possible by the soil bioturbating activity of earthworms (De Menezes et al., 2018).

Similar to bacterial communities, we also observed significant covariation between macrofauna and fungal community datasets including the positive association of *Annelida, Araneae* and *Chilopoda* with the fungal phyla Rozellomycota, as well as an association between *Coleoptera* (beetles) and Basidiomycota (Fig. 4.4b). This suggests that *Coleoptera* may be feeding on fungivores (e.g., Collembola), thereby reducing fungal predation and potentially stimulating fungal diversity by reducing grazing intensity of the fungi feeders (Tao et al., 2011; Crowther et al., 2012). Alternatively, these patterns may not be driven by biotic interactions, but these groups may just be responding to similar stimuli in the soil (e.g., crop residue presence/organic matter availability). While the specific mechanisms of macrofauna-microbial interactions are not fully elucidated, our results demonstrate that macrofauna and microbial communities are closely associated, such that one is influencing the other, or that they are responding to management in similar ways. Regardless, these findings corroborate the idea that macrofauna and microbial communities are strongly associated with each other, and further emphasizes the idea that

management for one particular aspect of soil health is likely to lead to complex and cascading effects on multiple soil taxa and environmental parameters (de Valença et al., 2017).

Conclusion

Our findings shed light on the complex and sometimes interactive effects of tillage and residue management on soil biological communities. In accordance with our first hypothesis, our findings suggest that soil biological communities are generally enriched and more diverse under continuous residue retention and this appears to be associated with an increase in SOM and overall C availability. While harvesting of residues may offer short-term financial gains and facilitate some aspects of management, there appear to be considerable consequences in the longer-term for soil biodiversity and a range of critical soil functions mediated by soil organisms. Meanwhile, our second hypothesis that tillage would reduce soil biological activity and diversity, and disproportionately affect larger organisms, was not well supported by our findings. In fact, tillage had only minimal impacts on soil macrofauna communities and actually increased the diversity of soil bacterial communities. These results, however, should be taken with some caution as the tillage employed in this study was relatively infrequent and not completely representative of more intensive tillage practices that are common in the region. Therefore, our conclusion that tillage may not be so deleterious for biological communities only extends to more conservative tillage strategies that avoid frequent and aggressive inversion of the topsoil. We also note that the effects of tillage tended to be more pronounced in the residue retention treatments, suggesting that residue management is important to consider when trying to predict tillage impacts. Finally, our data strongly supported our third hypothesis, that soil macrofauna would be closely associated with soil microbial communities. This finding is important as it suggests that strategies designed to optimize soil microbial communities must also consider larger soil invertebrates that can interact with and

regulate soil microbes both directly (e.g., though consumption) and through alterations to physical and chemical properties that shape microbial niches. In summary, our results suggest that residue retention is critical to promoting robust soil biological communities and associated soil health benefits in the semi-arid plains of eastern Colorado.

Table 4.1.

Mean values for soil physicochemical properties in surface soils (0-10 cm) under different tillage and residue management combinations: no-till + residue retention (NT-R), no-till + residue harvest (NT-RH), conventional tillage + residue retention (CT-R) and conventional tillage + residue harvest (CT-RH), within an irrigated corn system near Akron, Colorado. Numbers beneath each mean value and in parentheses represent the standard error of the mean. P-values for linear mixed models are reported at the bottom. Values in bold represent significant ($p < 0.05$) or marginally significant ($p < 0.10$) factor effects.

Management	**Bulk density (g cm^{-3})**	**Total N (%)**	**Total C (%)**	**POXC (mg kg^{-1})**	**GMC (%)**	**MWD (µm)**	**Available P (mg kg^{-1})**	**pH**	**EC (ds m^{-1})**
NT-R	1.19 (0.08)	0.17 (0.01)	1.66 (0.1)	615.4 (21.97)	22.18 (2.12)	675 (151.6)	1.56 (0.41)	7.3 (0.07)	0.24 (0.02)
NT-RH	1.29 (0.03)	0.13 (0.002)	0.99 (0.04)	485.3 (52.08)	18.72 (1.5)	409 (55.2)	1.25 (0.36)	7.2 (0.12)	0.15 (0.01)
CT-R	1.03 (0.05)	0.17 (0.01)	1.48 (0.05)	598.8 (22.81)	21.10 (0.58)	736 (177.1)	2.75 (0.51)	7.0 (0.15)	0.31 (0.04)
CT-RH	1.10 (0.03)	0.13 (0.01)	1.03 (0.04)	484 (53.64)	16.07 (1.68)	379 (48.9)	1.35 (0.31)	6.9 (0.16)	0.27 (0.04)
				P-values					
Tillage	**<0.001**	0.737	0.737	0.922	0.266	0.899	**0.051**	**0.025**	**0.008**
Residue	0.121	**<0.001**	**<0.001**	**<0.001**	**0.015**	**0.015**	**0.013**	0.597	**0.044**
Tillage*Residue	0.741	0.423	0.734	0.947	0.629	0.705	**0.090**	0.697	0.445

Table 4.2.

Mean values for soil earthworm and macroarthropod biomass and abundance in surface soils (0-10 cm) under different tillage and residue management combinations: no-till + residue retention (NT-R), no-till + residue harvest (NT-RH), conventional tillage + residue retention (CT-R) and conventional tillage + residue harvest (CT-RH) within an irrigated corn system near Akron, Colorado. Numbers to the right of each mean and in parentheses represent the standard error of the mean. P-values for linear mixed models are reported are reported at the bottom. Values in bold represent significant ($p < 0.05$) factor effects.

Management	**Earthworm biomass ($g\ m^{-2}$)**	**Earthworm abundance ($ind\ m^{-2}$)**	**Macroarthropod abundance ($ind\ m^{-2}$)**
NT-R	20.6 (5.0)	242 (76.2)	164 (22.3)
NT-RH	3.4 (1.5)	44 (22.8)	22 (6.0)
CT-R	14.3 (6.1)	134 (62.3)	162 (34.4)
CT-RH	1.41 (0.6)	22 (10.4)	54 (19.6)
		P-values	
Tillage	0.444	0.295	0.395
Residue	**<0.001**	**<0.001**	**<0.001**
Tillage*Residue	0.787	0.525	0.583

Table 4.3.

Indicator taxa and associate p-values or linear discriminant analysis (LDA) thresholds for surface soils (0-10 cm) under different tillage and residue management combinations: no-till + residue retention (NT-R), no-till + residue harvest (NT-RH), conventional tillage + residue retention (CT-R) and conventional tillage + residue harvest (CT-RH).

	Indicator taxa	**Management**			
		NT-R	**NT-RH**	**CT-R**	**CT-RH**
Macrofauna		p-values			
	Annelida	**0.04**	NI	NS	NS
	Aranaea	**0.04**	NS	NS	NS
	Chilopoda	**0.01**	NS	NS	NS
	Coleoptera	NS	NS	**0.01**	NS
Bacterial phyla		LDA threshold			
	Fibrobacteres	**>3.0**	NS	NS	NS
	Candidate division TM6	**>3.0**	NS	NS	NS
	Euryarcheaota	NS	NS	NS	**>3.0**
	Spirochaetae	NS	NS	**>3.0**	NS
	Chlamydiae	NS	NS	**>3.0**	NS
	Latescibacteria	NS	NS	**>3.0**	NS
	Cyanobacteria	NS	**>3.0**	NS	NS
	Thaumarcheaota	NS	**>3.0**	NS	NS
	Firmicutes	NS	**>3.0**	NS	NS
	Actinobacteria	NS	**>3.0**	NS	NS
	Gemmatimonadetes	NS	**>3.0**	NS	NS
	Armatimonadetes	NS	**>3.0**	NS	NS
	Candidate division SMF211	NS	**>3.0**	NS	NS
	Chloroflexi	NS	**>3.0**	NS	NS
	Verrucomicrobia	NS	**>3.0**	NS	NS
Fungal phyla		LDA threshold			
	Rozellomycota	**>4.0**	NS	NS	NS
	Basidiomycota	NS	NS	**>4.0**	NS

NS = non-significant indicator

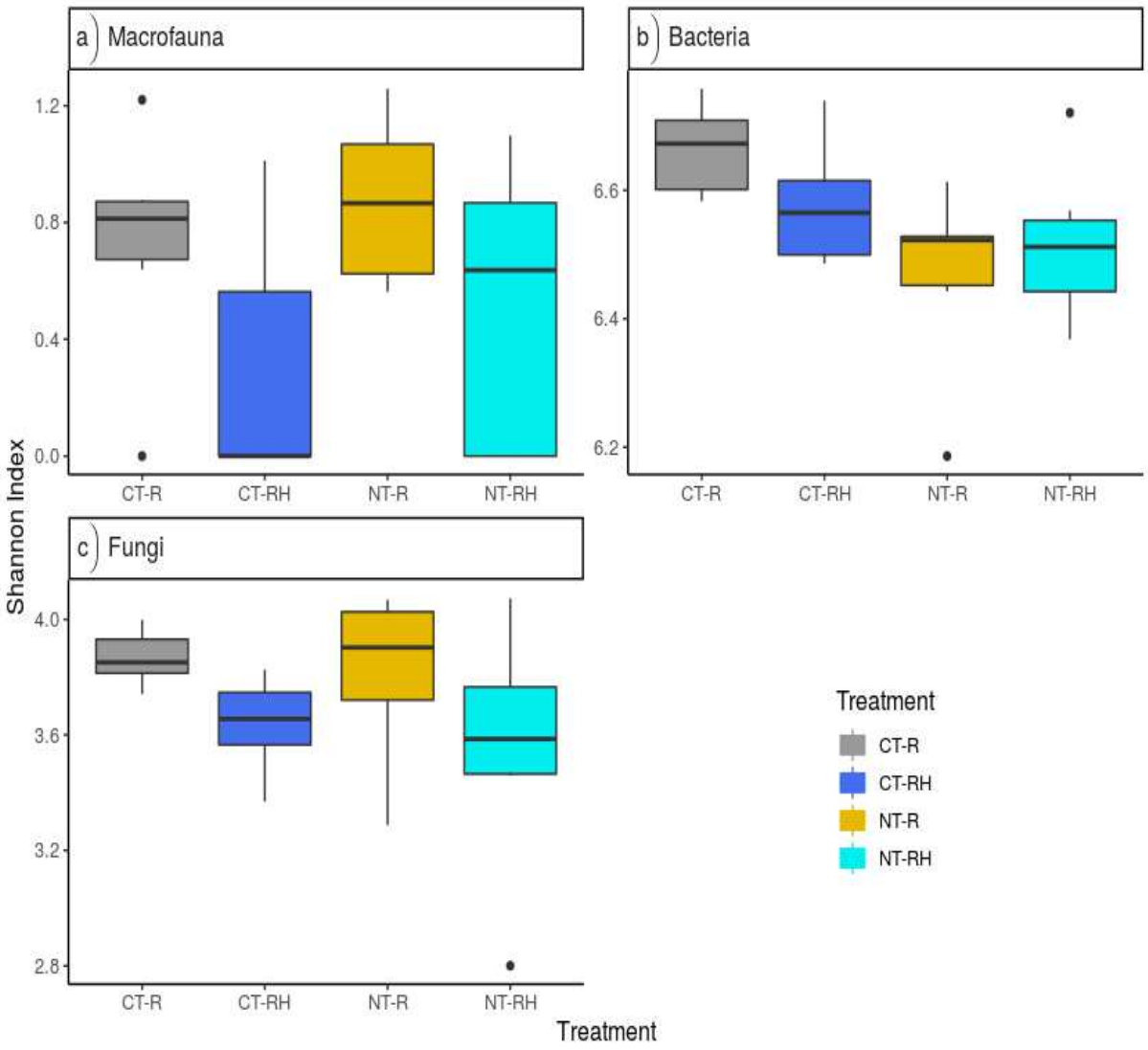

Figure 4.1. Boxplots indicating Shannon diversity index of a) macrofaunal,; b) bacterial; and c) fungal taxa in the topsoil (0-10 cm). Bold centerline within each boxplot represent median values. Treatment labels: no-till + residue retention (NT-R), no-till + residue harvest (NT-RH), conventional tillage + residue retention (CT-R) and conventional tillage + residue harvest (CT-RH).

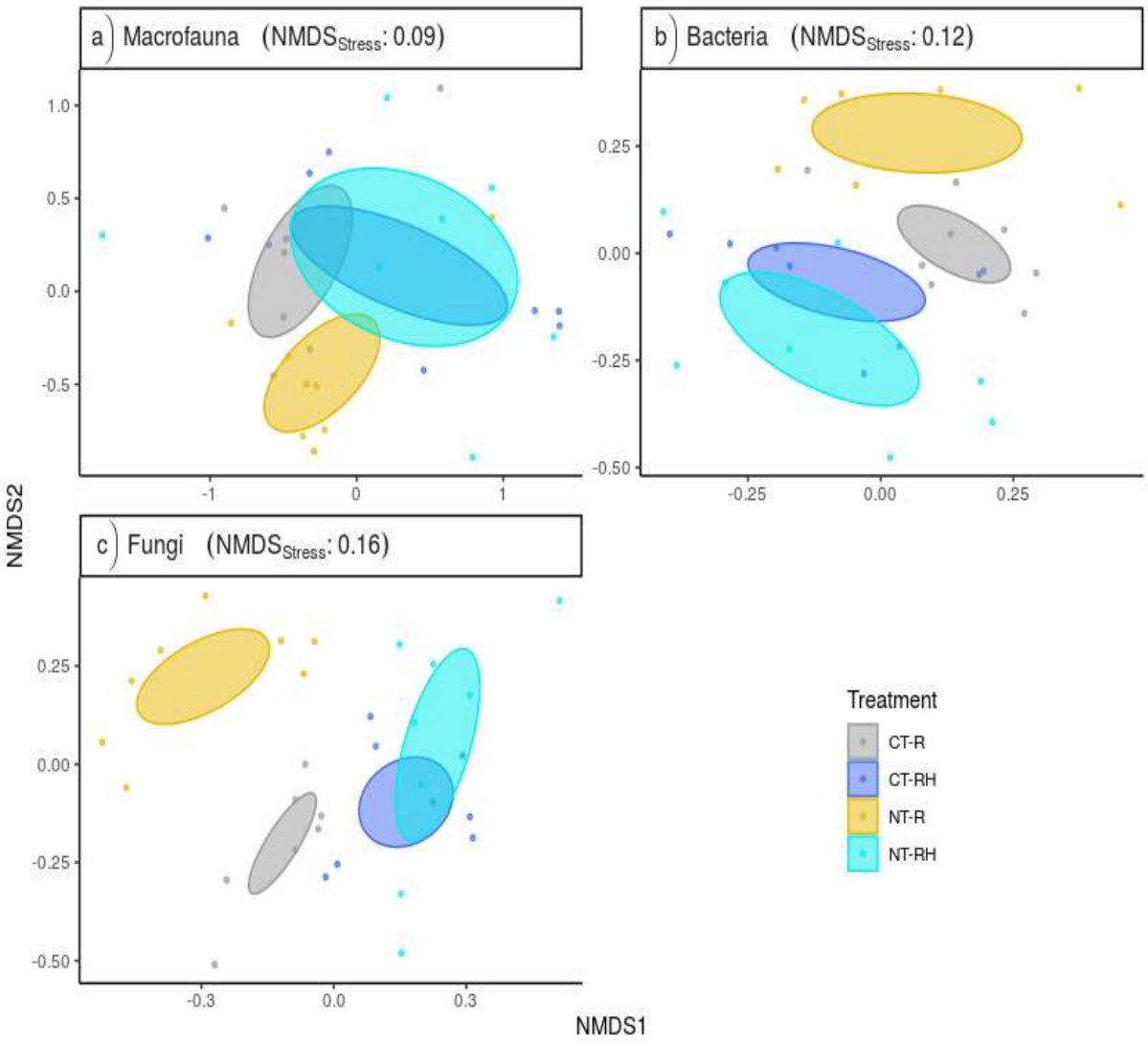

Figure 4.2. Nonmetric multi-dimensional scaled (NMDS) ordination plots of a) macrofaunal; b) bacterial; and c) fungal community structure in the topsoil (0-10 cm). Treatment labels: no-till + residue retention (NT-R), no-till + residue harvest (NT-RH), conventional tillage + residue retention (CT-R) and conventional tillage + residue harvest (CT-RH). Ordination represents Bray-Curtis dissimilarity distances.

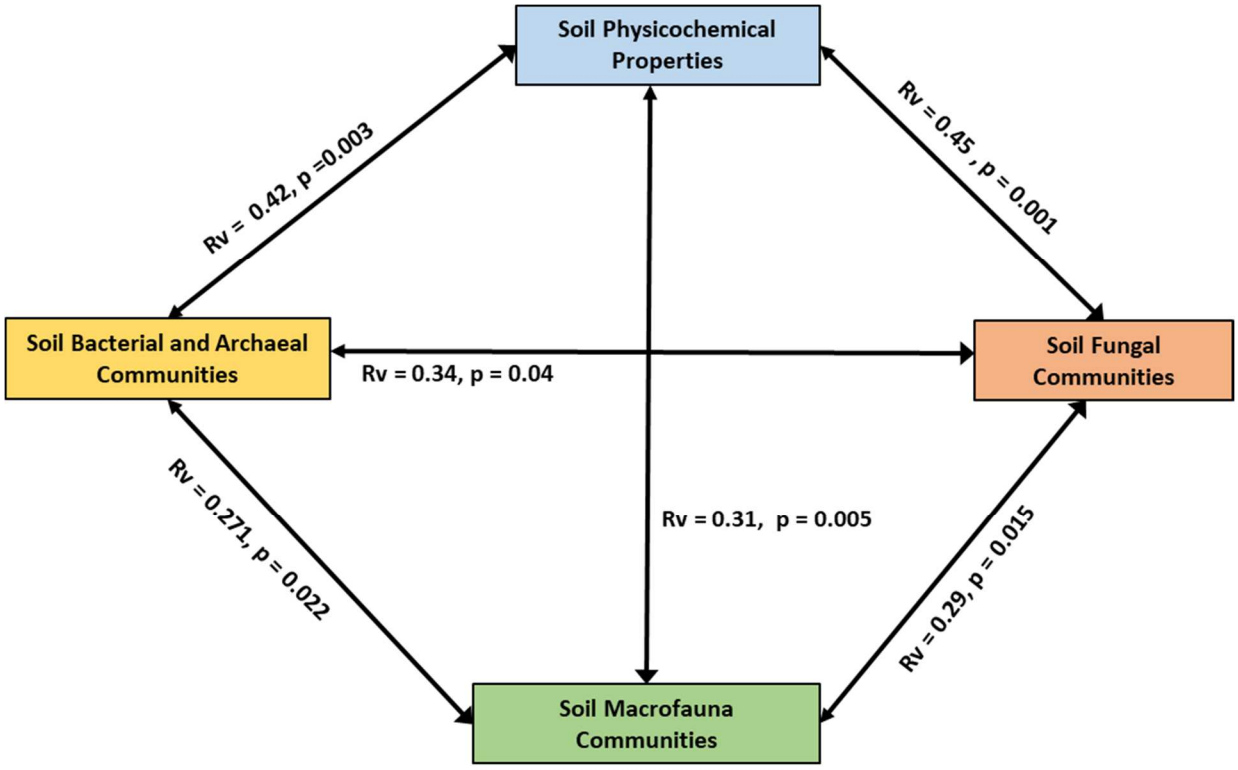

Figure 4.3. Schematic summary of the co-inertia analyses between four transformed datasets (soil physicochemical, macrofauna, fungal and bacterial data), with the Rv (matrix coefficient of covariation) and levels of significance (*P*-value) for each pair of data sets.

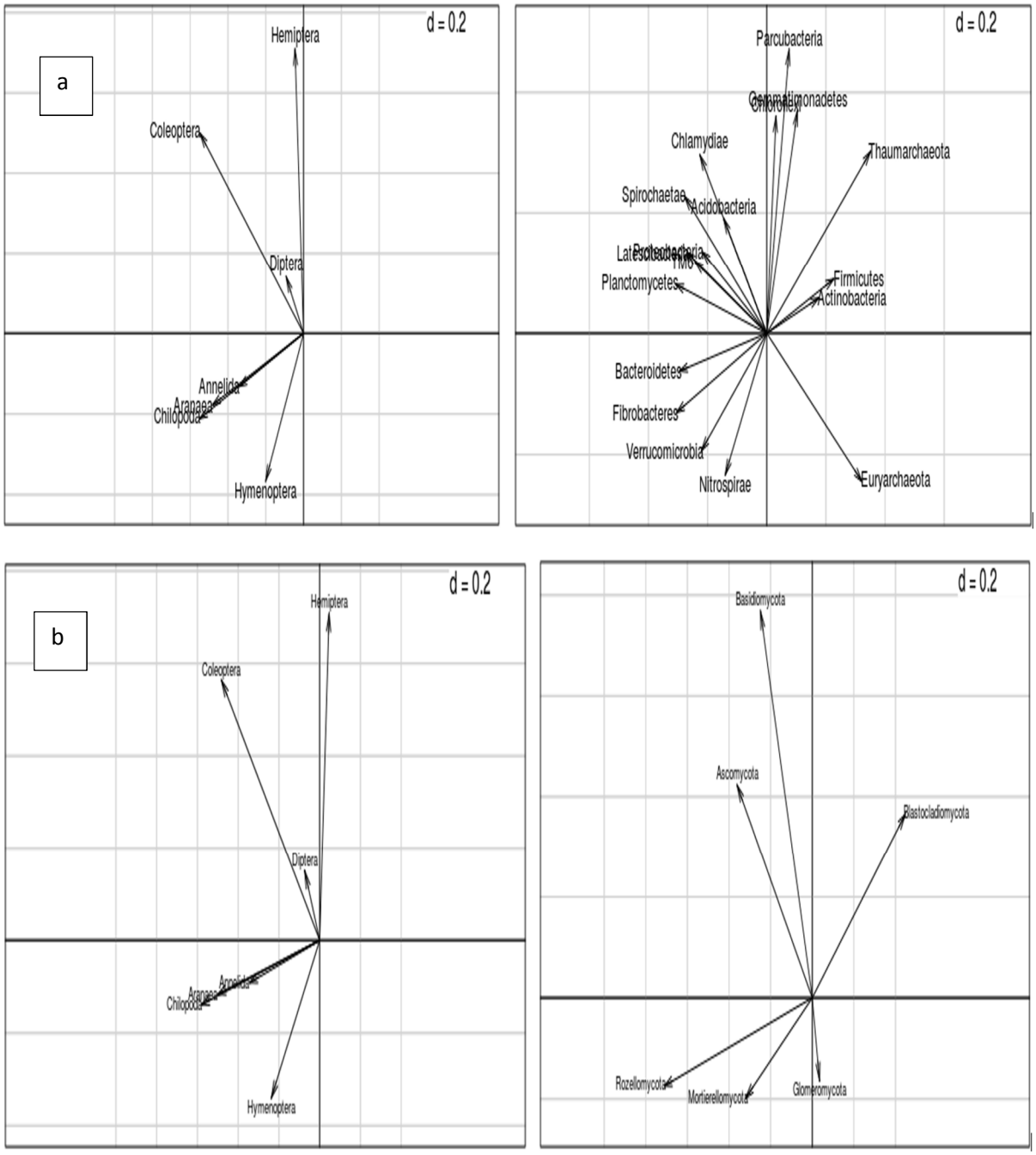

Figure 4.4. Coinertia analysis of (a) macrofauna taxa vs. versus bacterial phyla, (b) macrofauna taxa vs. fungal phyla. Covariation between all three data sets was significant (Monte Carlo permutation test, $P < 0.01$).

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CHAPTER 5: CONCLUSION

This dissertation was aimed at unravelling soil health outcomes under two different organic input management regimes involving a novel soil amendment derived from cheese manufacturing and corn residue management in semi-arid agroecosystems. The research questions that guided this body of work were:

1. What are the soil health benefits derived from the one-time, field application of a novel soil amendment derived from cheese manufacturing known as lactobionate?
2. What is the fate and persistence of lactobionate in soils and how are soil organic matter (SOM) fractions responding to lactobionate application with respect to C loss and stabilization?
3. Are there any linkages between soil biological and physicochemical health properties derived from the consistent field retention of corn residues and are these differences magnified when residues are left on the soil surface (no-till) versus incorporated into soils (conventional tillage)?

To answer the first question, field trials were conducted on wheat and corn to study the soil and crop benefits of the one-time, field application of lactobionate. Soil health benefits observed under lactobionate application for the wheat trial included higher soil water holding capacity, increased microbial biomass and a temporary immobilization of soil nitrate in microbial biomass with no negative effect on wheat grain yield and grain protein content. These benefits were observed mainly at the 5-15 cm soil depth. For the corn trial, no clear soil benefits were observed but a 14% increase in grain yield was observed. These findings suggest that feeding soils with lactobionate can yield soil health outcomes but the application rate and frequency, timing and mode of

application must be optimized for greater efficacy. Therefore, exploring alternative sources of high quality inputs such as food processing wastes and byproducts is highly recommended and can be a unique pathway to boost soil health particularly in low input agroecosystems such as my study site.

To study the fate of lactobionate in soils and its effect on priming and SOM dynamics, an 84-day laboratory incubation experiment using isotopically enriched lactobionate wasconducted. Lactobionate application stimulated soil CO_2 respiration and a priming effect that was negative for the first 14 days of the experiment and positive for the remainder of the incubation. As soil CO_2 respiration increased under lactobionate application, the examined SOM fractions responded to lactobionate application in different ways. The total and native C in the LF-POM fraction decreased while the total C in H-POM and MAOM fractions increased relative to unamended soils. While the WEOM fraction of the lactobionate-amended soils was greater than the unamended soils, this fraction declined exponential and quicker than other fractions. These findings demonstrated that positive priming occurs alongside a net decrease inLF-POM and an exponential decline in the WEOM fraction (the fraction with no C protection mechanisms). In addition, the increase observed in the H-POM and MAOM fractions shows the C-protection capacity of these fractions despite priming effects. More importantly, this work shows that priming can lead to the shuffling of C from less C-protected fractions to the more protected fractions. Thus, while feeding soils with lactobionate can induce priming effects, it can also increase the MAOM fraction in a relatively short period of time, a fraction known for its resistance to management.

Retaining residues in soils is another approach to feeding soils with high quality inputs. However, it remains unclear how the frequency of residue retention and the mode of retention (tillage vs no-till) can influence soil biological and physicochemical health properties. By manipulating residue

management alongside tillage through a 6-year field experiment, this study has attempted at resolving these knowledge gaps. This study showed that retaining residues (regardless of tillage mode) provided more soil health benefits when implemented consistently for 6 years but these benefits were mostly observed at the 0-10 cm soil depth (topsoil). The soil health properties enhanced under residue retention ranged from the physical (soil water content, soil aggregate stability), to the chemical (Soil C, N, active C, available P), to the biological (macrofauna and fungal alpha diversity). It was also clear that soil macrofauna and microbial communities respond in similar ways to residue and tillage management. Residue retention management stimulated a higher abundance of litter transformers (earthworms) and predators (beetles, spiders, centipedes) as well as C-degrading decomposers (Ascomycota, Basidiomycota, Bacteriodetes, Latescibacteria). Another key finding from the study was the strong association observed between soil physicochemical and biological properties indicating the linkages between soil health properties and the cascading effects of management on multiple soil properties targeting a single soil property. Hence, these findings demonstrate that consistently feeding soils with residue enhances soil biological, physical and chemical properties and that communities of soil macro- and microorganisms tend to respond in similar ways to management interventions.

Bringing these studies together, a number of inferences can be made on feeding soils with different inputs and their effects on soil health. First, there are clear spatial effects for soil health under input management as the top 10-15 cm of soil are clearly impacted to a greater extent than deeper profiles. Furthermore, time and management frequency are vital for soil health. The consistent implementation of residue retention for 6 years had a greater impact on soil health as compared to the one-time application of lactobionate. In addition, the quality of the inputs may have different impacts on soil health. While residues are plant-derived consisting of both labile and recalcitrant

constituents, lactobionate is derived from cheese production and dominated by labile, low molecular weight compounds. As demonstrated in the incubation study, lactobionate ends up quicker in the MAOM fraction due to its bioavailability to soil microbes but we can theorize that corn residues will be predominant in the POM fraction as shown in previous studies. Thus, the differences in quality of these inputs will have both convergent and divergent consequences for soil health. Another key inference is that certain soil health properties respond quicker to management as compared to others and there is a latent link between different soil properties. Hence, feeding soils consistently will usually lead to ripple effects on a wide range of soil health properties and outcomes.

APPENDIX I

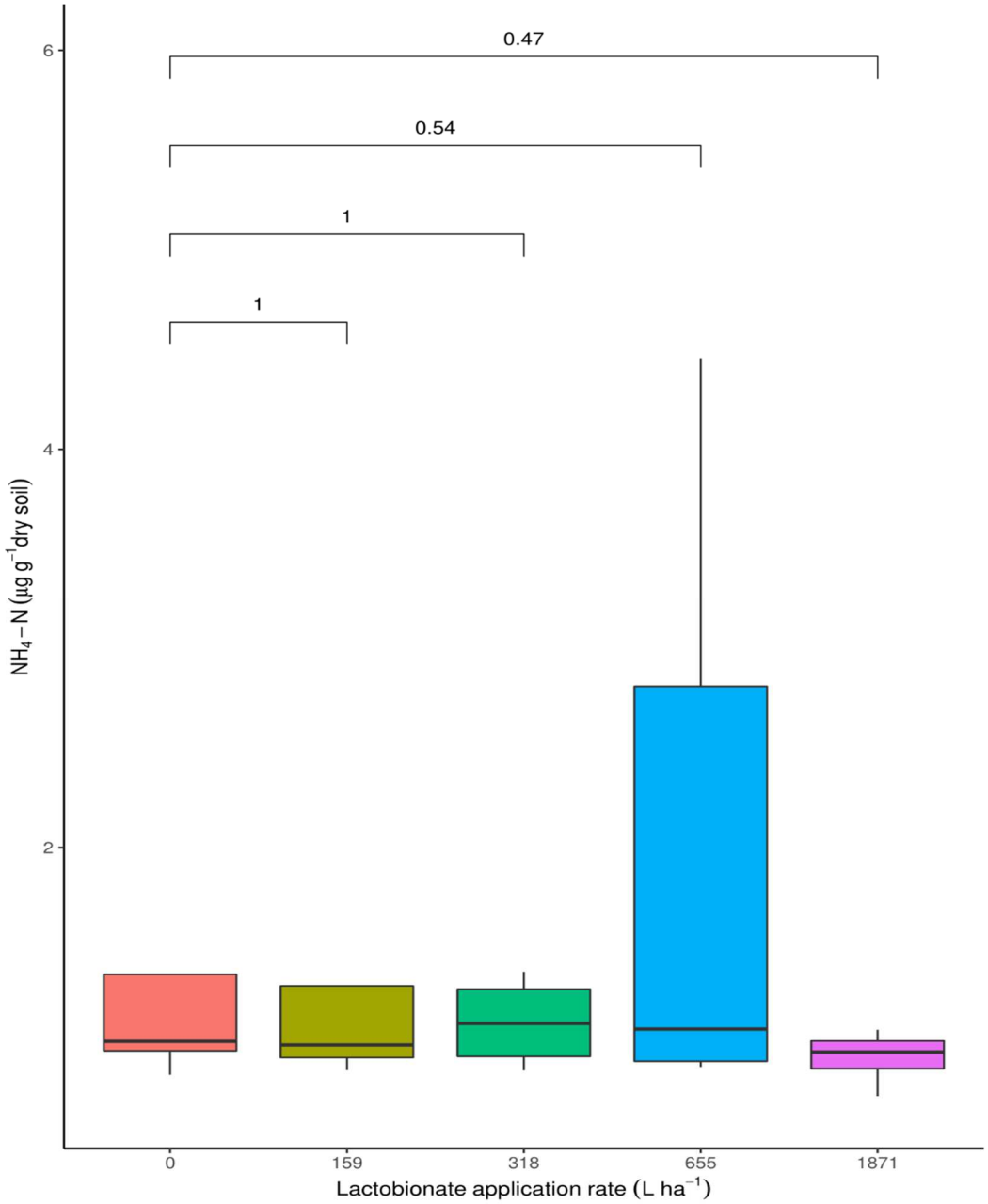


Figure S1.1. Effect of lactobionate application on soil ammonium-N for wheat trial (0-5 cm). Horizontal lines and p-values above each boxplot is the pairwise comparison of each treatment and control.

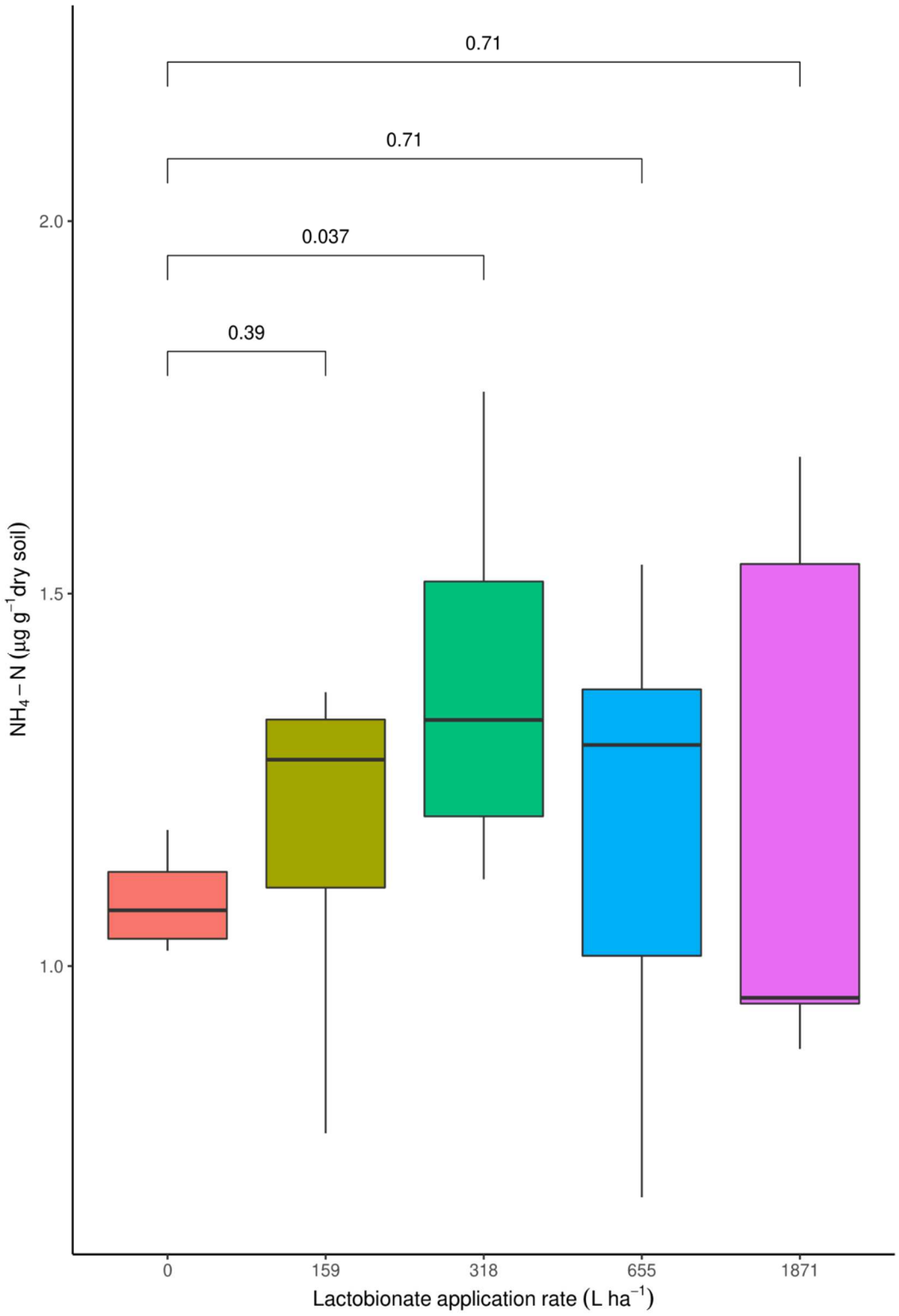


Figure S1.2. Effect of lactobionate application on soil ammonium-N for wheat trial (5-15 cm). Horizontal lines and p-values above each boxplot is the pairwise comparison of each treatment and control.

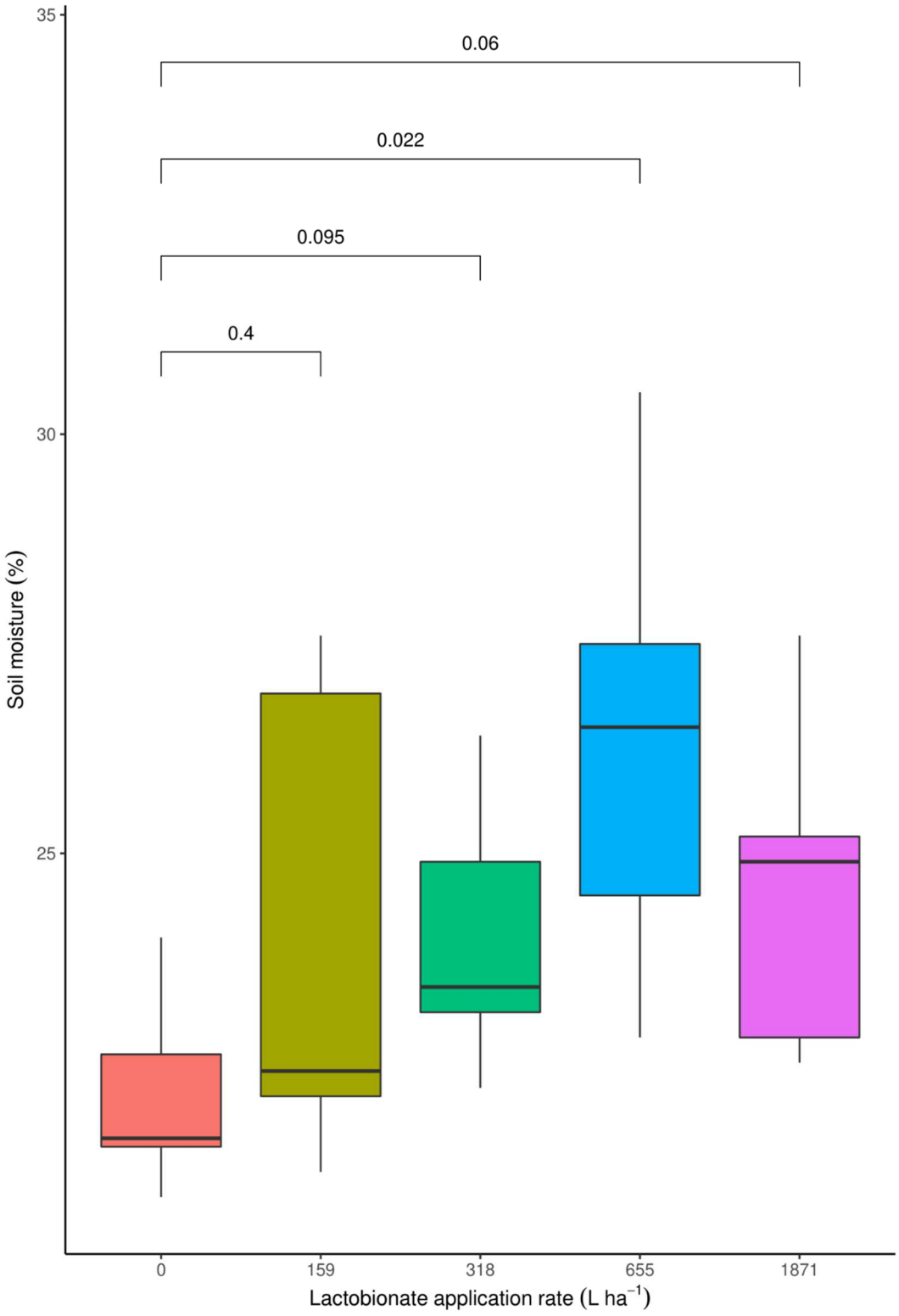


Figure S1.3. Effect of lactobionate application on volumetric soil moisture content for wheat trial (October 2017). Horizontal lines and p-values above each boxplot is the pairwise comparison of each treatment and control.

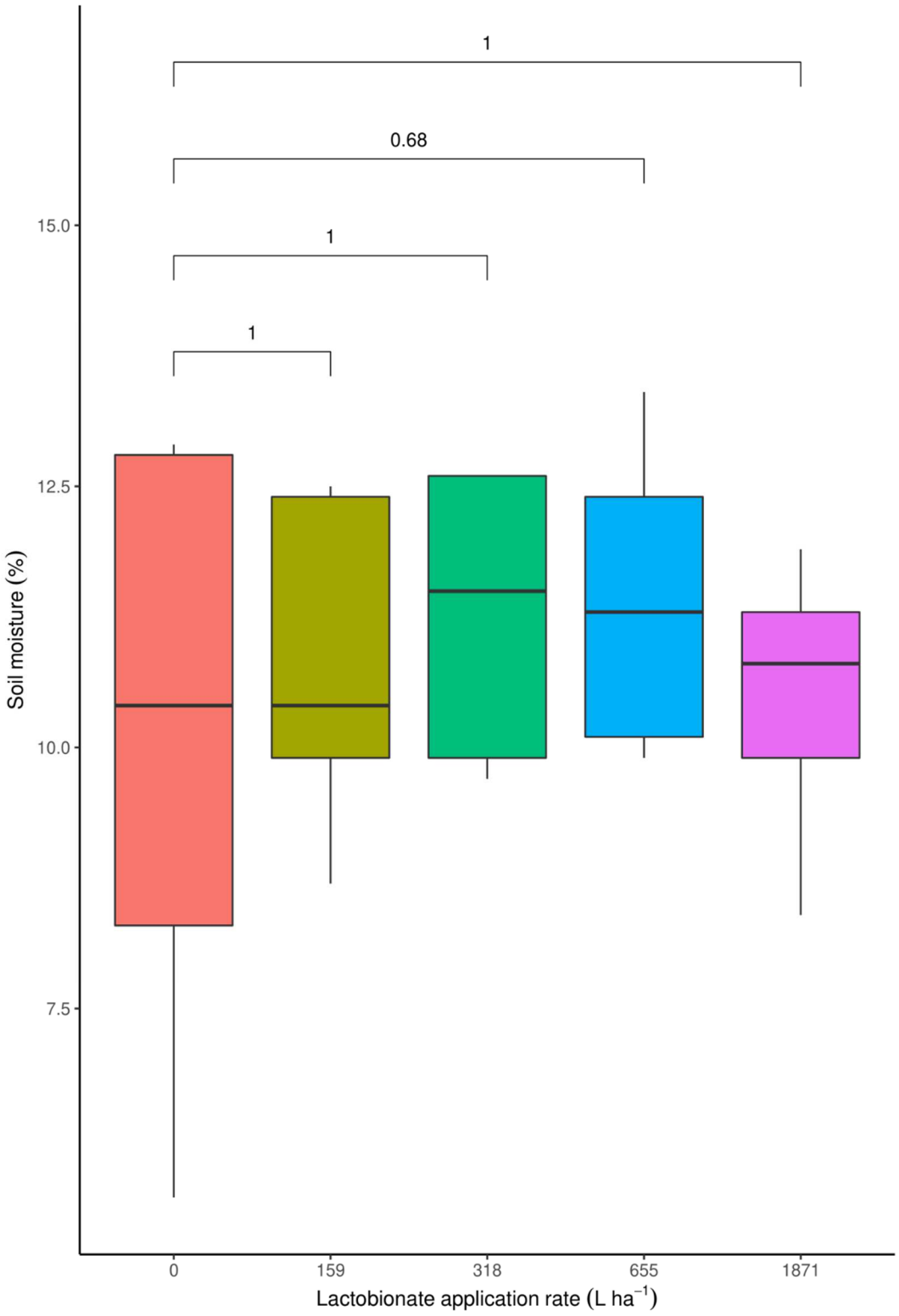


Figure S1.4. Effect of lactobionate application on volumetric soil moisture content for wheat trial (November 2017). Horizontal lines and p-values above each boxplot is the pairwise comparison of each treatment and control.

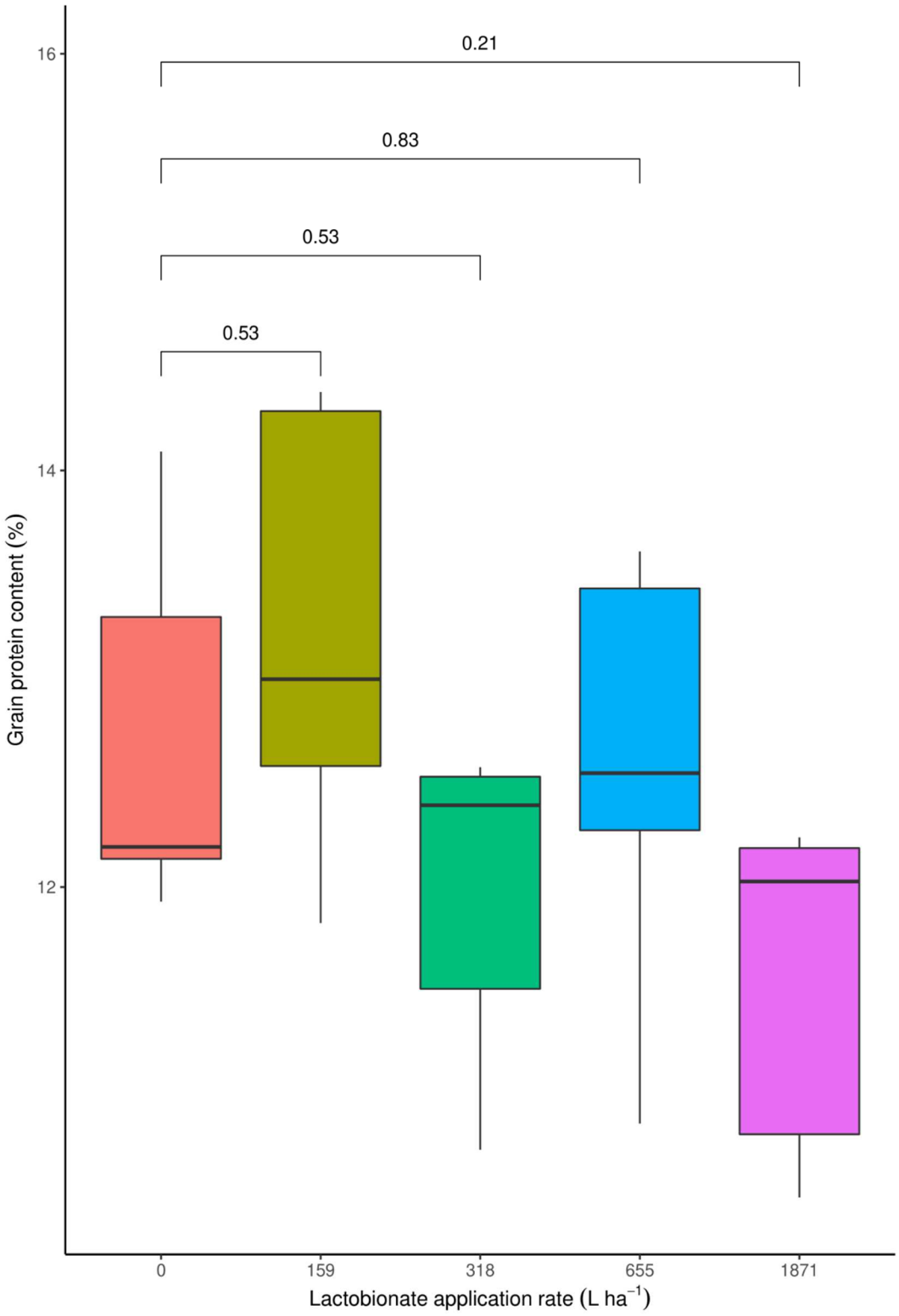


Figure S1.5. Effect of lactobionate application on wheat grain protein content. Horizontal lines and p-values above each boxplot is the pairwise comparison of each treatment and control.

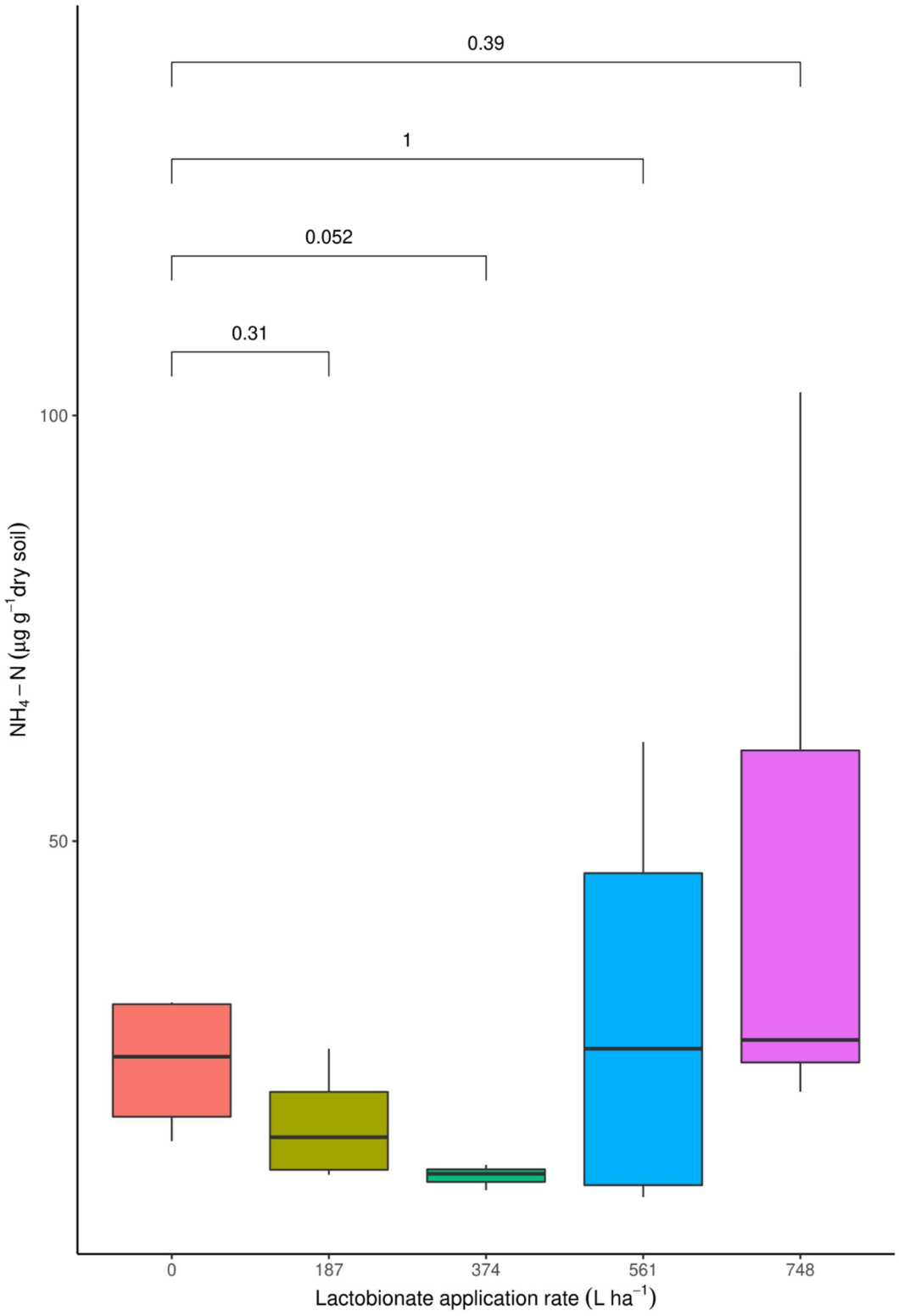


Figure S1.6. Effect of lactobionate application on soil ammonium-N for corn trial (0-5 cm). Horizontal lines and p-values above each boxplot is the pairwise comparison of each treatment and control.

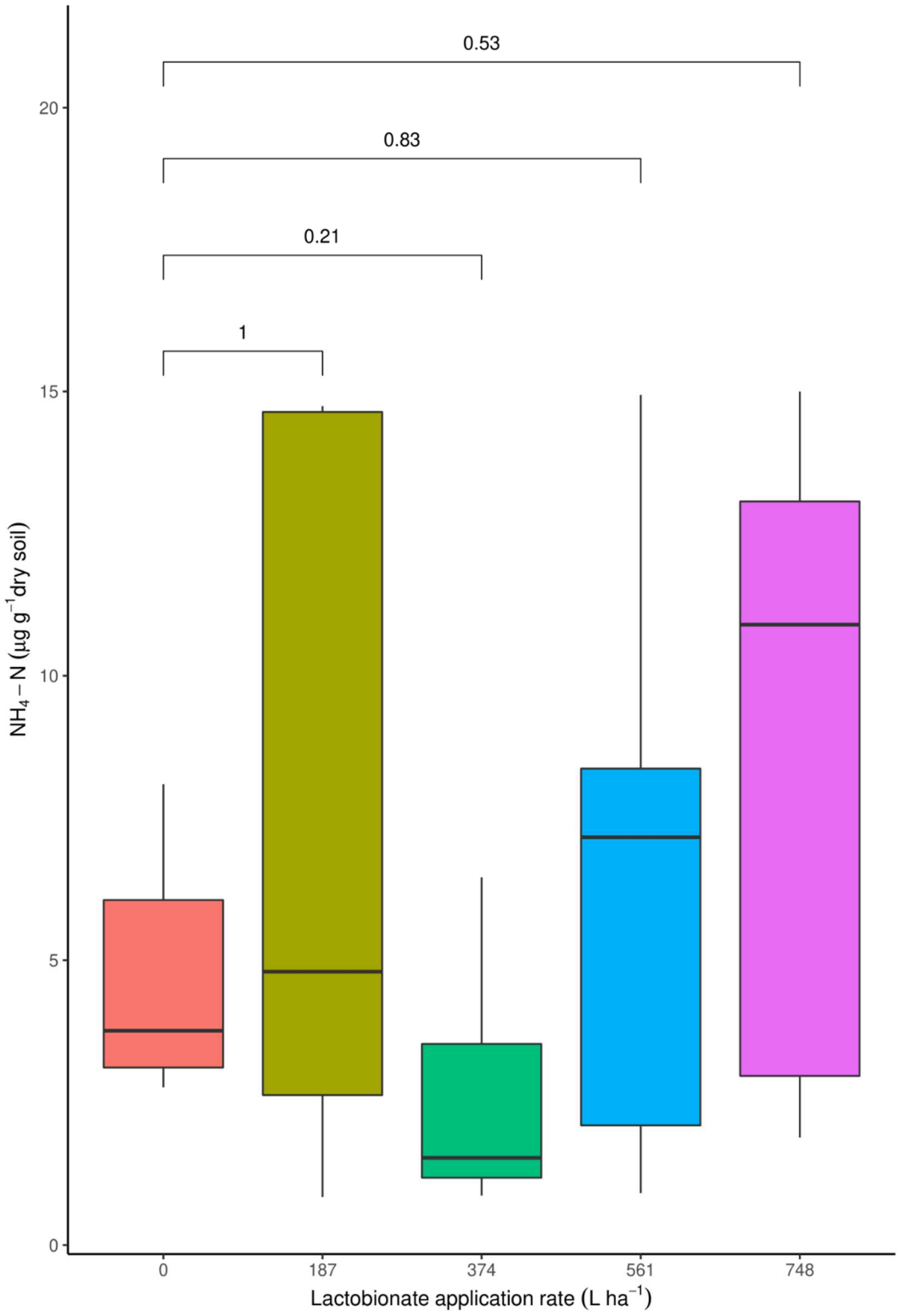


Figure S1.7. Effect of lactobionate application on soil ammonium-N for corn trial (5-15 cm). Horizontal lines and p-values above each boxplot is the pairwise comparison of each treatment and control.

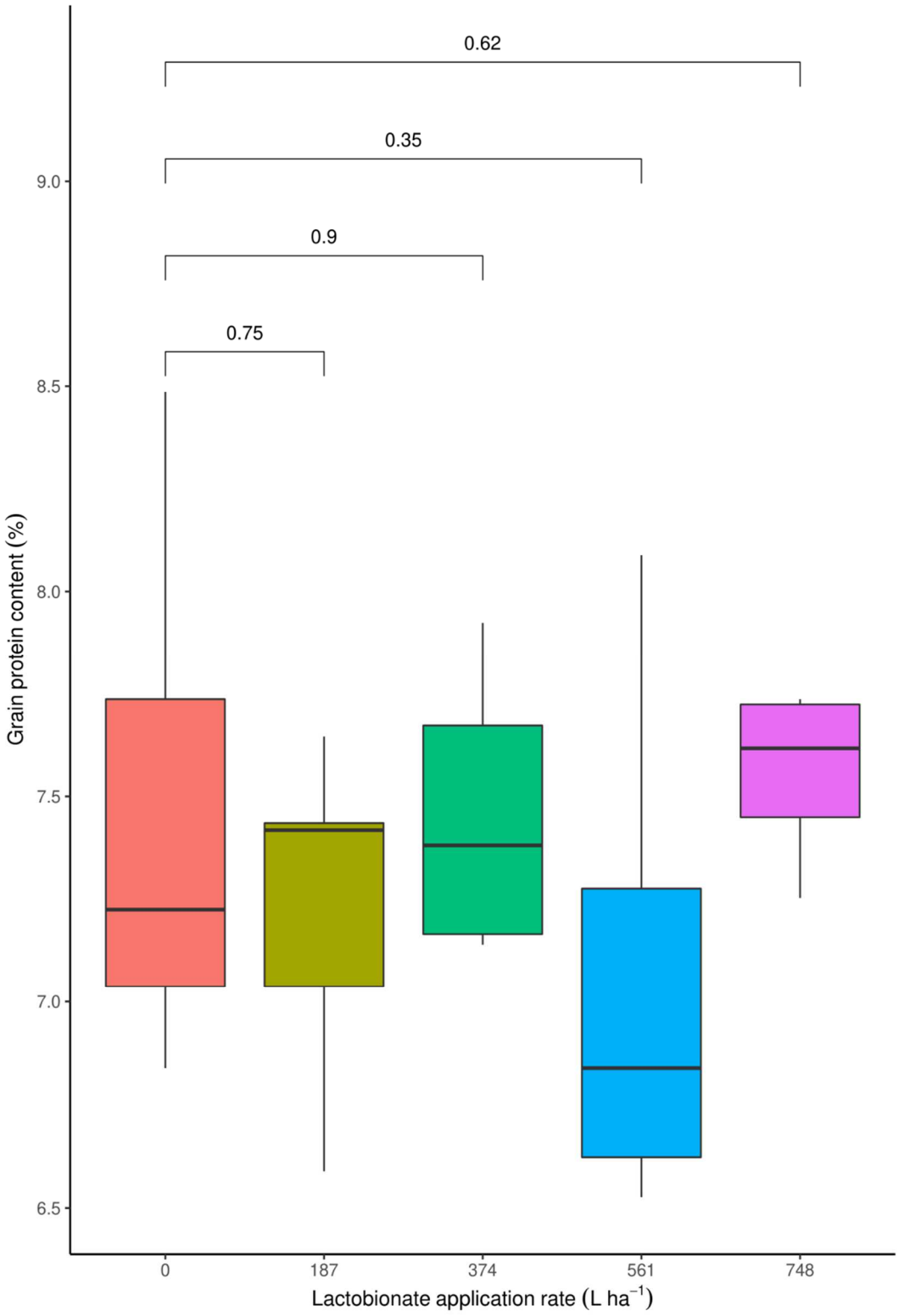


Figure S1.8. Effect of lactobionate application on corn grain protein content. Horizontal lines and p-values above each boxplot is the pairwise comparison of each treatment and control.

APPENDIX II

Table S2.1

$\delta^{13}C$-CO_2 values of unamended control, natural abundance (^{12}C) lactobionate-amended soils and ^{13}C lactobionate-amended soils for an 84-day incubation period.

Time (days)	Unamended control ($\delta^{13}C$-CO_2)	Amended soils with ^{12}C lactobionate ($\delta^{13}C$-CO_2)	Amended soils with ^{13}c lactobionate ($\delta^{13}C$-CO_2)
0	-10.062	-16.3626	113.0368
1	-12.8438	-22.0952	584.5774
2	-10.503	-23.6838	1131.176
3	-8.2757	-27.3489	1198.102
4	-13.7184	-24.9486	1194.686
5	-15.522	-30.7373	1154.989
6	-13.4794	-25.8832	1166.698
7	-14.9066	-23.2622	1162.852
8	-14.5518	-22.1696	1147.126
9	-15.411	-21.7908	1130.774
10	-16.2492	-21.2202	1083.062
12	-12.7432	-28.2402	1056.895
13	-10.4478	-21.3098	1016.6228
14	-13.3246	-20.9982	1002.1498
15	-13.1146	-20.6	951.114
17	-16.9964	-21.6264	892.674
19	-17.8346	-22.4192	835.7444
21	-17.9804	-21.0992	784.5154
24	-19.2546	-21.4424	725.0004
27	-18.7748	-20.3328	664.4096
29	-18.6984	-20.0902	619.3014
32	-19.8474	-20.858	580.9062
34	-19.505	-20.0904	539.9012
36	-17.9236	-20.577	484.5892

39	-19.2528	-21.1428	479.1714
41	-17.8584	-20.755	448.941
43	-18.1576	-21.2218	439.2434
46	-18.7882	-21.043	427.6394
49	-19.236	-20.2396	412.868
53	-20.1926	-20.3096	383.0238
55	-19.47648	-19.2494	350.5858
61	-21.0032	-20.7786	318.9392
64	-20.1066	-19.7654	290.8666
67	-20.3578	-19.454	272.7086
74	-21.697	-20.90925	258.3442
78	-21.213	-19.9515	242.3132
81	-20.971	-19.237	228.4846
84	-20.9462	-19.7045	215.4466

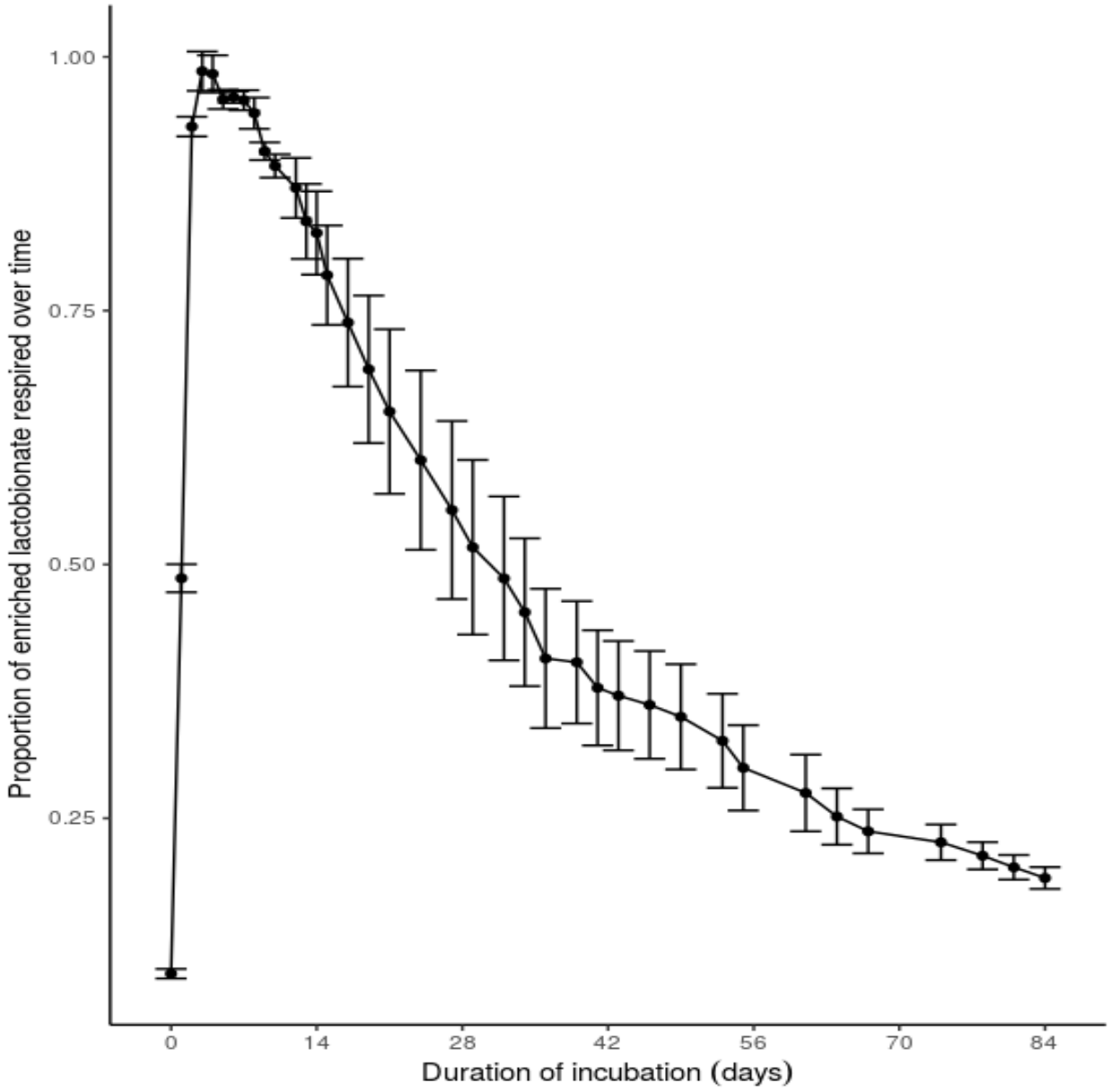


Figure S2.1. Mean proportion of respired lactobionate-carbon (^{13}C) during an 84-day incubation (n = 5, error bars are standard error of the mean).

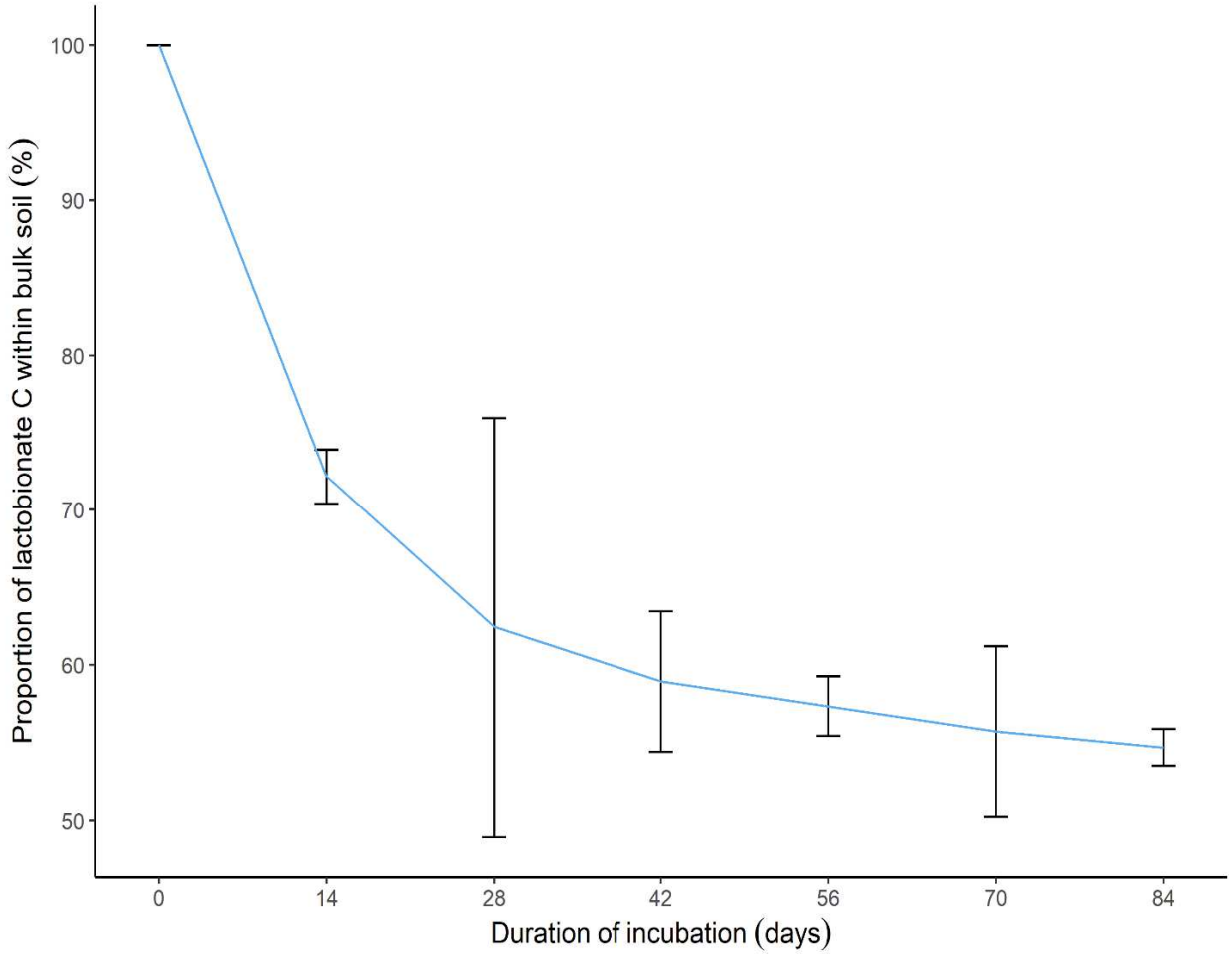


Figure S2.2. Mean proportion of lactobionate-carbon remaining in the bulk soil carbon relative to initial amount added during an 84-day incubation (n = 5, error bars are standard error of the mean).

APPENDIX III

Table S3.1

Mean soil physicochemical properties for subsoil (10-20 cm) under different tillage and residue management combinations: no-till + residue retention (NT-R), no-till + residue harvest (NT-RH), conventional tillage + residue retention (CT-R) and conventional tillage + residue harvest (CT-RH), within an irrigated corn system near Akron, Colorado. Numbers beneath each mean value in parentheses represent the standard error of the mean. P-values are reported at the bottom. Values in bold represent significant ($p < 0.05$) factor effects.

Management	Bulk density ($g\ cm^{-3}$)	Total N (%)	Total C (%)	POXC ($mg\ kg^{-1}$)	GMC (%)	MWD (μm)	Available P ($mg\ kg^{-1}$)	pH	EC ($ds\ m^{-1}$)
NT-R	1.39 (0.02)	0.11 (0.003)	0.89 (0.08)	378.4 (20.7)	17.8 (0.87)	343 (116.1)	0.71 (0.28)	7.4 (0.06)	0.16 (0.01)
NT-RH	1.28 (0.04)	0.11 (0.004)	0.73 (0.04)	395.4 (59.3)	16.6 (1.15)	306 (77.1)	0.22 (0.09)	7.3 (0.08)	0.13 (0.01)
CT-R	1.48 (0.01)	0.11 (0.007)	0.88 (0.05)	440.1 (79.7)	21.2 (1.78)	357 (74.3)	0.38 (0.09)	7.2 (0.13)	0.12 (0.02)
CT-RH	1.41 (0.03)	0.12 (0.003)	0.89 (0.03)	397.5 (9.1)	16.7 (2.36)	298 (50.8)	0.51 (0.12)	7.1 (0.1)	0.21 (0.03)
					p-values				
Tillage	**<0.001**	**0.081**	0.192	0.505	0.264	0.955	0.894	**0.011**	0.453
Residue	**<0.001**	0.191	0.172	0.798	**0.086**	0.552	0.236	0.472	0.162
Tillage*Residue	0.444	0.121	0.112	0.533	0.305	0.871	**0.041**	0.753	**0.008**

Table S3.2

Mean abundance of dominant soil macrofauna groups in the topsoil (0-10 cm) under different tillage and residue management combinations: no-till + residue retention (NT-R), no-till + residue harvest (NT-RH), conventional tillage + residue retention (CT-R) and conventional tillage + residue harvest (CT-RH), within an irrigated corn system near Akron, Colorado. Numbers to the right of each mean represent the standard error of the mean.

Management	Coleoptera	Chilopoda	Aranaea	Hemiptera	Hymenoptera	Diptera
NT-R	68 (12.70)	30 (6.37)	18 (8.25)	0 (0.00)	22 (19.8)	10 (10.20)
NT-RH	14 (5.44)	3 (2.29)	0 (0.00)	3 (2.29)	3 (2.29)	3 (2.29)
CT-R	128 (29.20)	12 (5.75)	7 (6.86)	10 (3.23)	0.0 (0.00)	5 (2.95)
CT-RH	40 (16.30)	6.0 (2.93)	0 (0.00)	4 (4.00)	0.0 (0.00)	4 (4.00)

Table S3.3

Mean earthworm biomass, abundance and total macrofauna abundance for subsoil (10-20 cm) under different tillage and residue management combinations: no-till + residue retention (NT-R), no-till + residue harvest (NT-RH), conventional tillage + residue retention (CT-R) and conventional tillage + residue harvest (CT-RH), within an irrigated corn system near Akron, Colorado. Numbers to the right of each mean represent the standard error of the mean. P-values are reported at the bottom. Values in bold represent significant ($p < 0.05$) factor effects.

Management	Earthworm biomass ($g\ m^{-2}$)	Earthworm abundance ($ind\ m^{-2}$)	Macroarthropod abundance ($ind\ m^{-2}$)
NT-R	8.80 (2.76)	116 (34.4)	16 (9.56)
NT-RH	0.44 (0.30)	10 (6.72)	10 (7.96)
CT-R	2.50 (1.70)	34 (19.20)	20 (7.86)
CT-RH	1.53 (0.76)	24 (12.82)	10 (5.18)
		p-values	
Tillage	0.132	0.111	0.791
Residue	**0.009**	**0.012**	0.313
Tillage*Residue	**0.036**	**0.033**	0.792

Table S3.4

Two-way ANOVA for bacterial and fungal phyla for topsoil (0-10 cm) under tillage and residue management. FDR adjusted P-values are reported. Values in bold represent significant ($p < 0.05$) factor effects and ns represent no significant difference.

Phyla	Tillage	Residue	Tillage*Residue
Proteobacteria	ns	**0.008**	ns
Acidobacteria	ns	ns	ns
Actinobacteria	ns	ns	ns
Bacteroidetes	ns	**0.001**	ns
Gemmatimonadetes	ns	ns	**0.071**
Chloroflexi	ns	ns	**0.073**
Verrucomicrobia	ns	ns	ns
Planctomycetes	ns	**0.001**	ns
Thaumarcheaota	ns	**<0.001**	**0.022**
Nitrospirae	ns	ns	ns
Latescibacteria	ns	**0.001**	ns
Spirochaetae	ns	**<0.001**	ns
Firmicutes	ns	**0.058**	**0.055**
Euryarchaeota	ns	**<0.001**	ns
Parcubacteria	ns	ns	ns
Fibrobacteres	ns	**0.001**	**0.047**
Chlorobi	ns	**0.094**	ns
Hydrogenedentes	ns	ns	ns
Candidate division TM6	ns	**0.002**	ns
Elusimicrobia	ns	ns	ns
Microgenomates	ns	**0.027**	ns
Chlamydiae	ns	**0.002**	ns
SHA109	ns	ns	ns
SM2F11	ns	ns	**0.009**
Woesearchaeota_DHVEG6	ns	ns	**0.031**
WCHB160	ns	ns	ns
Ascomycota	ns	**0.032**	ns
Basidiomycota	ns	**0.027**	ns
Mortierellomycota	ns	ns	ns
Rozellomycota	ns	**0.002**	**0.005**
Glomeromycota	ns	ns	ns
Blastocladiomycota	ns	ns	ns

Table S3.5

Mean bacterial and fungal Shannon diversity index for subsoil (10-20 cm) under different tillage and residue management combinations: no-till + residue retention (NT-R), no-till + residue harvest (NT-RH), conventional tillage + residue retention (CT-R) and conventional tillage + residue harvest (CT-RH), within an irrigated corn system near Akron, Colorado. Numbers to the right of each mean in parentheses represent the standard error of the mean. P-values are reported at the bottom. Values in bold represent significant ($p < 0.05$) factor effects.

Management	Bacterial Shannon diversity	Fungal Shannon diversity
NT-R	6.48 (0.02)	3.7 (0.11)
NT-RH	6.38 (0.06)	3.68 (0.15)
CT-R	6.52 (0.03)	3.66 (0.15)
CT-RH	6.46 (0.04)	3.68 (0.12)
	p-values	
Tillage	0.162	0.883
Residue	**0.042**	0.991
Tillage*Residue	0.544	0.882

Table S3.6

PERMANOVA output based on Bray–Curtis dissimilarities testing the effects of tillage and residue management on bacterial communities in the topsoil (0-10 cm).

Management	df	SumsOfSqs	MeanSqs	F.Model	R^2	Pr(>F)
Tillage	1	0.2473	0.24729	1.5329	0.0463	**0.025 ***
Residue	1	0.5095	0.50952	3.1584	0.0953	**0.001 *****
Tillage*Residue	1	0.2345	0.23448	1.4535	0.0439	**0.035 ***
Residuals	27	4.3557	0.16132		0.8146	
Total	30	5.3470			1.0000	

Table S3.7

PERMANOVA output based on Bray–Curtis dissimilarities testing the effects of tillage and residue management on fungal communities in the topsoil (0-10 cm).

Management	df	SumsOfSqs	MeanSqs	F.Model	R^2	Pr(>F)
Tillage	1	0.7985	0.79853	3.9726	0.1121	**0.001** ***
Residue	1	0.5218	0.52178	2.5958	0.0732	**0.002** **
Tillage*Residue	1	0.3789	0.37893	1.8851	0.0532	**0.013** *
Residuals	27	5.4273	0.20101		0.7616	
Total	30	7.1265			1.0000	

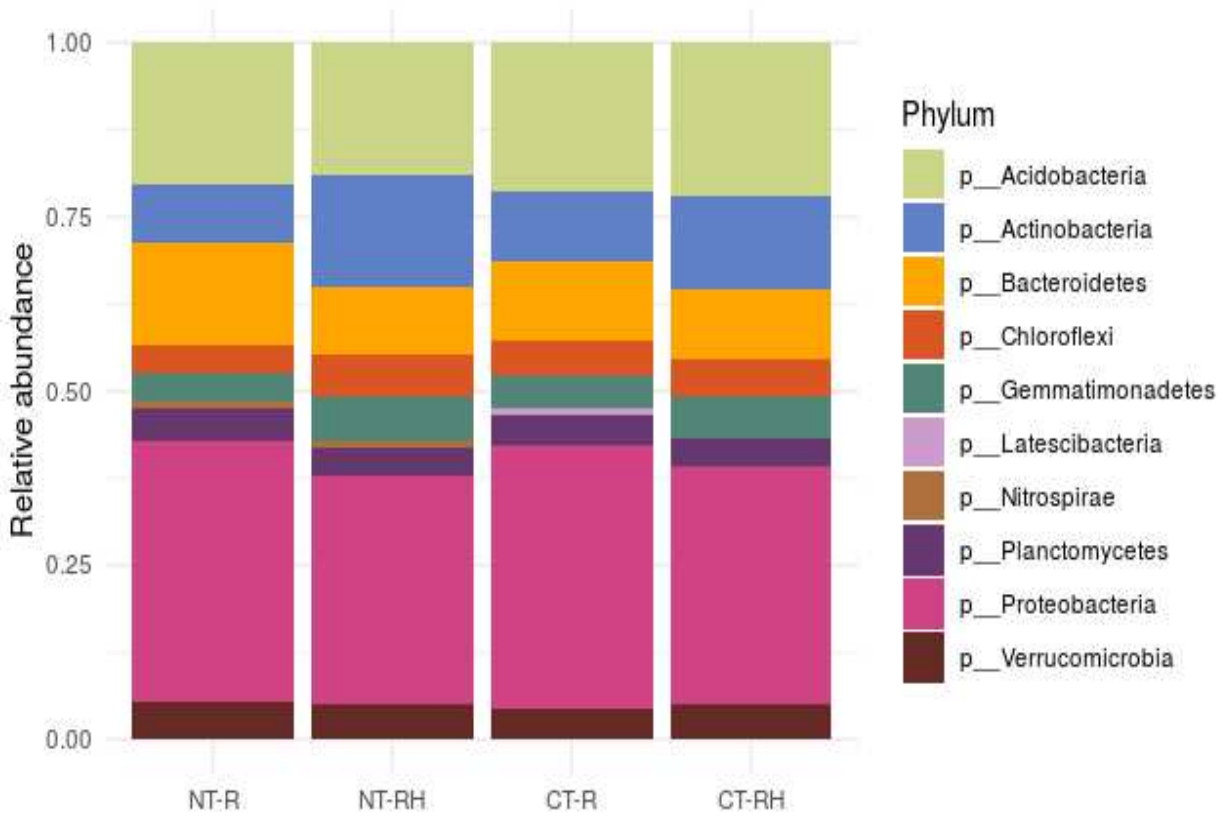


Figure S3.1. Relative abundance of bacterial phyla across different management practices in the topsoil (0-10 cm). Treatment labels: no-till + residue retention (NT-R), no-till + residue harvest (NT-RH), conventional tillage + residue retention (CT-R) and conventional tillage + residue harvest (CT-RH).

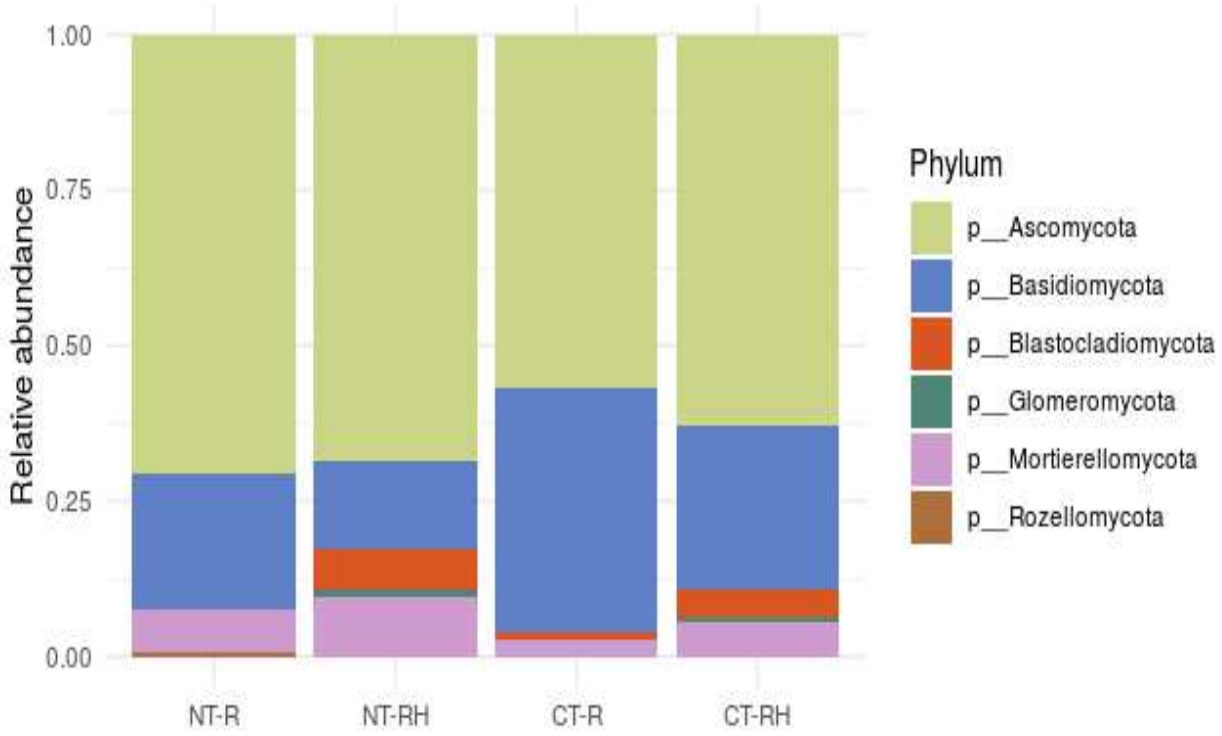
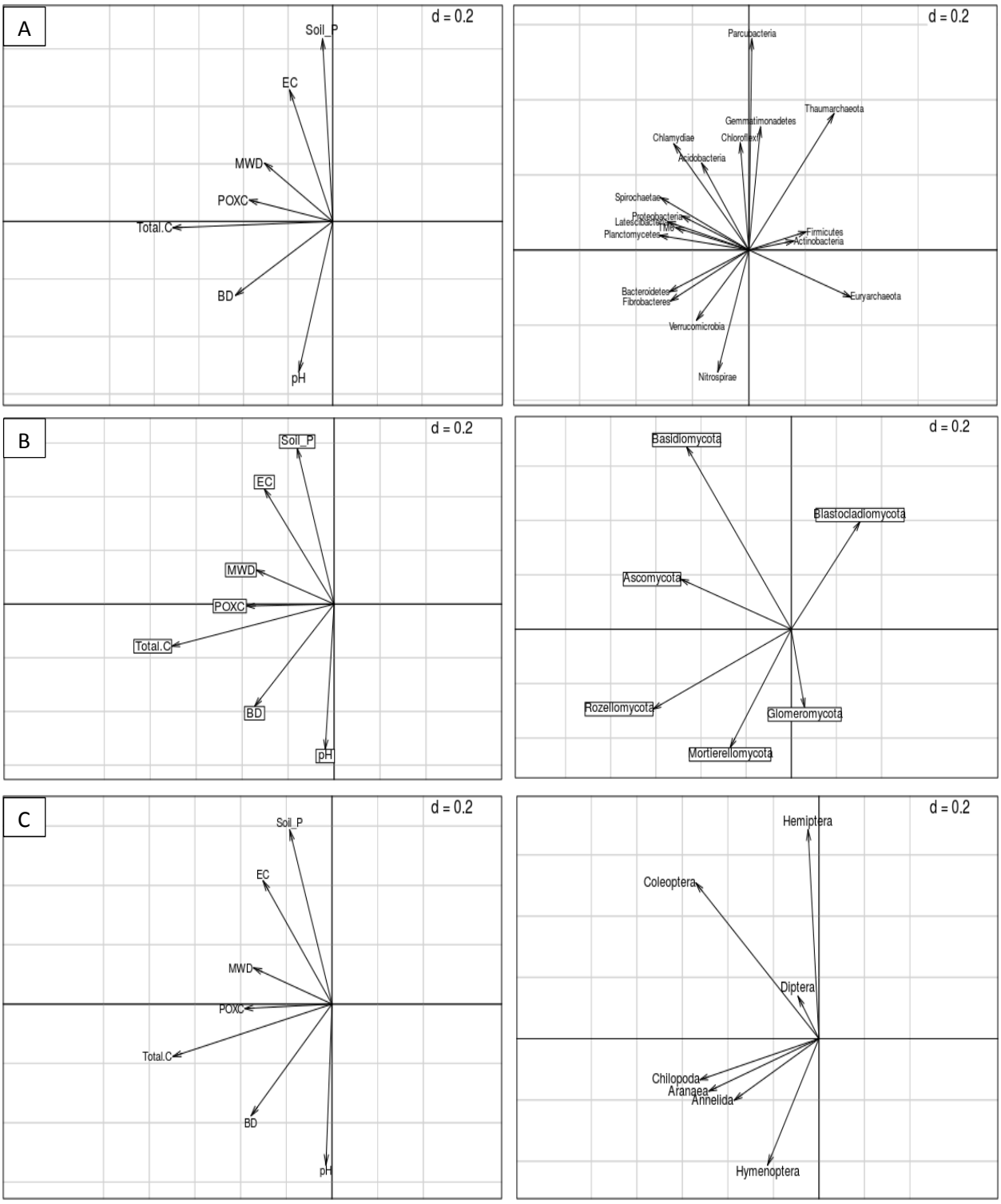


Figure S3.2. Relative abundance of fungal phyla across different management practices in the topsoil (0-10 cm). Treatment labels: no-till + residue retention (NT-R), no-till + residue harvest (NT-RH), conventional tillage + residue retention (CT-R) and conventional tillage + residue harvest (CT-RH).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Chlamydiae
Chloroflexi
Acidobacteria
Spirochaetae
Proteobacteria
Latescibacteria
Nitrospinae
Planctomycetes
Firmicutes
Actinobacteria
Bacteroidetes
Fibrobacteres
Euryarchaeota
Verrucomicrobia
Nitrospirae
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MWD
POXC
Total.C
BD
pH
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Blastocladiomycota
Ascomycota
Rozellomycota
Glomeromycota
Mortierellomycota
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MWD
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Diptera
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Annelida
Hymenoptera

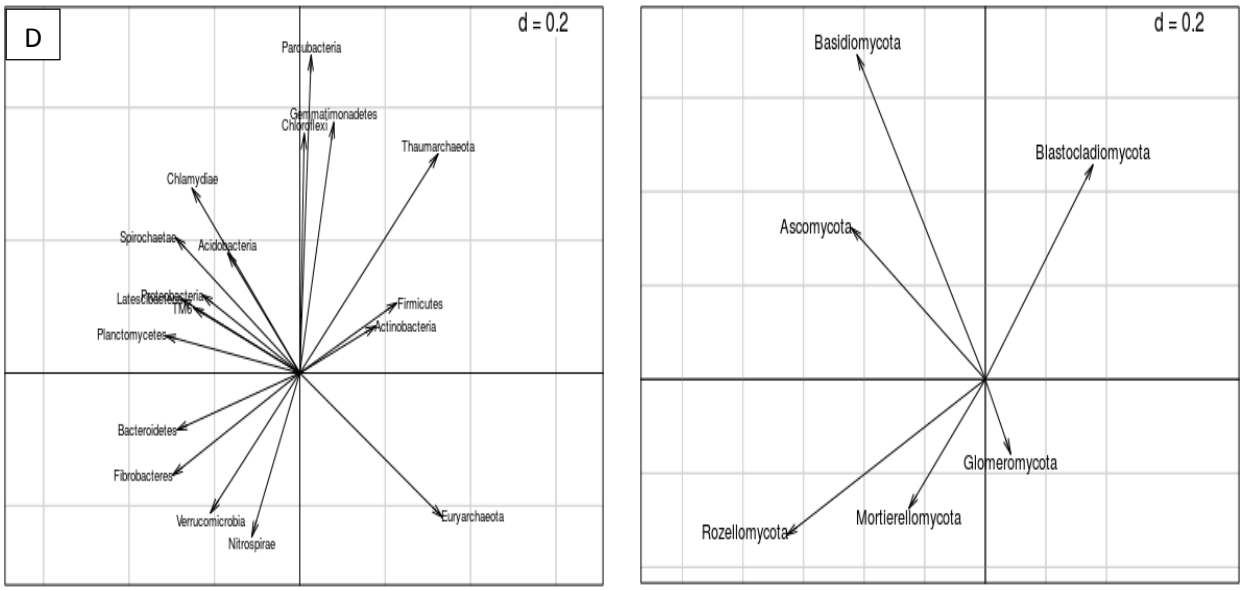

Figure S3.3. Coinertia analysis of (A) physicochemical properties vs. versus bacterial and archaeal phyla, (B) physicochemical properties vs. fungal phyla, (C) physicochemical properties vs. macrofauna taxa, (D) bacterial and archaeal phyla vs. fungal phyla. Covariation between all data sets was significant (Monte Carlo permutation test, $P < 0.01$).