

**Soil Health After 19 Years Under Organic and Conventional Agriculture and Restored
Prairie Grassland**

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Soil Health After 19 Years Under Organic and Conventional Agriculture and Restored Prairie Grassland

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Abstract

The Glenlea long-term study, located in Manitoba was established in 1992 to compare organic, conventional, no-input and restored prairie grass land management practices. Two 4-yr rotations exist under all agriculture management practices: annual-grain and forage-grain. The objective of the present study was to evaluate the effect of conventional, organic, and no-input agricultural management on soil health using restored grassland prairie as a biological benchmark for soil health. Microbial biomass carbon, microbial metabolic quotient (qCO_2), microbial biomass phosphorus, and microbial nitrous oxide quotient (qN_2O) were measured to evaluate soil health. Microbial biomass carbon, microbial biomass phosphorus, qCO_2 , and qN_2O were measured five times from May-October in 2010 under the *Triticum aestivum* (L.) (wheat) year in both rotations. Seasonal variation was significant for all measurements. Across all sample dates, MBC and activity were higher in the forage-grain (MBC= 1613 $\mu\text{g-MBC/g-dry soil}$; $qCO_2= 0.75 \text{ mg CO}_2\text{-C /g-dry soil/hr}$) rotation compared to the annual (MBC= 1124 $\mu\text{g-MBC/g-dry soil}$; $qCO_2= 0.60 \text{ mg CO}_2\text{-C /g-dry soil/hr}$). The forage-grain organic system (1718 $\mu\text{g-MBC/g-dry soil}$) had the highest MBC compared to its conventional (1476 $\mu\text{g-MBC/g-dry soil}$) counterpart and behaved most similarly to the restored grassland prairie (MBC= 1668 $\mu\text{g-MBC/g-dry soil}$; $qCO_2= 1.46 \text{ mg CO}_2\text{-C /g-dry soil/hr}$). Effects of rotation were significant ($P<0.0001$) for MBC, MBP, $CO_2\text{-C}$, qCO_2 , N_2O , and qN_2O suggesting rotation has a strong influence on soil microbial characteristics. The rotation and management interaction was statistically significant for MBC, CO_2 , and N_2O indicating that management effects microbial abundance and activity differently under annual rotations compared to rotations that include perennials, but does not affect the ratio of MBC to gas emissions. Rotation and fertility management practices, such as organic systems that include perennials, mimic natural systems like prairies and have the greatest capacity to sustain soil microbial life.

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1.1 Introduction

While large-scale conventional farms dominate the agricultural landscape, the number of certified organic acres in Canada increased from 1,304,227 in 2008 to 1,718,468 acres in 2009 (Macey, 2009; Macey, 2010). Increased participation in the production of organic goods and shift in availability of organic products from natural food stores to conventional supermarkets has increased availability to consumers. While many studies have compared conventional and organic yield potentials, nutrient cycling, and weed ecology, limited research is available on the long-term effects of agricultural practices on environmental health. As organic acres increase, it is important to evaluate the long-term impact of this growing sector on the environment, and compare these results with conventional agriculture practices. Long-term plots with both agriculture systems should subsequently be contrasted with a biological benchmark in order to determine the environmental impact of the practices.

Conventional and organic markets are dominated by different agricultural crops and this influences what is planted in the field, and ultimately impacts rotation length, weed and pest pressure, pesticides/chemical use, nutrient input, and environmental sustainability. Organic and conventional agriculture systems differ in approaches to managing crop rotation, soil fertility, weeds, and other pests. These agricultural management practices (rotation, weeds, pesticides etc.) directly influence environmental conditions and human health, particularly children and infants (Guillette et. al, 1998). (Implications for the effect of agriculture on human health should be investigated further but is not under the scope of this paper.)

Soil health is one indicator of the direct consequences of organic and conventional agricultural management practices have on the environment. While there are many definitions for soil health, the present study considers **soil health** to be the capacity of a soil to support biological life (active and passive) (Anderson and Domsch, 2010). Measuring the abundance and

activity of bacteria and fungi in soil is one way to determine the long-term implications of agricultural management on environmental health (Anderson and Domsch, 2010).

1.2 Literature Review

1.3 Bacteria and Fungi

From the terrestrial crust to aquatic environments, bacteria and fungi span Earth's massive network of ecological niches. As the first organisms, microbes form an interwoven labyrinth of energy and nutrient transformations at the base of the food web. With the ability to remain dormant for centuries, bacteria and fungi are the most widespread form of life on the planet and provide a robust reflection of environmental conditions.

Bacteria and fungi together are referred to as soil microbes. Microbial abundance and metabolic activity in agricultural soils is an important indication of carbon cycling, nutrient sustainability, and soil health (Chen, 2003). Abundance and activity of bacteria and fungi are used to measure soil health through microbial biomass carbon, and the microbial respiration quotient (qCO_2) (Anderson and Domsch, 2010). Microbial biomass phosphorus measurements can aid illustrations of microbial nutrient dynamics (Oehl et al., 2001). Microbial abundance and activity in soil characterizes the effect of agricultural management on carbon sequestration and mineralization-immobilization-turnover (Chen, 2003; Elser and Sterner, 2002).

Mineralization-Immobilization-Turnover is the conceptual model of biological nutrient cycling in the soil environment. *Mineralization* is the process of dead organic matter decomposition from large to small units that can be utilized by biological life. Physical and chemical mineralization are affected by soil texture, soil pH, climate, and weather conditions. Bacteria and fungi are the primary source of biological mineralization. Uptake by microbes

results in the *immobilization* of nutrients. During immobilization nutrients are tied up in biological organisms. *Turnover* is the conversion of immobilized nutrients to biologically available forms in the soil. Energy and nutrient transformations in agricultural soils are directed by the ecological process of mineralization-immobilization-turnover (Elser and Sterner, 2002).

As a result of mineralization-immobilization-turnover and all metabolic processes, gas is emitted as a byproduct. Potential metabolic byproducts at all stages of mineralization-immobilization-turnover include greenhouse gasses such as CO₂, N₂O, and CH₄. Soil microbes are the primary participants in decomposition and carryout the majority of mineralization-immobilization-turnover processes; therefore they are also the major contributors to greenhouse gas emissions into from soil (Elser and Sterner, 2002).

Microbial abundance measured by microbial biomass carbon, and microbial activity measured by gas emissions can be used together to reflect long-term soil health (Anderson and Domsch, 2010). The combination of these measurements as a fraction reflects the active and passive fraction of soil microbial populations. Active microbial populations are desirable for nutrient cycling. However, over-active microbes can increase the release of stable carbon from soil ultimately decreasing the capacity to sequester carbon from the atmosphere. Measuring microbial abundance and activity in long-term agricultural studies can increase understanding about how management practices mitigate the fine balance of biological life in soil. Biological benchmarks, like natural or restored landscapes, offer an environmental status quo for soil health and function as the standard of health for comparing other soils.

1.4 Microbial Biomass

The sum of bacterial and fungal mass is defined as microbial biomass. Knowledge about microbial biomass has many useful implications. Comparing microbial biomass dynamics in

natural and agricultural systems can assist our understanding and development of sustainable land management practices. In natural systems, animal-plant interactions and environmental conditions are the major influence on biological transformations, and provide important references for microbial biomass measurements. Agricultural management imposes these and additional variables like fertilizer, tillage, chemicals, which influence soil bacterial and fungal activity (Fliessbach et al., 2007). Different management practices result in distinct microbial ecological niches unique to each landscape (Mader et al., 2002). Microbial biomass abundance and activity measurements reflect the sustainability of management practices to support biological life in soil.

In addition to reflecting the soil's capacity to support life, microbial biomass is also related to nutrient cycling. Microbial biomass is correlated with carbon, nitrogen, and phosphorus transformation (Luxhøi, 2006; Oehl, 2001) and respiration rates in soil (Anderson and Domsch, 2010). Mineralization and immobilization of C and N are highly correlated with respiration ($R^2=0.87$) (Luxhøi, 2006), and indicate a direct relationship between microbial activity and nutrient cycling. Microbial biomass activity is also the driving force behind phosphorus mineralization-immobilization reactions (McLaughlin et al., 1988). Measurements of the relationship between microbial biomass and respiration (Anderson and Domsch, 1993) have been used world-wide to compare the effect of agricultural management practices on soil nutrient transformations and soil health (Heinze, 2010; Fliessbach, 2007; Bohme, 2005; Chen, 2003).

Agricultural management practices such as nutrient input, tillage and chemical use affect microbial communities differently. Comparing the long-term effect of conventional and organic agriculture on microbial biomass carbon, phosphorus and respiration can help optimize management strategies and reduce negative effects on soil health.

1.5 Microbial Substrates and Soil Organic Matter

In agricultural systems, manure, composted manure, root exudates, and plant residues returned to the soil are the primary source of organic carbon inputs. As plants photosynthesize during growth, fixed carbon from above ground vegetation is translocated to the root and either respired as carbon dioxide (CO₂), or is excreted into the soil as organic carbon sugars, amino acids, organic acids, and enzymes (Rovira, 1959). The amount of carbon exuded from the roots of a plant is closely related to the amount of carbon fixed by that plant through photosynthesis (Robinson and Scrimgeour, 1995), i.e. higher rates of photosynthesis lead to higher rates of root exudation. Root excretions in the rhizosphere from photosynthetically active plants and carbon from dead roots are the major source of microbial substrates (Foster, 1986; Watt et al., 2006). Microbes also feed on incorporated plant residues, organic carbon from manure and compost, but these are not the major sites of microbial colonization (Watt et al., 2006). Together these inputs supply the organic carbon required to support microbial life in agricultural soils. Therefore, microbial activity is directly related to primary plant production.

The rhizosphere is generally defined as the zone within 5mm of the root in soil. Root excretions are exuded in the rhizosphere and directly impact microbial organisms in the soil as the dominant energy source for survival and reproduction. The rhizosphere environment is not static and provides energy flow within the soil profile through time and space as the plant develops physiologically (Rovira, 1956). The *rhizosphere effect* (effect of root exudates in the rhizosphere) stimulates microbial survival and reproduction due to organic carbon compounds provided by root excretions (Rovira et al., 1976). Agricultural rotations that include perennial crops may have the potential to amplify the *rhizosphere effect* and extend the duration that roots

stimulate microbial growth due to the presence of roots over longer periods of time compared to annual rotations.

When plant litter from crop residues is introduced to the soil system it goes through a series of chemical and physical weathering processes before utilization by fungi and bacteria (Fig. 1.0). Litter from primary producers (plants) is not completely utilized by microbes within the first year (Anderson and Domsch, 1985). Soil organic matter of various degrees of stability will accumulate in soil over years. Recent additions of plant residue, compost, or manure replenish accumulated stable carbon pools and microbes utilize these aged pools of readily decomposable, slowly decomposing, and moderately recalcitrant litter (van Veen, 1984). Microbes also contribute to old and active pools of organic matter. Biomass from bacteria and fungi are decomposed and returned to mineral nutrient pools (van Veen, 1984) and stable soil organic matter.

Bacteria and fungi are the primary participants in the decomposition of organic matter (Elser and Sterner, 2002). Microbial processing of carbon from plants, manure, and compost is a key element in the soil food web and nutrient cycling. As microbes decompose carbon in metabolic processes, they convert that carbon into soil organic matter and respire CO₂ into the atmosphere (Figure 1.0). The high percentage of carbon in organic matter supplies food for other microbes. Decomposition of organic matter is related to carbon sequestration and nutrient cycling (Elser and Sterner, 2002), which are essential to soil health and productive agriculture systems since decomposition releases nutrients to plants. The microbial process of decomposition is particularly important for organic agriculture, where crop production depends on mineralization-immobilization-turnover cycling for plant available nutrients.

Microbial utilization of soil carbon and subsequent conversion to soil organic matter is critical for sustaining soil quality. Soil organic matter is composed of 40% carbon and vital for good tilth (Wander, 2004). Soil organic matter is a key indicator of soil quality due to characteristics that reduce soil erosion, increase buffering capacity, provide habitat and substrate for microorganisms, and facilitates CO₂ sequestration (Koen, 2009). Soil organic matter reduces erosion by increasing aggregation which reduces the susceptibility of soil to wind and water erosion. Humus, the stable form of organic matter, has negative charges on the surface due to carboxylate groups (COO⁻) which increases the capacity of a soil to buffer changes in pH. Carboxylate groups also exchange cations increasing CEC (cation exchange capacity) and nutrient holding capacity in soil.

While it is important to build soil organic matter because it provides a large pool of potential energy for biological life, this energy can only be realized through decomposition with carbon dioxide gas lost as a byproduct (Janzen, 2005). Microbial biomass carbon and microbial respiration together reflect the efficiency of microbes to utilize energy from carbon (Anderson and Domsch, 2010). Efficient microbial utilization of C substrates increases soil organic carbon as a result of sequestering CO₂ that was fixed through photosynthesis, either directly from plants or indirectly through animal waste (from feed that was produced by plants). If large amounts of carbon are being lost as CO₂ relative to the amount of carbon sequestered in the microbial biomass, soils are losing soil organic matter carbon at an unhealthy rate. Agricultural systems must balance carbon pools of soil organic matter with carbon stored in microbial biomass and CO₂ emitted from respiration in order to generate healthy soils.

Consistent rates of carbon inputs should be reflected by consistent levels of microbial biomass and respiration over the long-term. Temporary increases or decreases in the soil organic

matter carbon pool will be echoed by short term, temporary increases or decreases in microbial biomass. If nutrient sources are exhausted in the soil through plant uptake or other processes, decreases in microbial biomass carbon can be expected (Anderson and Domsch, 1985). Nutrient limitation forces microbes to use endogenous carbon sources for energy, thus the microbial biomass becomes smaller. Environmental variables like extreme wet or dry conditions may temporarily increase or decrease microbes' access to nutrients, or their capacity to metabolize substrates, which can impact the size of microbial biomass in soil (Anderson and Domsch, 1993). Microbial biomass is a function of long- and short-term trends in carbon inputs and an important indication of resilience to extreme environmental conditions.

Balancing carbon in agricultural fields is demands considerations of a number of factors. Agricultural practices like rotation length, plant diversity and, use of annuals or perennials should be considered since these management decisions influence the size of microbial biomass pools and their relative activity as a result of decomposing carbon in soil organic matter. Management practices like nutrient source, pest management, tillage, and water conservation also affect carbon gains and losses. Systems that are lowest risk for carbon losses include additions of composted manure to soil in the absence of synthetic fertilizers (Fleissbach et al., 2007). Manure soil amendments increase the risk of carbon loss relative to compost additions (Fleissbach et al., 2007). Soils without additions of manure or composted manure and with synthetic fertilizer are at the highest risk for carbon losses (Fleissbach et al., 2007). Balancing SOM in agricultural systems through management decisions is essential for maintaining and increasing healthy soils and nutrient cycling.

1.6 Microbial Biomass Carbon (MBC)

Microbial biomass carbon (MBC) is the amount of carbon contained in bacterial and fungal cells. Carbon makes up 90% of the total dry microbial biomass (Elser and Sterner, 2002). If a soil's capacity to support biological life represents soil health, then larger MBC values reflect healthier soils.

The literature currently lacks a standard definition for an acceptable range of MBC values. Comparison studies provide a platform for contrasting microbial biomass carbon values. Soils with native, non-agricultural vegetation can also provide biological benchmark values for comparison with experimental treatments.

1.6.1 Changes in MBC

Microbial biomass carbon can increase, decrease, or stay the same over time. Changes in MBC depend on vegetation (van Veen, 1985), nutrient status, temperature, pH (Anderson and Domsch, 1985), and moisture (Moat and Foster, 2002) of soil. Agricultural practices like rotation and management (conventional, organic) have different effects on these soils properties and therefore impact MBC and soil health directly.

1.6.1.1 Vegetation

The presence of plants and their associated rhizosphere is the single most important factor contributing to microbial biomass carbon in soils (Watt et al., 2004). Agricultural management practices that incorporate perennials, long crop rotations (4+ years), and high diversity (3+ crops) can sustain larger amounts of MBC compared to short rotations (≤ 2 years) or monocultures (Vinther et. al, 2004). Perennials increase the presence of the rhizosphere

temporally and spatially, which increases substrate availability and increases MBC. Long crop rotations increase plant diversity, which increases substrate diversity. Crops like peas, oats (Rovira, 1956), and wheat (Rovira, 1969) excrete different amino acids, sugars, and organic acids in different ratios as plants develop physiologically. Substrate diversity stimulates a wider variety of microbial niches, increasing MBC in soil (Mäder, 2007). Incorporating mixed perennials in rotation is one way to increase both diversity and presence of the rhizosphere, which can enhance MBC and soil health.

1.6.1.2 Nutrient status: Carbon

Microbial biomass carbon can increase, decrease, or stay the same in the presence of carbon, hydrogen, and oxygen alone. Carbon is the single most important nutrient for sustaining microbial biomass carbon. Anderson and Domsch (1985) first demonstrated the theory of a *zero growth maintenance demand*, which illustrates that consistent inputs of carbon substrates induce consistent MBC values.

Increases or decreases in carbon supply relative to the zero growth maintenance demand of a microbial population is echoed by increases or decreases in MBC values (Anderson and Domsch, 2010). If carbon substrates are higher than the zero growth demand, MBC will increase until the carbon source is substantially consumed at which point MBC will begin to decline. In order to maintain levels of MBC in agricultural soils, carbon must be consistently added to soils at a constant rate. To increase MBC in agricultural soils, carbon amendments must consistently exceed the present zero growth maintenance demand (Anderson and Domsch, 2010). Rotation and soil organic matter management will determine carbon substrate availability to microbes and therefore drive maintenance, increases, and decreases in MBC values in soil.

If access to carbon substrates is limited by supply, oxygen, or water, microbial populations undergo a physiological change in mass. When access to carbon is diminished, microbial biomass carbon declines to a constant mass with low, constant respiration rates. This state of microbial activity is called *self-induced dormancy* and is a stress response (Anderson and Domsch, 1985). The literature does not contain any studies demonstrating direct measurements of zero growth maintenance demands or self induced dormancy in agricultural soils.

Quantity and frequency of soil carbon amendments, as well as quality (C stability) and other nutrient concentrations (N, P, S etc.) of organic C additions are variables that influence MBC growth and maintenance in soils (Dawes and Sutherland, 1992). Inputs of carbon to agricultural soils differ in quality and nutrient concentration depending on the source of C. For example, carbon additions from main crop residues, manure, composted manure, green manure, and cover crops have different stabilities and ratios of carbon:other nutrients (Mohanty et al., 2011). All inputs of carbon have the potential to increase MBC by increasing substrate availability, but the degree of beneficial effects depends on the specific C input.

Carbon from synthetically fertilized main crop residues, manure, or legume plant residues incorporated into agricultural soils are not equally accumulated, and decomposed into soil organic matter (Drinkwater et. al, 1998) by microbes. In some experiments, synthetically fertilized soils that incorporate plant residues have not demonstrated the ability to significantly increase SOM (Drinkwater et. al, 1998). Therefore, additions of main crop residues alone may not maintain MBC values and likely cannot increase MBC in soil. Conversely, manure inputs in addition to crop residues have been able to increase soil organic matter (Drinkwater et. al, 1998) and can be useful for maintaining or increasing MBC. Similarly, incorporation of legume plant

residues can also increase soil organic matter (Drinkwater et. al, 1998). Soil organic matter management practices must consider variables like carbon stability (lignified=recalcitrant, humified=slowly decomposing, sugar-fresh residue=readily decomposed) and nutrient concentration (lignified=high C ratio, humified=moderate C ratio, fresh residue=low C ratio). Proper soil organic matter management will result in maintenance or consistent increases in MBC. Consistent decreases in MBC may indicate imbalanced carbon cycling.

1.6.2 MBC in Long-term Organic-Conventional Comparison Studies

Several studies around the world from countries like Switzerland, Denmark, Italy, and the United States have compared the long-term effects of organic and conventional agriculture management on MBC. These long-term experiments represent a variety of soil types, rotations, and climatic regions. While these research sites embody a range of environmental variables, the results demonstrate that organic systems illustrated a superior ability to sustain MBC than corresponding conventionally managed systems (Table 1.1).

One major difference between organic and conventional agriculture management is the type and source of nutrient inputs, or soil amendments. Organic and biodynamic systems, for example, are required by certification standards to use composted manure. As a result of composting manure, nutrients in the compost are stable and do not readily leach into the soil environment. The nutrients tied-up in compost must first be mineralized by microorganisms before they can enter soil solution and become plant available. Therefore, organic and biodynamic agriculture systems depend heavily on microbes for the process of mineralization-immobilization-turnover for the release of nutrients held in composted manure.

In contrast to organic and biodynamic agriculture systems, conventional systems are allowed to use raw manure or synthetic fertilizer as soil amendments and nutrient inputs. Nutrients from raw manure can be more labile compared to composted manure, and can readily enter into soil solution where they become available for crop uptake (Munoz et al., 2008), or leach through the soil profile. The labile nature of nutrients from raw manure allows them to enter soil solution more easily and may not depend entirely on the microbial process of mineralization-immobilization-turnover, and therefore decreases the reliance of conventional agriculture on microbial activity. However, labile carbon from raw manure is an important substrate for microbes (Aira and Dominguez, 2008) and as they consume raw manure, nutrients are also released into the soil environment (van Veen, 1984). Subsequently, microbial dynamics are also pertinent to conventional agriculture. The addition of raw manure in conventional systems has been shown to increase MBC compared to the exclusive use of synthetic fertilizers (Yuan et al., 2011).

Many conventionally managed soils exclusively use synthetic fertilizers as the main source of nutrients to agricultural crops. Synthetic fertilizers are labile and readily available to plants in the presence of water, and microbial activity cannot increase the availability of nutrients to plants under these circumstances. As microbes consume synthetic fertilizers the nutrients become immobilized and unavailable to plants (Zaman et al., 1999). The exclusive use of synthetic fertilizers without additions of carbon will decrease microbial populations (Anderson and Domsch, 2010). Research world-wide demonstrates that excluding carbon inputs from composted or raw manure, together with the use of synthetic fertilizers has decreased MBC in conventional systems (Table 1.1).

Previous research from long-term experiments illustrates distinct differences in the effect of management practices on soil health via microbial biomass carbon. Management systems in each experiment are primarily distinguished by fertilization amendments like composted manure, raw manure, and synthetic fertilizer. Results from these long-term comparison studies provide a global characterization of organic and conventional agricultural management practices on soil health.

Table 1.1 Comparison of microbial biomass carbon ($\mu\text{g MBC/g soil}$) across long-term agricultural management practices from 1984-2010 under annual rotations and rotations that include perennials.

	Annual						Perennial	
Agricultural Management	¹ Darmstadt	² FiBL (1998)	³ FiBL (2005)	⁴ PA, USA	⁵ UK	⁶ Pisa, Italy	⁵ UK	⁷ Northunberland
No Fertilizer		235 c			158		569-1627	1826
Biodynamic	149 a	360 a	470 a					
Organic+ Compost		313 ab	430 a	351		33.4		
Conventional+ Farmyard manure	149 a (compost)	267 bc	310 b		342			2104
Conventional + synthetic fertilizer	136 b	218 c	230 c	231	190	30.3	99-1379	1877

¹(Heinze et al., 2010); ²(Fleissbach et al., 2007); ³(Joergenson et al., 2010) ⁴(Raupp et al., 2006); ⁵(Brookes et al., 1984); ⁶(Mazzoncini et al, 2004); ⁷(He et al., 1997)

1.6.2.1 FiBL- The Swiss DOK Trial

The DOK Trial is a long-term experiment initiated in 1978 near the city of Basil, Switzerland. This study compares stockless organic and conventional agriculture management typical of Swiss agriculture (Fleissbach, 2007). MBC measurements from this experiment indicate that organically managed soils have a greater capacity to support microbial life and therefore have greater health than conventionally managed soils under the same rotation.

The DOK Trial represents four management systems: biodynamic (composted manure + biodynamic preparations); organic (composted manure); conventional farmyard (farmyard manure + pesticides); and conventional synthetic (synthetic fertilizer + pesticides). Each system is under a seven year rotation that includes 2-3 years of mixed grass-clover forage, and the remaining years are in small grains. In addition to main crops, green manure, cover-crops, under-sowing/intercropping, and catch crops are used throughout the rotation (Fleissbach, 2007). Fertilizer and pesticides are applied as needed and at recommended rates based on the demands of each management system and its regulations.

Measurements of soil health from the DOK trial show evidence that biodynamic and organically managed soils have a greater abundance of MBC compared to both conventional farmyard and synthetic treatments. Soils were analyzed for MBC first in 1998 and again in 2005. Results demonstrate that biodynamic and organic systems increased their MBC values, creating a significant distinction from both conventional systems (Table 1.0). Significant differences were also found in 2005 between conventional + raw manure, and conventional + synthetic fertilizer. This result indicates that additions of raw manure with conventional management can develop better soil health compared to the exclusive use of synthetic fertilizers. However, raw manure amendments do not amplify MBC to the extent of either biodynamic or organic systems using composted manure. Accordingly, soil health is augmented by increases in MBC due to biodynamic and organic management practices.

1.6.2.2 MASCOT Trial, Italy

The MASCOT long-term experiment began in the autumn of 2001 at the University of Pisa, Italy. This study compares two stockless agriculture systems: organic and conventional.

Each system is under the same rotation of durum wheat, red clover, sunflower, pigeon bean, common wheat, red clover, and maize. Management treatments were distinguished by fertilizer and pesticide applications. Organic systems received composted manure and the conventional system received synthetic fertilizer and pesticides (Mazzoncini et. al, 2004). Results from this study illustrated no significant differences in MBC values for the different management systems. In addition to no significant differences, MBC values were one or two orders of magnitude less than all other reported values of MBC from other long-term trials (Table 1.1). This long-term experiment, having been established for only three years, may be too young for comparisons with other long-term experiments.

1.6.2.3 Darmstadt- Hessia, Germany

The Darmstadt long-term fertilization trial in Hessia, Germany was established in 1980. This study compares biodynamic (composted manure + biodynamic preparations), conventional (composted manure), and conventional (synthetic fertilizer + pesticides) management systems. All systems were under a rotation of red clover or lucerne, spring wheat, potato or carrot, and winter rye (Heinze, 2010). Results from Darmstadt indicate biodynamic and conventional+compost management systems have a greater capacity to support MBC compared to conventional management. These results agree with previous results from the Swiss DOK Trial (Table 1.1).

1.6.2.4 Southwest Research and Outreach Center- Minnesota, USA

The Southwest Research and Outreach Center in Lamberton, Minnesota was established in 1989. This site is home to the Variable Input Crop Management Systems (VICMS) research plots. The study evaluates the effect of four management systems under 2-year and 4-year crop

rotations (Kuratomi et al., 2004). No input, reduced input, high input, and organic systems are compared in this study with: corn-soybean (2 yr) and corn-soybean-oat/alfalfa-alfalfa (4 yr) rotations. Results from MBC measurements demonstrate increased capacity for organic and reduced input systems to improve soil health (Kuratomi et al., 2004).

Rotation and management influenced MBC values in the VICMS research plots. The longer, four year rotation performed substantially better than the two year rotation with greater MBC values, except in the case of the reduced input system. Within the four year rotation, the organic system achieved the highest values of MBC, which are comparable to the two year reduced input MBC values. These results demonstrate the interaction of rotation and management on soil health (Kuratomi et al, 2004).

1.6.2.5 Long-term analysis of Agriculture Rotation

Microbial biomass carbon responds significantly to agricultural rotation i.e. annual, inclusion of perennial, permanent grassland. Five years after the establishment of a long-term study in Denmark, results showed that rotations including perennials support more microbial carbon than annual rotations (Vinther et al., 2004). While this study is young, significant differences between rotations were already apparent. Comparison of 15 different soils demonstrated similar results, illustrating perennial soils can hold more MBC by an order of magnitude (Brookes et al., 1984). Brookes et al. (1984) compared unfertilized fields under annual rotation which measured 158 $\mu\text{gMBC/g soil}$, while perennial soils sustained 569-1627 $\mu\text{g/g MBC}$. Agriculture systems including perennials have reported MBC values that are one or two orders of magnitude greater than annual rotations. One study from Northumberland demonstrated that perennial grassland without fertilizers could sustain 1826 $\mu\text{gMBC/g soil}$,

compared to the highest MBC value from annual rotations 351 $\mu\text{gMBC/g soil}$ (Table 1.1). This research clearly shows longer rotations that include perennials can improve soil quality by sustaining more microbial biomass carbon.

These long-term experiments comparing organic and conventional agriculture on soil health illustrate the beneficial consequences of organic systems that utilize long rotations and include perennials. Intentionally applied composted manure (as a carbon source) and perennial rotations have the potential to collaboratively increase long-term soil health. Agricultural management practices that add organic carbon and include perennials therefore have the greatest capacity to benefit soil health.

1.7 Microbial metabolic quotient ($q\text{CO}_2$)

The microbial metabolic quotient is a measurement used to assess the efficacy of carbon sequestration in terrestrial landscapes. The microbial metabolic quotient ($q\text{CO}_2$) is defined as the CO_2 respired per unit of microbial biomass, per hour, per gram of dry soil: $\mu\text{g-CO}_2/\text{g-MBC/hr}$ (Anderson and Domsch, 1985). This measurement takes into consideration soil respiration (microbial activity), microbial biomass carbon (microbial abundance), and time (rate of microbial activity relative to the size of the population).

The microbial metabolic quotient was a result of Anderson and Domsch's (1985) eco-physiological approach to environmental assessment and was developed as a consequence of long-term ecosystem research projects developed world-wide in the 1980's (Anderson and Domsch, 2010). At the time, interest in element and energy transfers in soil increased research emphasis on the heterotrophic activity responsible for mineralizing organic substrates and recycling nutrients (Anderson and Domsch, 2010). The microbial metabolic quotient, together

with microbial biomass carbon, and soil respiration represents the capacity and efficacy of soils to sustain life (Anderson and Domsch, 2010).

The microbial metabolic quotient was the first attempt to demonstrate a relationship between carbon pools like microbial biomass carbon and respiration, in relation to soil C substrates (Anderson and Domsch, 1993). The quotient (qCO_2) should always be interpreted considering MBC and CO_2 respiration individually and together as the metabolic quotient. It is necessary to consider these parameters separately and together because, for example, a large and small microbial population can have the same values for qCO_2 but the abundance of MBC can be significantly different. In addition, two soils might have different qCO_2 and it is important to know which factor, MBC or CO_2 , is contributing to that difference i.e. is the MBC the same with different rates of CO_2 ? Or, is CO_2 the same but the size of MBC different? Considering qCO_2 measurements in this way provides information about the relative carbon exchange dynamics performed by microbial populations in soil.

1.7.1 Stability and Changes in qCO_2

Stable carbon inputs produce stable microbial respiration and microbial respiration is correlated with microbial biomass C (Anderson and Domsch, 1978), therefore microbial biomass carbon stability and abundance are directly related to C inputs. Microbial qCO_2 may change over time, in response to temperature, C inputs, and due to the size of the microbial biomass itself (Anderson and Domsch, 1985; 1993). Agricultural management and rotation can also influence qCO_2 (Anderson and Domsch, 1990). These variables will affect stability or changes in qCO_2 .

As soils mature, qCO_2 decreases. Mature soils are considered to have at least 15 years under similar management/rotation. Over time, soils under consistent agricultural management are shown to decrease qCO_2 as the age of the plot increases ($y=0.000834e^{-0.01x}$) (Anderson and

Domsch, 1990). A study of 41 arable soils (20 monoculture and 21 crop rotation plots) that were analyzed for $q\text{CO}_2$ and plotted against the maturity of soils showed a negative relationship between plot age and $q\text{CO}_2$. These results demonstrate that consistent management over time reduces $q\text{CO}_2$ as soils mature, creating healthier soils.

Anderson and Domsch (1985) also demonstrated that with rising temperatures, respiration and maintenance energy carbon demands increased. The shift in $q\text{CO}_2$ due to environmental change indicated this measurement could quantify stress on microbial communities (Anderson and Domsch, 2010). Shifts in metabolic quotients should be interpreted together with basal soil respiration (CO_2 emitted per unit of soil) and microbial biomass carbon since soils can have low $q\text{CO}_2$ and with high rates of respiration if MBC is also high.

Metabolic quotients have been shown to increase with increasing microbial biomass (Anderson and Domsch, 1985). A comparison of three soil types (parabrown, chernozem, rendzina) demonstrated that smaller values of MBC were accompanied by lower $q\text{CO}_2$ values (Anderson and Domsch, 1985). As MBC increased, so did $q\text{CO}_2$. Theoretically, this result implies that higher MBC increases the active fraction of that population (Anderson and Domsch, 1985). The increased active fraction of the microbial population is reflected by increases in CO_2 emissions from soil relative to MBC in soil and therefore $q\text{CO}_2$ should also increase (Anderson and Domsch, 1985). A microbial population that is large with a small $q\text{CO}_2$ value may only actively supporting a small fraction of its mass, which could be related to substrate availability, water or temperature (Anderson and Domsch, 1985)

Fluctuations in $q\text{CO}_2$ that are a result of stress can be identified by increasing CO_2 respiration from soil microbes, or a decrease in MBC (Anderson and Domsch, 1985).

Fluctuations in MBC or CO₂ accompanied by stable qCO₂ do not imply stress responses and may be explained by higher trophic level predation on soil microbes (Anderson and Domsch, 1985). Little is known about qCO₂ fluctuations in agricultural systems. Knowledge regarding trends in microbial C dynamics in soil can assist our understanding of nutrient cycling, SOM management, and the affect of agricultural management and rotation on soil health.

1.7.2 Range of qCO₂ in Agricultural Soils

Agricultural practices like SOM management and rotation have significant effects on qCO₂ and soil health. The healthy range of qCO₂ for agricultural soils is 0.5-2.0 mg-CO₂-C/g-MBC/hr (Anderson, 2003). Mature soils that measure values higher than 2.0 mg-CO₂-C/g-MBC/hr for qCO₂ can be considered critical (Anderson, 2003). Critically high qCO₂ values reflect microbial populations that are emitting CO₂ at rates higher than their maintenance requirement, which reduces the capacity of a soil to sequester carbon. Values below 0.5 mg-CO₂-C/g-MBC/hr can be considered predominantly dormant microbial communities that are not participating in nutrient cycling or carbon sequestration. Attention to SOM management and rotational diversity are crucial for establishing health soils that fall within the acceptable range of qCO₂ values for agricultural soils.

Management decisions that increase SOM like manure and compost additions and high rotational diversity can increase soil health by reducing qCO₂. Investigation of metabolic quotients from 41 arable soils in long-term research experiments showed that microbial populations responded differently to soils under annual monoculture and soils with continuous annual crop rotation (Anderson and Domsch, 1990). The qCO₂ of the monoculture (qCO₂ 6.0-17.0 mg-CO₂-C/g-MBC/hr) was higher than the soil managed under continuous two year annual

rotation (qCO_2 1.7-12.0 mg- CO_2 -C/g-MBC/hr) (Anderson and Domsch, 1990). Lower quotients reflect reduced maintenance energy demands for soils with increased diversity of primary producers under continuous annual rotation (Anderson and Domsch, 1990). Still, the soils presented by Anderson and Domsch (1990) exhibit high values for qCO_2 , indicating that mature soils under monoculture and two year crop rotation may not increase soil health. Therefore, maturity alone does not imply healthy soils. While maturity reduces qCO_2 values, maturity alone may not lead to healthy ranges of qCO_2 . Consequently, in order to generate healthy soils with good metabolic quotients, SOM addition and high plant diversity should be incorporated into agricultural management practices. Increased quality and diversity of organic residue inputs can also reduce metabolic quotients (Anderson and Domsch, 1990).

1.7.3 qCO_2 in Long-term Organic-Conventional Comparison Studies

Microbial metabolic quotients are significantly influenced by agricultural management and rotation. Typically, management systems that use only synthetic fertilizers without manure additions over a long period of time, demonstrate the highest values for qCO_2 (Table 2.1). Consistent additions of manure in conventional systems have the potential to reduce qCO_2 values. Finally, steady additions of compost over time can increase soil health beyond that of conventional soils with or without manure amendments. These management strategies, along with rotation have long-term effects of microbial metabolic quotients in agricultural soils.

1.7.3.1 FiBL- The Swiss DOK Trial

Soils under the same seven year rotation from the DOK Trial in Switzerland demonstrated significant differences between management treatments with no-fertilizer, biodynamic, organic, conventional+raw manure, and conventional+synthetic fertilizer after 27

years (Fleissbach et al, 2007). Results from this study showed the highest qCO_2 under conventional management with synthetic fertilizers (1.66 mg- CO_2 -C/g-MBC/hr), the lowest qCO_2 under the biodynamic treatment (1.10 mg- CO_2 -C/g-MBC/hr), and the conventional+ raw manure treatment fell in the middle (1.30 mg- CO_2 -C/g-MBC/hr). The organic management system (1.16 mg- CO_2 -C/g-MBC/hr) was not significantly different from either no-fertilizer, biodynamic, or conventional+raw manure treatments, but was significantly lower than the conventional+synthetic fertilizer system. After 27 years of management, the biodynamic, organic, and conventional+raw manure treatments demonstrated greater soil health compared to the conventional+synthetic fertilizer soil.

Considering MBC and qCO_2 together, MBC was higher in the biodynamic (360 μ g-MBC/g soil) and organic systems (313 μ g-MBC/g soil), and these treatments had the lowest qCO_2 . This result illustrates that larger microbial populations from the biodynamic and organic systems are emitting less CO_2 per unit of MBC, and more carbon is being stored in the microbial biomass. Carbon stored in the microbial biomass increases the capacity of soils to sequester carbon and ultimately increase soil health. The Swiss DOK Trial demonstrates that conventional systems using only synthetic fertilizer have a reduced power to store carbon in the microbial biomass, which decreases soil health.

Table 2.1 Measurement of microbial metabolic quotient (qCO_2) across long-term agricultural management practices under annual rotations.

Agricultural Management	Annual Rotations				
	¹ Darmstadt, DE (mg/g/hr)	² FiBL, CH (mg/g/hr)	³ Bad Lauchstadt (mg/g/hr)	³ Keszthely (mg/g/hr)	⁴ Pisa, Italy (mg/mg/d)
No Fertilizer		1.24bc	9.01c	6.83c	
Biodynamic	.0015ab	1.10c			
Organic+ Compost		1.16bc			0.24
Conventional+ Farmyard manure	.0014a (compost)	1.30b	5.43a	5.70a	
Conventional + synthetic fertilizer	.0017b	1.66a	7.38b	6.24b	0.23

¹(Heinze et al., 2010); ²(Fleissbach et al., 2007); ³(Böhme et al., 2005); ⁴(Mazzoncini et al., 2004)

1.7.3.2 Darmstadt Hestia, Germany

Soils under the same rotation with biodynamic, organic, and conventional+synthetic fertilizer had significantly different qCO_2 after 17 years (Heinze et al., 2010). Results from this long-term trial demonstrated similar trends in qCO_2 compared to the Swiss DOK Trial. Soils from Darmstadt, Germany had significantly lower qCO_2 in the biodynamic (.0015 mg-CO₂-C/g-MBC/hr) and organic (.0014 mg-CO₂-C/g-MBC/hr), which were not significantly different from each other. Both biodynamic and organic were statistically lower than the conventional+synthetic fertilizer treatment (.0017 mg-CO₂-C/g-MBC/hr). Low MBC values in conventional+synthetic fertilizer (136) and the highest value for qCO_2 indicate this soil has the poorest health. Soils under organic (149 μ g-MBC-g soil) and biodynamic treatments (149 μ g-MBC-g soil) from Darmstadt had higher MBC and lower qCO_2 also indicating increased soil health compared to the conventional treatment. However, these values for qCO_2 fall below the healthy range of metabolic quotients (0.5-2.0 mg-CO₂-C/g-MBC/hr) for agricultural soils.

Authors of the study did not compare their results with Anderson and Domsch's researched values.

Soils from the long-term trial in Darmstadt, Germany follow similar trends to the DOK Trial for $q\text{CO}_2$ and MBC, but do not fall within the healthy range of $q\text{CO}_2$ values. One possible explanation for this could be the difference in rotational management. While the DOK Trial has a seven year rotation with cover crops, catch crops, and frequent legume inputs, the Darmstadt rotation consists of four year red clover, spring wheat, potatoes or carrots, and winter rye. The shorter rotation at Darmstadt, exclusion of cover/catch crops and less frequent use of legumes is one possible explanation for the difference in soil health.

1.7.3.3 MASCOT Pisa, Italy

The long-term MASCOT Trial in Italy comparing organic and conventional+synthetic fertilizer management systems did not show significant differences for $q\text{CO}_2$ values (Mazzoncini et al, 2004). The $q\text{CO}_2$ values for the organic (.24 $\text{mg-CO}_2\text{-C/g-MBC/hr}$) and conventional (.23 $\text{mg-CO}_2\text{-C/g-MBC/hr}$) systems both fell below the acceptable range (0.5-2.0 $\text{mg-CO}_2\text{-C/g-MBC/hr}$) for agricultural soils. Considering these $q\text{CO}_2$ results with MBC values for the organic (33.4 $\text{mg-CO}_2\text{-C/g-MBC/hr}$) and conventional (30.3 $\text{mg-CO}_2\text{-C/g-MBC/hr}$) treatments, the low quotients might be attributed to low MBC. One possible explanation for low $q\text{CO}_2$ and MBC values could be the age of the experiment under these treatments (5 years). The sandy soil texture of the experimental site (Mazzoncini et al, 2004), which has low surface and high porosity, may reduce the potential of the soil to provide protected habitat for microbial life. Results from this study should be compared to future measurements of $q\text{CO}_2$ and MBC to assess how these systems impact soil health over longer periods of time.

1.7.3.4 Bad Lauchstadt, Germany and Keszthely, Hungary

In a study conducted by Böhme et al (2005) researchers compared two long-term experiment sites from Germany (initiated 1902) and Hungary (initiated 1963). Both sites had similar soil types, nutrient status and annual crop rotations including small/large grains and potatoes (*Solanum tuberosum* L.)/sugar beet (*Beta vulgaris* L). Three management treatments exist including no-fertilizer, conventional+raw manure, and conventional+synthetic fertilizer. Similar to previous studies, soils amended with organic inputs had the lowest qCO_2 values.

Values for qCO_2 from conventional+raw manure treatments in Germany (5.43 mg- CO_2 -C/g-MBC/hr) and Hungary (5.70 mg- CO_2 -C/g-MBC/hr) were the lowest compared to conventional+synthetic fertilizer (7.38, 6.24 mg- CO_2 -C/g-MBC/hr respectively). The no-fertilizer treatments were the highest for both Germany (9.01 mg- CO_2 -C/g-MBC/hr) and Hungary (6.83 mg- CO_2 -C/g-MBC/hr). These results demonstrate the beneficial qualities of organic matter additions to soil. However, all qCO_2 values from this study fall outside of the healthy range (0.5-2.0 mg- CO_2 -C/g-MBC/hr) for agricultural soils. These results indicate soils are losing carbon at an accelerated rate, decreasing the potential to store carbon in the microbial biomass. Measurements of MBC would help interpretation of these results, in addition to measurements of qCO_2 and MBC over time that would reflect increases or decreases in the microbial biomass.

1.7.4 Synthesis of qCO_2 in Long-term Organic and Conventional Comparison Studies

Results from various long-term agricultural experiments demonstrate the beneficial effects of organic carbon amendments on long-term soil health. Lower qCO_2 values in biodynamic, organic, and conventional+raw manure management consistently show soils to be

healthier compared to conventional systems using only synthetic fertilizer. Lower $q\text{CO}_2$ values in biodynamic and organic systems compared to conventional+raw manure (Fleissbach et al, 2007) illustrate the enhanced benefit of composted manure on soil health. Therefore, the quality of organic amendments has an impact on soil health over time represented by low $q\text{CO}_2$ and high MBC values. Consequently, soils under biodynamic and organic management have the potential to sequester larger amounts of stable carbon in the soil as microbial biomass and subsequent humified organic matter.

1.8 Microbial Nitrous Oxide Quotient $q\text{N}_2\text{O}$

The microbial nitrous oxide quotient ($q\text{N}_2\text{O}$ mg- N_2O -N/g-MBC/hr) has not been previously reported in the literature and is a variation of the microbial metabolic quotient ($q\text{CO}_2$). Instead of looking at the carbon released from aerobic respiration per unit of MBC, the $q\text{N}_2\text{O}$ evaluates the nitrous oxide emitted per unit of MBC as a result of anaerobic respiration. The interpretation of this variable is slightly different from $q\text{CO}_2$. While $q\text{CO}_2$ reflects emission and sequestration of carbon, $q\text{N}_2\text{O}$ represents emission of N_2O and carbon sequestered. This variable ($q\text{N}_2\text{O}$) should also be interpreted with MBC values. Talking these measurements into consideration together demonstrates the abundance of microbial biomass contributing to nitrous oxide emissions. Larger populations with low N_2O values would be valuable because the soil can sustain high numbers of microbes that are not depleting N supplies or releasing harmful green house gasses. Small microbial populations with high $q\text{N}_2\text{O}$ values would therefore be the least valuable since the soil cannot support large numbers and the microbes that are present deplete N supplies and release high amounts of green house gasses relative to its size.

Nitrous oxide emissions from soil are mediated by anaerobic microbes. To better understand how agricultural management affects N₂O emissions, it is necessary to look at the organisms doing this work. N₂O is an anaerobic metabolic process carried out by bacteria, which exerts energy from anaerobic respiration. Like aerobic respiration, anaerobic respiration has the potential to sequester carbon while it releases N₂O as a result of electron transfers from metabolic activity. Considering nitrous oxide emissions in terms of the microbial population provides new observations about agricultural management and rotation, and their impact on green house gas emissions, carbon sequestration, and the balance of aerobic and anaerobic microbial respiration in soils.

1.9 Microbial biomass phosphorus (MBP)

The addition of synthetic, inorganic P fertilizer can offset the depletion of plant available phosphorus in the soil of conventional systems. Organic farming however, depends on phosphorus mineralization-immobilization-turnover by microorganisms for rudimentary conversion of unavailable P to plant available phosphate (Richardson et al., 2011). Microbial biomass is the principle site for soil organic phosphorus mineralization from recalcitrant to labile forms that are plant available (Brookes, 1982). Organic phosphorus soil amendments like compost, manure and rock phosphate are decomposed by microbes along with soil organic matter (Richardson et al., 2011). Utilization of organic P requires extracellular hydrolysis by phosphatase enzymes and uptake occurs through high affinity transporters (Richardson et al., 2011). Significant amounts of P, up to 45- 84 ppm, can be cycled through the microbial biomass in one year, influencing plant available phosphorus and P uptake (Sugito et al., 2010; Seeling and Zasoski, 1993).

1.9.1 Microbial Phosphorus Processes: The Basics

Microbial biomass phosphorus is an important pool of potentially plant available phosphorus in agricultural systems. Conventional and organic management practices depend on MBP as a nutrient source to different degrees. Conventional agriculture has the capacity to supplement soil P reserves with synthetic fertilizers, which are immediately available to crops. Therefore conventional systems do not necessarily depend heavily on MBP as a source of P. MBP in organic agriculture however, has a profound effect on available P to crops. Phosphorus can only be applied through composted manure or rock phosphate in organic farming, and this form of P must go through mineralization-immobilization-turnover to become plant available. Since MIT is carried out by micro-organisms, measures of MBP may be useful for predicting potential P availability in agricultural systems that depend on this process to supply P nutrients to crops.

Microbial biomass phosphorus includes the amount of PO_4^- ions and polyphosphate chains inside the cell membrane of soil microbes. These forms of inorganic phosphorus account for 90% of microbial P (Brookes, 1982), and represent a large pool of labile nutrients. MBP is an important reserve of nutrients that is protected from the environment and can become available to crops over the growing season. Phosphorus that is contained in the microbial biomass may be unavailable to plants periodically but has the potential to become available to crops (Richardson et al., 2011) as a result of mineralization-turnover through the growing season.

Microbes that store polyphosphate chains can utilize these polymonomers as a substitute for ATP in microbial metabolic pathways (Kulaev et al., 1985). Usually, ATP-phosphate groups provide the energy needed for metabolism to function inside microbial cells. Microbes can store

high polymeric polyphosphates that can substitute for ATP phosphate in energy transfers (Kulaev et al., 1985). Phosphorus, therefore, influences the amount of carbon microbes can accumulate by regulating the energy required to metabolize carbon. High-polymeric polyphosphates are the most important activated reserves of P in microbes (Kulaev et al., 1985). These polyphosphates are innocuous and, osmotically and electrostatically inert (Kulaev et al., 1985), which enables microbes to hold onto accumulated polyphosphates under water stressed conditions. The ability to store PO_4^{3-} polymonomers gives microbes more independence from environmental conditions (Kulaev et al., 1985). Microbes that depend entirely on environmental phosphorus can build surplus polyphosphates (Kulaev et al., 1985) and may be more resilient to changing conditions.

The ability to store P in different forms, and hold onto this nutrient under variable environmental conditions is important for organic agricultural systems that depend on MBP reserves for plant available nutrients. Organic systems can build P in the microbial biomass, where it is resilient to drought and flooded conditions. The advantage of storing P in microbial biomass is that it is not susceptible to leaching, like synthetic P fertilizers. Phosphorus contained in microbes might be able to help mitigate the negative effect of P run-off into aquatic ecosystems. Manure and composted manure contain C and P, which have been shown to accumulate linearly in the microbial biomass (Brookes et al, 1982) have reduced qCO_2 , thereby increasing soil health (Fleissbach et al, 2007; Böhnme et al, 2005). Therefore, in order for conventional systems that use only synthetic fertilizer to take advantage of the MBP nutrient pool, organic C and P must be added to soil simultaneously. The addition of organic C and P inevitably occurs in organic agriculture through compost that tends to have high P concentrations.

Organic agriculture systems depend on environmentally stable nutrients and may have the ability to accumulate large quantities of phosphorus in the microbial biomass. Microbes that rely on P from compost or rock phosphate must mineralize these organic compounds using phosphatase enzymes. Microbes in conventional systems using synthetic fertilizer, which is already readily available, do not need to exert enzymes to acquire phosphorus. Theoretically, the microbes in the conventional system do not sense a need to build polyphosphates due to surplus quantities, while the microbes in the organic system do sense the need to store inert forms of P as a result of the extra energy required to accumulate this P using enzymes. If microbes accumulate more polyphosphates in organic systems, these soils have a greater capacity to maintain protected pools of P in their microbial biomass. Consequently, organic soils would have a greater potential to withstand flood/drought conditions due to the inert nature of polyphosphate chains.

1.9.2 Competition for Phosphorus by Plants and Microbes

During the growing season, plants and microbes compete for inorganic phosphate in soil. Immobilization of P into microorganisms is an important reason for low P availability to plants in organic systems. Microbial synthesis and mineralization of organic phosphorus affects P equilibriums in soil solution by changing the concentration of inorganic P and releasing organic P compounds into soil (Seeling and Zasoski, 1993). In organic systems, where P is only available to plants after mineralization-immobilization-turnover, microbes might have a competitive advantage for this nutrient. Immobilization of P into microbial biomass may be less significant for conventional crop production where synthetic fertilizer additions give plants and microbes equal access to inorganic P in the soil environment, and microbes do not have a competitive advantage.

1.9.3 Stress Response to Low Soil Phosphorus Availability

Microbial phosphorus is dynamic and susceptible to significant changes due to environmental temperature, moisture and carbon supply (Richardson et al., 2011). In phosphorus deficient environments, plants and microbes respond to stress by increased expression of phosphatase enzymes and high affinity P transporters (Richardson et al., 2011). This starvation stress response is a scavenging strategy and persistence eventually results in smaller, hardier and more metabolically efficient microbial cells (Moat and Foster, 2002), which reduces MBC. In addition, low P availability can trigger plant exudations of organic anions in the rhizosphere, which increases the concentration of P in soil solution by solubilizing minerals and desorbing phosphate from soil particles (Richardson et al, 2011). These stress responses can increase P availability but also cost energy to microbes, which increases respiration and reduces MBC.

1.9.4 MBP in Long-term Organic-Conventional Comparison Studies

Low-input and organic agriculture systems can become deficient in phosphorus due to high P removal rates and slow rates of P mineralization from inputs (Welsh et al, 2009). Organic rotations that include forage and remove large amounts of nutrients several times through the season have higher risks for P deficiencies in plants because phosphorus is likely being extracted faster than it can be mineralized. Most organic systems in Canada include forages (Macey, 2010) and are likely removing phosphorus faster than it can be solubilized and replaced in the soil environment.

If organic agriculture systems have a greater potential for storing microbial carbon, they should also have higher potential for storing phosphorus compared to conventional soils. Soils with high capacities for microbial carbon also have the potential to store large inorganic

phosphorus pools inside microbial biomass (Brookes, 1982). However, in P limited environments, which can occur in organic systems, basal phosphorus mineralization (mineralization of P with no recent additions of soil amendments) may not be closely related to carbon (Oehl et al., 2004). It is necessary to maintain adequate levels of soil organic carbon *and* phosphorus in order to store large quantities of P in the microbial biomass. This is particularly important for organic systems with high P removal rates.

Agricultural management influences microbial biomass phosphorus dynamics over time (McLaughlin et al., 1988). Microbial biomass phosphorus is lower in systems that use synthetic fertilizer, compared to un-amended soils (Seeling and Zasoski, 1993). Soils with manure, compost, biodynamic additions, or no fertilizer demonstrate higher values for MBP (Table 3.1). Comparison of MBP in long-term studies provides valuable information about the effects of organic and conventional management on the capacity of soils to store P in the microbial biomass.

1.9.4.1 FiBL- The Swiss DOK Trial

In the DOK Trial, biodynamic (17.6 $\mu\text{g-MBC/g soil}$) and organic (16.5 $\mu\text{g-MBC/g soil}$) management systems had significantly higher microbial biomass compared to synthetically fertilized conventional (9.0 $\mu\text{g-MBC/g soil}$) soils (Oehl et al, 2004). These soils also sustained great microbial biomass carbon (Fleissbach et al, 2007). Currently there are not any parameters for acceptable ranges of MBP in agricultural soils. However, this study supports that higher MBP is found in soils with higher MBC.

1.9.4.2 Darmstadt- Hestia, Germany

Research from Darmstadt supports previously mentioned trends with higher microbial biomass phosphorus in soil amended with manure (28.9 $\mu\text{g-MBP/g soil}$) or compost, and higher microbial biomass carbon in the same soil (Heinze et al., 2010). While unfertilized plots under annual rotations have lower MBP, unfertilized plots and composted plots under perennials had higher base values for MBP.

1.9.4.3 Various Long-term studies-United Kingdom

Fifteen soils from the UK demonstrated that MBP was lowest where synthetic fertilizer was used (Brookes et al, 1984). In addition, this study compared annual and perennial agricultural soils. Higher values were found for MBP under permanent perennial fields (Table 3.1). These soils followed the same trend for MBP and MBC in both annual and perennial soils i.e. higher values of MBP were coupled with higher values of MBC. As a result, perennial soils have a higher capacity to store nutrients in the microbial biomass.

Table 3.1 Comparison of microbial biomass phosphorus ($\mu\text{g MBP/g soil}$) across long-term agricultural management practices under annual rotations and rotations that include perennials.

Agricultural Management	Annual			Perennial	
	¹ FiBL ($\mu\text{g-MBP/g soil}$)	² UK ($\mu\text{g-MBP/g soil}$)	³ Darmstadt ($\mu\text{g-MBP/g soil}$)	² UK ($\mu\text{g-MBP/g soil}$)	⁴ Northumberland, UK ($\mu\text{g-MBP/g soil}$)
No Fertilizer	8.0	6.0		48.6-87.6	37
Biodynamic	17.6a		7.2		
Organic+ Compost	16.5b			24.8 (bonemeal)	
Conventional+ Farmyard manure		28.9	8.0(compost)		193
Conventional + synthetic fertilizer	9.0c	5.3-7.0	7.0	12.0-106.0	88

¹(Oehl et al., 2004); ²(Brookes and Powlson, 1984); ³(Heinze et al., 2010); ⁴(He et al., 1997)

1.9.4.4 Palace Leas Meadow Hay Trial- Northumberland, UK

The Palace Leas long-term hay trial was established in Northumberland, UK in 1897. The site represents management treatments with no fertilizer, farmyard manure, and synthetic fertilizer. Similar to other studies, the soils with synthetic fertilizer (88 $\mu\text{g-MBP/g soil}$) had lower MBP values compared to soils with organic carbon amendments of raw manure (193 $\mu\text{g-MBP/g soil}$) (He et al, 1997). In addition, the soils in this study were under long-term perennial production and demonstrate higher MBP compared to annual cropping systems (Table 3.1). This research demonstrates the positive interaction of management and rotation on the capacity of a soil to store nutrients in the microbial biomass.

1.9.5 Synthesis of MBP

Values for microbial biomass phosphorus in rotations that include perennials have a higher bottom line compared with annual rotations under all management systems (Brookes, 1982). Organic soils also have an increased capacity to store P in the microbial biomass. Organic and perennial soils with organic C additions also show a greater capacity to support microbial biomass carbon. As a result, MBC and MBP collectively accumulate in soil. Therefore, in order to improve soil health and capitalize on the beneficial aspects of microbial P physiology, agricultural systems must manage soil organic carbon and phosphorus together.

Currently few studies have focused on the long-term effects of soils under different agriculture management and rotation on microbial biomass phosphorus over time. Research comparing the effect of different fertilizers (organic/inorganic), management and rotation on MBP over time can benefit nutrient management in all agriculture systems. The potential for building phosphorus in the microbial biomass could enhance crop production through increased understanding of environmentally protected P supplies. Phosphorus stored in microbial biomass may also have potential for reducing the negative effects of PO_4^{3-} leaching, which has been a problem for Lake Winnipeg in Canada.

1.10 Objectives and Hypotheses

The first objective of this research project was to evaluate the effect of annual and perennial rotations under organic, no-input, and conventional agricultural management on soil health, compared to restored grassland prairie. The microbial metabolic quotient ($q\text{CO}_2$), microbial biomass carbon, microbial biomass phosphorus, and nitrous oxide quotient ($q\text{N}_2\text{O}$)

measurements were used to address hypotheses in this study. The second objective was to evaluate these measurements over time to observe seasonal trends.

Hypotheses: 1) If agricultural rotation and management affect soil health, then qCO_2 will be significantly different in the order: annual-organic > annual-conventional > annual-no-input > perennial-organic > perennial-no-input > perennial-conventional > restored grassland prairie. 2) If agricultural rotation and management affect soil health, then microbial biomass carbon will be significantly different in the order: restored grassland prairie > perennial-organic > perennial-no-input > perennial-conventional > annual-organic > annual-conventional > annual-no-input. 3) If agricultural rotation and management affect soil health, then microbial biomass phosphorus will be significantly different in the order: restored grassland prairie > perennial-organic > perennial-no-input > perennial-conventional > annual-organic > annual-conventional > annual-no-input. 4) If agricultural rotation and management affect soil health, then qN_2O will be significantly different in the order: annual-conventional > perennial-conventional > perennial-organic > annual-organic > perennial-no-input > annual-no-input > restored grassland prairie. 5) Seasonal trends for all measurements will increase and peak during anthesis of crops followed by a decrease during and after harvest.

2.1 Materials and Methods

2.2 Experimental Site

The Glenlea long-term rotation located in Glenlea, Manitoba was established in 1992 to compare organic, conventional, no-input and restored prairie grass land management practices. The rotation has transformed over the years (Welsch, 2008). Since 2007, two 4-yr agriculture rotations exist: grain-only (*Triticum aestivum* (L.) wheat, *Linum usitatissimum*(L.) flax, *Avena sativa* (L.) oats, *Glycine max* (L.) soybean/*Glycine max* (L.), *Pisum sativum* (L.) pea, *Hordeum vulgare* (L.) barley green manure) and grain-forage (wheat, flax, *Medicago sativa* (L.) alfalfa, *Trifolium pretense* (L.) red clover, *Dactylis glomerata* (L.) orchard grass, and *Phleum pretense* (L.) Timothy forage yr1, forage yr2). Restored prairie grassland includes native perennial grasses (*Andropogon gerardii* Vitamin var. (L.), *Sorghastrum nutans* (L.), *Panicum virgatum* (L.), *Agropyron smithii* (Rydb.), *Elymus lanceolatus* (Scribn. and Smith) Gould., *Elymus trachycaulus* (Link) Gould ex Shinnars). Plot map in Appendix.

All years of both rotations are planted every season across all management practices. Restored grassland plots are perennial and have been burned every 4-5 years, most recently in June 2011. For the objective of this study only wheat plots were sampled because it is a common crop in both rotations and widespread through the Canadian Prairies.

2.3 Soil Classification and Properties

The site is 238 meters above sea level. Mean precipitation is 541.1 mm/yr with 432.4 mm of rainfall/yr (MAFRI Ag-Weather). Mean temperature for Glenlea during the 2011 summer season was 19.5° C and 513.7 mm precipitation reported by Statistics Canada. Soils at the experimental site are from the Blackearth zone (Manitoba Soil Survey, 1975). The Red River

association with two sub-classifications: Red River clay and Osborne Clay is found in all plots. Red River clay is well to intermediately drained. The typical “A” horizon is very dark grey to black clay, 20-25 cm thick; weakly prismatic macrostructure breaks readily into granular aggregates; very plastic and very sticky when moist, hard when dry; neutral in reaction (Manitoba Soil Survey, 1975). Native vegetation for Red River clay is tall prairie grasses, and meadow prairie grasses and herbs; aspen willow may occur on the intermediately drained sites. Natural fertility is high and soils are well suited for grain and grass-alfalfa mixtures. Slow drying of soil in wet seasons makes management difficult (Manitoba Soil Survey, 1975).

Osborne Clay is a poorly drained associate. The “A” horizon is very dark grey clay, 6-15 cm thick; amorphous appearing mass of feebly expressed granular aggregates; very sticky and very plastic when moist, very hard when dry; alkaline (Manitoba Soil Survey, 1975). Tongues into “Ca” horizon. Native vegetation consisted of swale grasses, and sedges. Aspen, black poplar, willows and dogwood grew east of the Red River. Natural fertility is moderate. Can be utilized for grain production and livestock but requires adequate drainage. Grasses and legumes that do well in spring moisture may not kill out. Osborne Clay is the least productive of the Red River soils. Management is difficult in wet conditions (Manitoba Soil Survey, 1975).

2.4 Agricultural Management Treatments

On May 19th, 2011 hard red spring wheat (cv. Waskada) was direct-seeded into conventional plots; organic and no-input plots were rotor-tilled and harrowed immediately prior to seeding. Fertilizer and herbicide rates were applied based on soil test and product recommendation (Table 4.1). Mature wheat was harvested with a plot combine on August 29, 2011. All plots were rotor-tilled post-harvest on September 20, 2011.

Table 4.1 Management treatments for wheat plots across annual-grain and forage-grain rotations for no-input, organic and conventional systems in 2011.

Management	Seeding Date/Rate	June 10	Tillage	Fertilizer	Herbicide
No-input	May 19 112 kg/ac	1 plot reseeded in the annual rotation	May 19/ Sept 20	-	-
Organic	May 19 112 kg/ha	1 plot reseeded in the annual rotation	May 19/ Sept 20	Compost 2007 10 t/ha conv cattle manure) N:2.52% P: 0.50% K: 2.45% S: 0.25% Dry matter: 49%	-
Conventional	May 19 112 kg/ha (Direct- seeded)	½ plot reseeded in the annual rotation	Sept 20	At seeding, 84 kg/ha urea and 39 kg/ha P were hand broadcast onto plots	Horizon (May 26 low rate not effective; June 24 re- applied recommended rate)

2.5 Soil Sampling and Analysis

Samples from all treatments were collected at intervals during the 2011 growing season. Soil cores were analyzed for CO₂ and N₂O gas emissions. Then soil cores were evaluated microbial biomass carbon and phosphorus. In addition, microbial *free-NH₄⁺* (the amount of NH₄⁺ that is not bound to another molecule) was quantified.

Soil sampling was repeated five times from May to October in 2011 (Table 5.1). Sampling dates were timed to evaluate soil health under pre-seed, tillering, soft dough, maturity, and post-harvest tillage conditions. Sample times were selected to capture microbial dynamics during a typical wheat growth cycle.

Table 5.1 Dates and wheat physiological stage during sample periods 1-5.

Sampling Date (GDD)	May 17 (127.2)	June 21	July 25	August 18	October 11
Wheat Physiological Stage	Pre-seed	Tillering	Dough	Maturity	Post-harvest

Since conventional plots were twice the size of organic and no-input plots, only half of each conventional plot was sampled on each date. A new random sample pattern was generated for every sampling period. All plots were sampled in the same pattern within a given date. Three intact soil cores were taken from each plot for a total of nine cores (5x15cm²) per treatment.

Before each soil core was removed from the plot, wheat and weed biomass along a length of 25 cm *within* one row and *between* one row-space was collected. Plant biomass was separated into wheat and weeds and mass was recorded. No above ground biomass was collected pre-seed and post-harvest. Once above ground plant biomass was removed, the soil core was removed. In prairie plots, soil samples were taken completely randomly. While observation indicates more weeds and less wheat in organic and no input plots, the sample unit size (25 cm of crop in one row and one space between the row was removed before each soil core was taken and separated into crop and weed biomass) was not large enough to render the data of any practical use.

Three intact soil cores were taken with an AMS Soil Core Sampler in each plot. While plants were developing, each core was extracted *directly* in the 25 cm where above ground plant biomass was removed. Half of the core was placed in the crop row and the other half of the cylinder rested between the rows. This placement was chosen in order to accurately represent the distinct differences between inter row-space in the three management and two rotation systems. When plants were not growing, proximity to the row was not considered.

Immediately after soil samples were removed from the corer, they were placed in plastic bags and stored in iced coolers until sampling was completed. Sampling typically began at sunrise and was completed within six hours. Soil cores were transported to the Soil Ecology Laboratory at the University of Manitoba where they underwent analysis.

2.6 Incubation Measurements (CO₂ and N₂O)

Intact soil samples were brought from coolers in the field to a growth chamber set at 21°C. Coolers were opened, ice packs were removed, and cores were allowed to reach equilibrium with the chamber temperature. Individual cores were placed in separate 1.5 L jars and sealed with a lid, with a rubber septum inserted in the top. After two hours, 20 mL samples were drawn with a 23 gauge needle and additional syringe. Samples were inserted into 12 mL evacutainer vials for CO₂, and N₂O analysis. Following incubation, intact soil cores were placed back in plastic bags and into coolers where they were stored at 4°C overnight. Gas was stored at room temperature and analyzed using gas chromatography within two months.

All subsequent lab procedures were performed within eight consecutive days from initial sampling. When samples were not being weighed or processed they were stored at 4°C. The day after sampling, soil was broken by hand using powder free gloves. Soil protocol recommends breaking soil into pieces smaller than 1cm³, which was almost always accomplished. On occasion, some pieces of soil were too dry to break apart without using excessive force and time, which I decided to be a poor use of time management. Breaking up soil samples typically extended over 7-9 hours with two people working; time depended on soil moisture.

Individual soil cores were broken down to the appropriate size within the original bag and then sealed and placed directly back into the cooler. Individual soil samples were kept in their

original bags throughout analysis. Soils were kept in the order they were taken out of the ground and all analysis was performed in the same order at every point throughout all procedures.

2.7 Microbial Biomass Carbon

The day after breaking soil in pieces $<1 \text{ cm}^3$, samples were prepared for microbial biomass carbon and free- NH_4^+ analysis. Microbial biomass carbon and free- NH_4^+ were measured using chloroform-fumigation-direct-extraction (Vance, 1987; Jenkinson, 1981). Field moist soil from individual samples was weighed into 25g portions. Two 25g portions were weighed into fumigated (F) and un-fumigated (UN) groups for each soil sample (2x63). Samples were extracted in glass, square, French bottles with #5 stoppers. French bottles were labeled permanently to correspond with individual samples so that the same bottle was used for the same core out of the same plot every sample period. Some bottles broke and were replaced.

Soil samples were extracted using 50 mL 0.5M K_2SO_4 . Before shaking un-fumigated samples, fumigation was set-up for fumigated samples (this process was generally completed in 1.5-2hrs). Fumigation is thought to dissolve microbial cell membranes, spilling their guts into the soil where they can be extracted. Fumigated samples were placed in vacuum sealed desiccators with 40 mL ethanol free chloroform (CHCl_3) in a 100 ml beaker with boiling chips. Moist paper towels lined the inside walls of the desiccators. Chloroform was boiled under reduced vacuum pressure for 2-3 minutes and then desiccators were sealed and covered with aluminum foil for dark conditions. Fumigation lasted 24 hours (Carter and Gregorich, 2008).

Un-fumigated samples were extracted immediately after completing the fumigation set-up. Fifty milliliters of extraction solution was added to each of the un-fumigated samples; bottles were sealed with a stopper and shaken vigorously by hand before shaking on a horizontal shaker

for two hours at 150 rpm. Each bottle was then poured through an 150 mm Whatman 934-AH glass microfiber filter paper (folded into a cone) into a 20 mL scintillation vial (Carter and Gregorich, 2008). Extracts were frozen immediately.

After 24 hour fumigation, moist paper towels were removed and desiccators were evacuated for two 1-hour periods to remove residual CHCl_3 . Fumigated samples were then immediately shaken for two hours with 50ml 0.5 M K_2SO_4 . Samples were then poured through 150 mm Whatman 934-AH glass microfiber filter papers into 20 mL scintillation vials and frozen immediately (Carter and Gregorich, 2008).

Extracts were analyzed using the Technicon Auto-Analyzer II System. Carbon was determined colorimetrically. Determination involves a pretreatment to remove inorganic C by subjecting the acidified solution to a high velocity stream of C-free air to remove carbonate-derived CO_2 . Next the sample is mixed with 0.5 M H_2SO_4 and 4% (w/v) potassium persulfate, and subjected to UV radiation. The result is CO_2 derived from the organic C. This CO_2 is dialyzed through a silicone rubber membrane and reacted with a weakly buffered phenolphthalein indicator. Finally, the decrease in the intensity of the color of the indicator is measured at 550 nm and is proportional to the organic C content (Carter and Gregorich, 2008).

Free-NH₄⁺ was analyzed using the Technicon Auto-Analyzer II System. The oxidation step for total microbial biomass nitrogen was not performed. Nitrogen was measured using Cd reduction followed by the Griess-Ilosvay reaction (Carter and Gregorich,). The result is NH_4^+ as a result of fumigation.

Gravimetric moisture was measured for use in microbial biomass carbon and phosphorus calculations. GMC measurements were taken between carbon and phosphorus procedures so that

moisture approximated moisture levels during both extractions. Moisture did not appear to change much over time based on observation.

Calculation:

$$\text{MBC } (\mu\text{g-MBC/g-soil}) = (C_F - C_{UF}) / K_{EC} \quad (1)$$

$K_{EC} = 0.25$ and is the microbial biomass carbon recovery constant (Carter and Gregorich, 2008).

2.8 Microbial Biomass Phosphorus

Microbial biomass phosphorus was measured by chloroform-fumigation-direct-extraction according to the technique developed by Brookes et al., (1984). Five grams of soil from each subsample (63 total) was weighed into fumigated (F), un-fumigated (UN), P-spiked groups (SPK). Un-fumigated samples were spiked with 0.5ml de-ionized water and extracted immediately with 100ml 0.5M NaHCO_3 pH=8.5, after shaking for one hour. P-spiked samples received 0.5ml 250 $\mu\text{g/g}$ PO_4^{3-} and extracted immediately with 100ml 0.5M NaHCO_3 pH=8.5, after shaking for one hour. After 24 hour fumigation (method described earlier), fumigated samples were spiked with 0.5ml de-ionized water and extracted immediately with 100ml 0.5M NaHCO_3 pH=8.5, after shaking for one hour. All extracts were filtered through Whatman No. 40 filter papers and frozen immediately (Carter and Gregorich, 2008).

Extracts were analyzed using the Technicon Auto-Analyzer. Phosphorus concentration was determined by the method of Murphy and Riley (1962). In this method, ammonium molybdate and antimony potassium tartrate react in an acidic environment with phosphate to form an antimony-phospho-molybdate complex. Ascorbic acid is used to reduce the antimony-phospho-molybdate complex, which results in an intense blue-colored complex. The absorbance

was determined at 890 nm and is proportional to the phosphate concentration (Carter and Gregorich, 2008).

Calculation:

$$\text{MBP } (\mu\text{g-MBP/g-soil}) = [(P_F - P_{UF})/K_{EP}] \times (100/R) \quad (2)$$

$K_{EP} = 0.40$ and is the microbial biomass phosphorus recovery constant; and $R = 100[(P_i \text{ spiked soil} - P_{UF})/P_{250} \text{ spike}]$ and is the inorganic phosphorus recovery for each sample (Carter and Gregorich, 2008).

2.9 Microbial Metabolic Quotient and Microbial Nitrous Oxide Quotient

The metabolic quotient is calculated from the carbon dioxide emitted and microbial biomass carbon measurement from the same soil sample.

Calculation:

$$q\text{CO}_2 \text{ (mg-CO}_2\text{-C/g-MBC/hr)} = \text{CO}_2/\text{MBC} \quad (3)$$

The nitrous oxide quotient is calculated from the nitrous oxide emitted from the soil and microbial biomass carbon measurement from the same soil sample.

Calculation:

$$q\text{N}_2\text{O (mg-N}_2\text{O-N/g-soil/hr)} = \text{N}_2\text{O/MBC} \quad (4)$$

2.10 Statistical analysis

Statistical analysis for response variables were performed using Statistical Analysis System 9.2 (SAS Institute, 2002-2008) software. Analysis of variance ($\alpha = 0.05$) for microbial

biomass carbon, microbial biomass phosphorus, microbial metabolic quotient, and basal soil CO₂ emission responses measured multiple times on the same experimental unit were analyzed using a repeated statement in the mixed procedure of SAS. Fixed effects were management, rotation, and time. The experimental design was treated as a 2x3 factorial with a completely randomized design, and was decided to be the most appropriate design for this experimental site. Prior to analysis, the data did not meet the test for normality (Shapiro-Wilks). Data were subsequently log₁₀ transformed, after which all data were verified for normality of residuals (Shapiro-Wilks). Outliers were removed manually. Constant variance of error terms was visually tested while plotting the residuals versus the fitted values. Horizontal alignment across upper and lower values suggested constant variance. Means were separated using the LSD test (pdiff option in SAS).

A separate analysis was conducted to compare each treatment to the grassland prairie plots. This analysis was run assuming the experimental design resembled a completely randomized design without repeated measures. Therefore, seven main effects were analyzed (prairie, FG no-input, FG organic, FG conventional, AG no-input, AG organic, and AG conventional). SAS was used to compare treatments using estimate statements in GLM. To compare qN₂O, only data from date1 was statistically analyzed. GLM analysis was run using contrast statements.

3.1 Results and Discussion

Analysis of variance resulted in significant main effects and significant interactions of main effects. Table 3.0 illustrates results of the complete ANOVA for all main effects and interactions. Rotation effects were always found to be significantly different between treatments indicating that perennial rotations have a distinctly different impact on soil health compared to annual rotations. The effect of date also differed significantly illustrating that seasonal dynamics heavily influence microbial biomass activity and therefore soil health. The interaction of rotation and management significantly affected microbial carbon but not phosphorus signifying that while microbial carbon is susceptible to change due to rotation and management, microbial phosphorus may not be sensitive to the same conditions. Rotation, management, date, and interactions between treatments offer support that soil health is significantly affected by different agricultural practices over time.

Table 6.1 Analysis of variance table for dependent variables across all sample dates (*qN₂O values are for date1 only). Letters represent significant differences (LSD; $\alpha = 0.05$) within each column. Treatments include: organic (Org), no-input (NI), and conventional (Conv) management, under forage-grain (FG) and annual-grain (AG) rotations.

	MBC ($\mu\text{g/g}$)	CO ₂ (mg/kg/hr)	qCO ₂ (mg/g/hr)	*N ₂ O (mg/g/hr)	*qN ₂ O (mg/g/hr)	MBP ($\mu\text{g/g}$)	MBC/ MBP
Rotation (R)							
Forage-Grain	1613a	1.17a	0.75a	4.98E-04a	6.33E-04a	53a	30
Annual-Grain	1124b	0.65b	0.60b	1.11E-04b	1.09E-04b	36b	34
Management (M)							
No-Input	1336ab	0.86	0.67	3.48E-04a	1.24E-04c	48	32
Organic	1384a	0.91	0.68	1.41E-04b	2.34E-04b	43	29
Conventional	1319b	0.86	0.67	2.65E-04a	6.22E-04a	41	34
Date							
1	1499a	1.27b	0.99b	-	-	37bc	40ab
2	1450ab	1.73a	1.19a	-	-	41b	37b
3	1251c	0.63d	0.50d	-	-	27d	51a
4	1166d	0.43e	0.37e	-	-	32cd	39ab
5	1396b	0.87c	0.92c	-	-	129a	11c
RxM							
FG-NI	1648a	1.20a	0.76a	4.04E-04ab	3.33E-04	49	33
FG-Org	1718a	1.35a	0.81a	3.80E-04ab	7.08E-04	65	26
FG-Conv	1476b	1.00b	0.70ab	8.05E-04a	1.07E-03	47	31
AG-NI	1080d	0.62d	0.59c	3.00E-04b	4.62E-05	37	32
AG-Org	1115d	0.62d	0.57c	5.20E-05c	7.73E-05	35	33
AG-Conv	1179c	0.73c	0.64bc	8.76E-05c	3.60E-04	36	36
ANOVA							
Rotation	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	ns
Management	0.0355	ns	ns	<.0133	<.0001	ns	ns
Date	<.0001	<.0001	<.0001	-	-	<.0001	<.0001
RxM	<.0001	0.0002	0.0513	<.0047	ns	ns	ns
RxD	ns	<.0001	<.0001	-	-	0.0043	ns
MxD	ns	ns	ns	-	-	0.0533	ns
RxMxD	ns	ns	ns	-	-	ns	ns

3.2 Soil Moisture

Volumetric soil moisture was measured at each sample date. Management, rotation, and date were significant main effects. Interactions of main effects were not statistically significant.

Soil moisture was significantly the highest on the first sample date across all treatments (Figure

1.1). Soil moisture followed the same *seasonal* trends as microbial biomass carbon, CO₂, and qCO₂, across all treatments. Therefore, one of the important factors affecting these parameters was the water content of the soil.

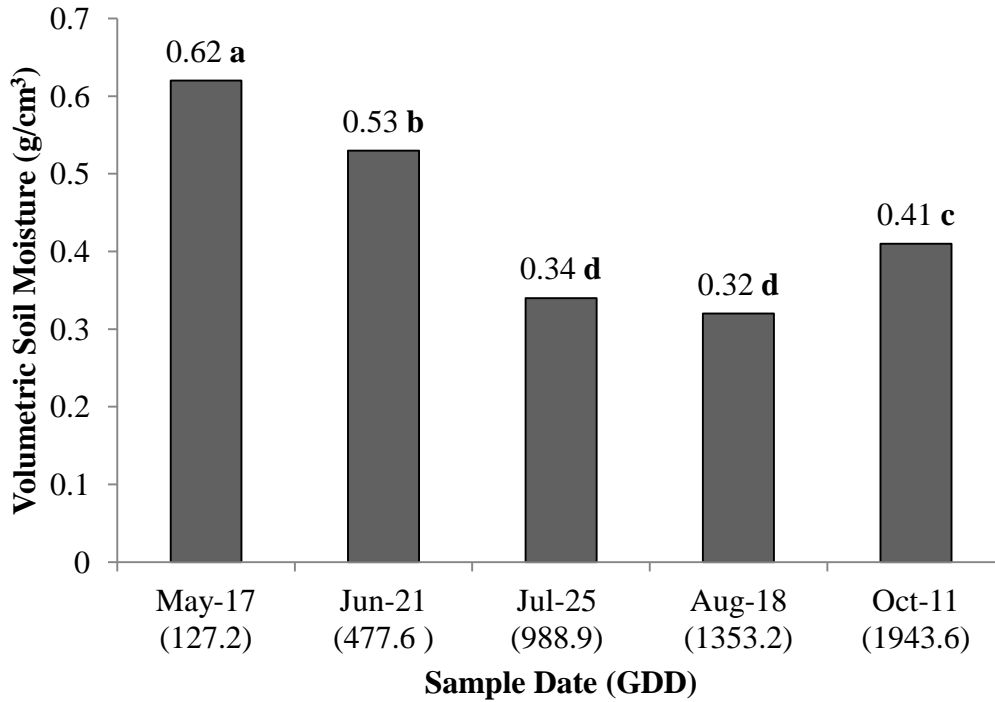


Figure 1.1 Volumetric soil moisture (g/cm³) by date across all treatments. Letters represent significant differences across sample dates ($\alpha = 0.05$).

3.2.1 Soil Moisture: Main Effects

Due to heavy spring rain, soil moisture was the greatest during the first two sample periods. Soil moisture significantly decreased from date 2 to date 3 and date 4. These two sample dates (3 & 4) took place during long periods without precipitation and were conducted under extremely dry conditions. By date 5 however, soil moisture significantly increased due to fall precipitation.

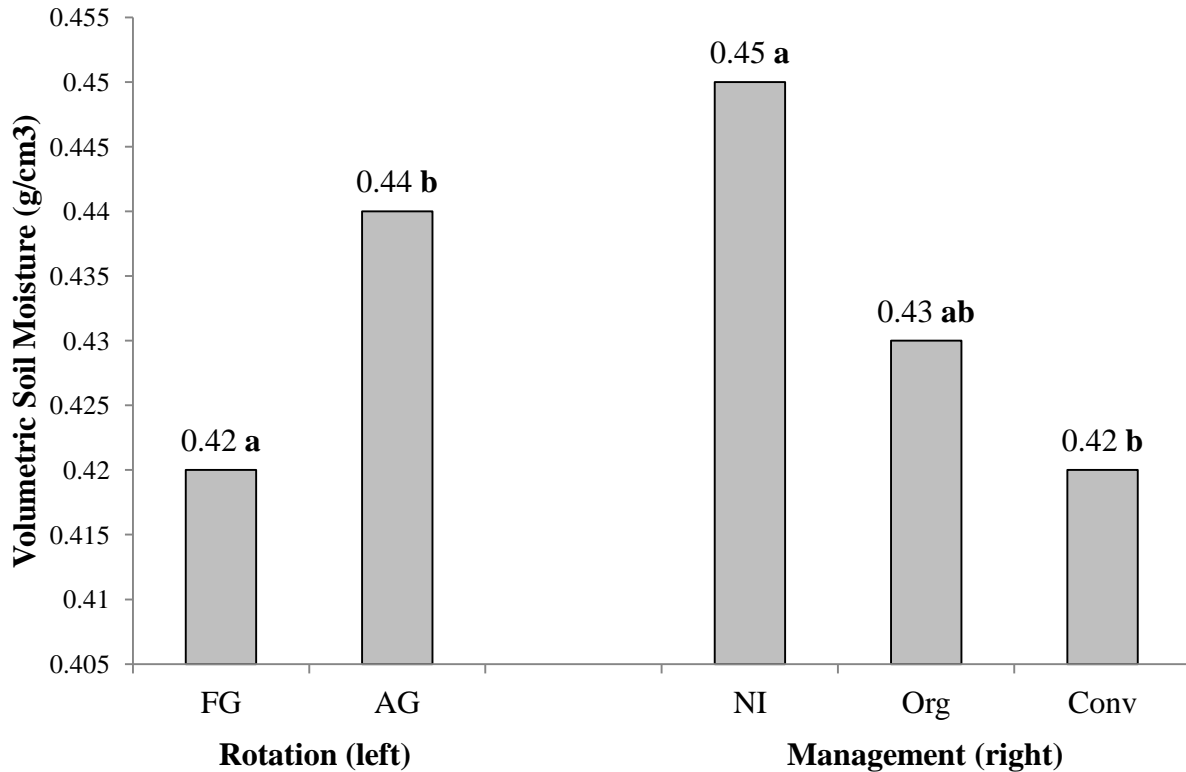


Figure 2.1: Volumetric soil moisture (g/cm³) for main effects of rotation (FG: forage-grain; AG: annual-grain) and management (NI: no-input; Org: organic; Conv: conventional) across all sample dates. Letters represent significant difference within each main effect ($\alpha = 0.05$).

Volumetric soil moisture was significantly higher in the AG rotation compared to the FG rotation. Lower moisture content of forage-grain soils can be attributed to increased water use from uptake by perennial roots in the previous fall (Ward et al, 2002), before forages were terminated. These results indicate that when soil moisture was high the FG rotation mitigated water better than the AG system. However when moisture was low, the FG system had no beneficial effect on soil moisture indicated by the absence of significant interactions between rotation and date.

Management also had a significant effect on soil moisture. The no-input system had the highest water content, and was significantly greater compared to the conventional treatment. The organic cropping system was not different from either no-input or conventional treatments. Since

moisture has a significant impact on soil respiration (Yuste, 2007), soil water content as an effect of cropping system is integral for interpreting biological parameters.

3.3 Microbial Biomass Carbon

3.3.1 MBC: Main Effects

Microbial biomass carbon was significant for agricultural rotation and management effects. MBC was higher under the forage-grain rotation (1613 $\mu\text{g-MBC/g soil}$) compared to the annual-grain rotation (1124 $\mu\text{g-MBC/g soil}$). Literature values from long-term studies support MBC values found in the present study for perennial rotations. Literature values for MBC from perennial rotations range from 1379-2104 $\mu\text{g-MBC/g soil}$ (Brookes et al, 1982; He et al, 2007). However, MBC values from Glenlea were much higher than literature values for other long-term annual rotations. MBC values range from 30.3-470 $\mu\text{g-MBC/g soil}$ according to previous literature (Heinz et al, 2010; Joergenson et al, 2010; Mazzoncini et al, 2004). Higher values found in the present study could be attributed to the high percent of clay in the Glenlea soil, which has higher surface areas compared to the loams and sands measured in the literature.

MBC was significantly higher in the organic (1384 $\mu\text{g-MBC/g soil}$) system compared to the conventional (1319 $\mu\text{g-MBC/g soil}$) system, while the no-input (1336 $\mu\text{g-MBC/g soil}$) system was not statistically different from either. Literature trends are similar with organic management systems sustaining higher MBC than their conventional counterparts (Joergenson et al, 2010; Fliessbach et al, 2007). The results for management and rotation effects are therefore consistent with current literature trends.

3.3.2 MBC: Interactions

3.3.2.1 MBC: Rotation x Management

Significant interactions were found for rotation and management. While microbial biomass carbon was significantly different for the management x rotation interaction, (Table 3.0), trends for this interaction were different within each rotation (Figure 3.1). Within the FG rotation, the trend was organic>no-input>conventional; while in the AG rotation the trend was conventional>organic>no-input.

The organic (1718 $\mu\text{g/g}$) and no-input (1648 $\mu\text{g/g}$) treatments in the FG rotation were not statistically different (Table 3.0). However, soils under conventional management within the FG rotation supported significantly less MBC (1476 $\mu\text{g-MBC/g soil}$) compared to organic and no-input treatments in the same rotation. MBC measurements demonstrated opposite trends in the AG rotation compared with the FG rotation. Within the AG rotation, conventional microbial biomass carbon (1179 $\mu\text{g-MBC/g soil}$) was significantly higher than both organic (1115 $\mu\text{g-MBC/g soil}$) and no-input (1080 $\mu\text{g-MBC/g soil}$) treatments. Organic and no-input treatments within the AG were statistically the same. Within each rotation, no significant differences between organic and no-input were found. However, conventional management within each rotation was always significantly different.

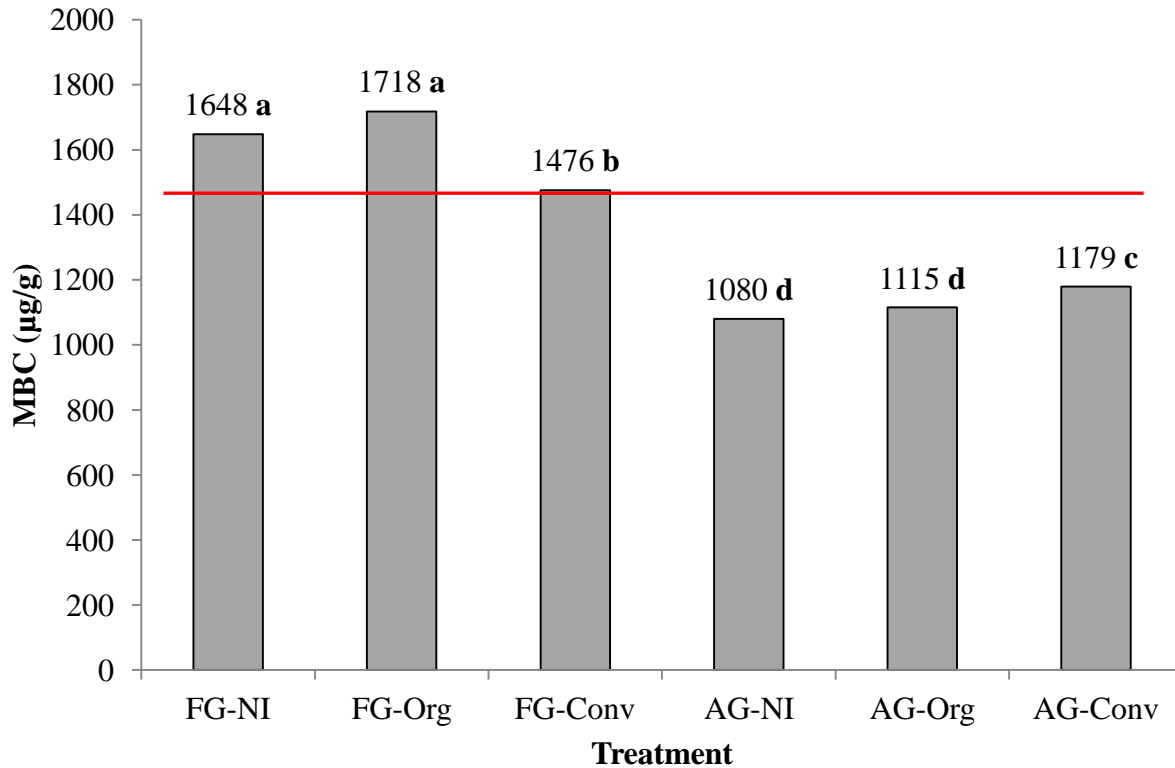


Figure 3.1 Microbial biomass carbon measurements ($\mu\text{g MBC/g soil}$) under organic (Org), no-input (NI), and conventional (Conv) management treatments for forage-grain (FG) and annual-grain (AG) rotations across all sampling dates. Red line represents grassland prairie average.

In the present study, this perennial system demonstrated significantly greater ability to sustain microbial life compared to the annual-grain (AG) rotation. This trend agrees with previous literature that demonstrates perennial rotations have higher carrying capacities for microbial biomass carbon than annual rotations. Higher MBC in organic vs conventional systems observed in the literature (Fließbach et al., 2007) also supports results from the present study. However, the AG rotation does not agree with this literature trend since the organic system had significantly less MBC than the conventional treatment in the present results. Published research shows that with the addition of organic amendments in annual rotations, MBC increases compared to the use of synthetic fertilizer (Table 1.1). For example, the Swiss DOK Trial applied farmyard manure to a conventional annual rotation and this soil had significantly higher MBC

than the conventional system using only synthetic fertilizers. The annual-grain rotation in the present study had significantly more MBC in the conventional system, contradicting previous research.

The major difference between soil micro-environments across treatments is the presence of roots throughout the growing season. The rhizosphere is the principal site of microbial colonization in soil environments (Watt et al., 2006). Differences in microbial biomass carbon values across treatments could be related to the presence of the rhizosphere and consequent rhizodepositions of readily utilizable carbon substrates from plants to microbes. In addition to the presence of roots, diversity of plant roots and exudations might be contributing factors to differences in MBC across treatments.

The difference between conventional and organic and no-input treatments in the annual-grain rotation follows the difference in the presence of the rhizosphere environment. The lowest MBC values in the annual-grain no-input and organic systems could be explained by the early termination (terminated in July at anthesis) of the green-manure year in the annual rotation. Terminating the green-manure also terminates the presence of any rhizosphere. The conventionally managed soil in the annual-grain rotation had significantly more MBC than organic and no-input. In the AG-conventional system, soy-beans coincide with the green-manure year in the organic and no-input rotations. Soy-beans are typically harvested last, around September or even into early October. The presence of soy-bean in the AG-conventional system extends the duration of the rhizosphere environment at least 2 months longer than its organic and no-input counterparts. This result suggests that long-term effects of rotation and management interactions on soil health may be related to the presence of plants and their roots.

The forage-grain rotation demonstrated significantly higher microbial biomass carbon compared to the annual-grain rotation. The reason for this could be that perennial plants substantially increase the presence of the rhizosphere environment, which persists through winter months for two years of the four year rotation. In addition, more plant diversity is found in the FG rotation since the forage years are composed of four species. Increasing plant diversity also increases diversity of root exudations and can stimulate different microbial communities (Rovira, 1956). Including forages in rotation may increase MBC as a consequence of persistent rhizodepositions and due to the increased plant diversity provided by the mixed forage.

Considering that roots are present for the same duration under no-input, organic and conventional management systems in the forage-grain rotation, significant differences under these treatments are more likely due to management itself. The major difference in management between conventional, organic, and no-input is the use of synthetic fertilizer and source of carbon input. Higher MBC in the forage-grain organic system compared to the forage-grain conventional cannot be attributed to compost addition since the forage-grain no-input was also significantly higher than forage-grain conventional and not statistically different from forage-grain organic soils.

An additional explanation for the increased MBC in the forage-grain no-input and forage-grain organic system could be that increased weed persistence in the organic and no-input treatments increases the presence and diversity of the rhizosphere in soil. Consequently, biomass returned to the soil from forage-grain no-input and forage-grain organic soils are also more diverse and could potentially stimulate more ecological niches where microbial communities can survive and reproduce.

Microbial biomass carbon was not different between organic and no-input management in either rotation. Considering that the half-life of beneficial compost effects on microbial biomass carbon has been recorded as 6 years (Reeve et al., 2011), some nutrient *other* than carbon might be limiting microbial growth in the organically managed soils. Or, the benefits of the compost have diminished and this compost had a shorter half-life.

3.3.2.2 MBC: Management x Time

A significant interaction between cropping system and time indicated that rotation and input effects for MBC differed across the growing season. No differences were observed between treatments on the first, third, and fourth sampling dates. However, significant differences for MBC were observed on date 2 and date 5 for organic, no-input and conventional management treatments (Table 6.1; Figure 4.1).

On date 2 in June, the organic treatment showed significantly higher MBC compared to no-input, but not compared with conventional management. All treatments significantly decreased from date1 and date2 to date3 in July, when moisture became limiting. MBC stayed the same from date 3 to date 4 for all treatments. From date 4 to date 5 when soil moisture increased, organic and no-input MBC values increased significantly to statistically similar values as on date 1. However, the conventional treatment did not significantly increase from date 4 to date 5 and maintained values significantly lower than MBC on date 1, decreasing MBC over the growing season. The conventional treatment on date 5 was therefore significantly lower than both organic and no-input cropping systems. By the end of the season in October, both organic and no-input MBC demonstrated significantly greater capacities to support microbial life.

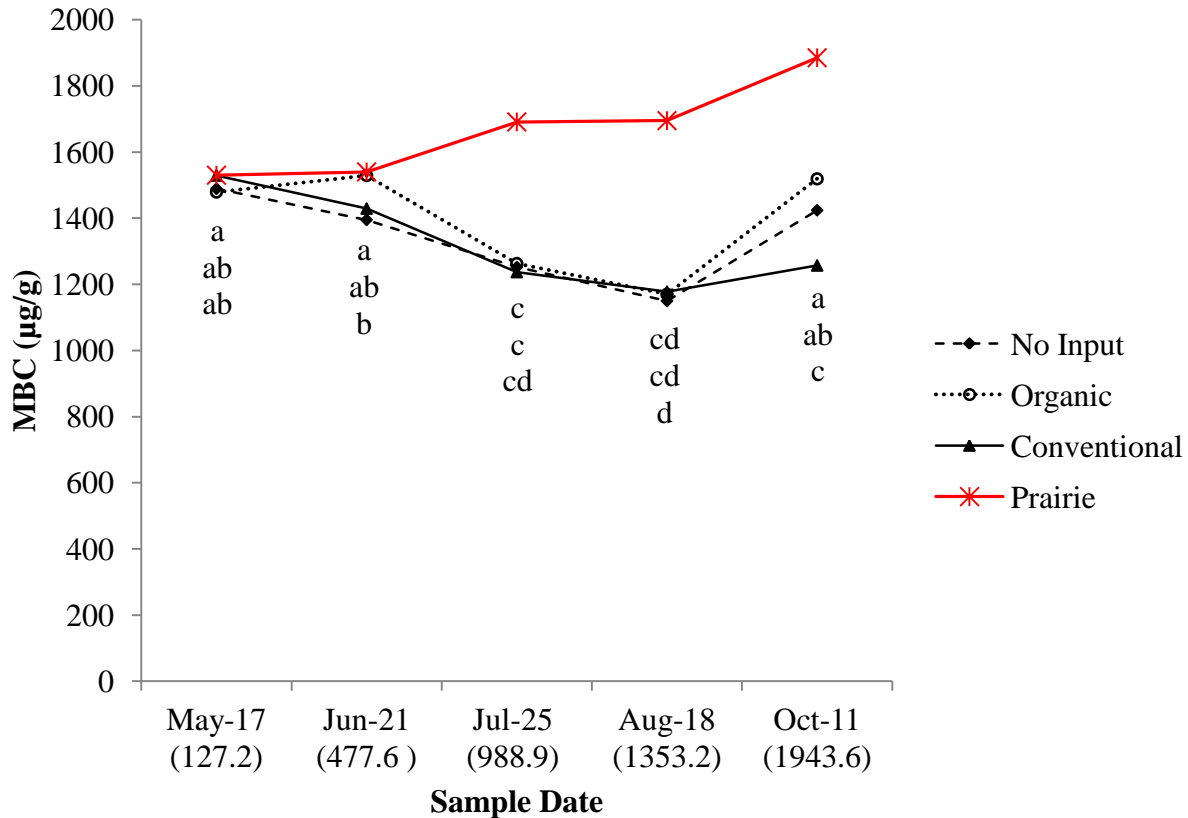


Figure 4.1 Seasonal microbial biomass carbon measurements ($\mu\text{g MBC/g soil}$) under organic, no-input, and conventional management, and prairie grassland across five sampling dates. Letters signify differences ($\alpha = 0.05$) between and across management and date, but **do not** apply to prairie grassland (red line).

Comparing management treatments between date 1 and date 5 illustrates that seasonal dynamics of MBC are affected by agricultural practices. Organic and no-input systems were not statistically different on date 1 in May and date 5 in October. Although MBC for organic and no-input significantly decreased on date 3 and date 4, these treatments demonstrated the ability to build soil microbial carbon back up to initial values. In contrast, conventional management on date 5 was significantly lower than date 1. As a result, conventional systems did not demonstrate the ability to build soil microbial carbon back up to initial spring values. There are not any published studies for comparing these seasonal dynamics between long-term conventional, organic, and no-in-put systems.

While all management systems and the grassland prairie demonstrated equal MBC on date 1 of the growing season, by date 5 the conventional soils had significantly decreased MBC. Organic and no-input MBC was statistically the same on date 1 and date 5 demonstrating the ability to maintain and sustain significantly more MBC. The seasonal transition from extremely wet to extremely dry, followed by re-wetting in the fall, may imply that conventional soils were not resilient to these changing environmental conditions. Organic and no-input soils were resilient to these extreme changes demonstrated by the maintenance of the initial microbial biomass carbon. Other studies also showed organic and no-input agriculture systems are more resilient to extreme weather conditions and plant diversity can increase drought resilience (Sanaullah, 2011) compared to conventional soils. Since no-input and organic systems maintained MBC over time, the maintenance of MBC is not due to the input of compost. The decrease in MBC in the conventional soils is *not* due to withholding organic carbon additions or the no-input system would have decreased, which it did not. The decrease in MBC in the conventional soil might be explained by the use of synthetic fertilizers and pesticides by reason of deducting other variables.

The increasing trend of MBC in the prairie grassland suggests that these soils never underwent a state of self-induced dormancy. Self-induced dormancy can be recognized by decreased microbial biomass carbon, respiration, and values for qCO_2 below $0.50 \text{ mg } CO_2\text{-C/g-MBC/hr}$. While all other treatments demonstrated characteristics of self-induced dormancy over the growing season, the prairie grassland did not. Observations of extremely dense root masses in prairie soils offer an explanation for increased MBC as a consequence of abundant carbon substrates from living and dead roots and better water buffering capacity. Other studies have

demonstrated that natural systems like prairie grassland support the healthiest soils and are resistant to periods of extremely dry conditions (Williams, 2007).

3.4 Microbial Respiration (CO₂)

3.4.1 CO₂: Main Effects

Results for CO₂ emissions from soil were significant for rotation but not management (Table 6.1). Respiration was significantly higher in the forage-grain (1.17 mg-CO₂-C/kg-soil/hr) rotation compared to the annual (0.65 mg-CO₂-C/kg-soil/hr) rotation. The forage-grain rotation also had higher MBC, which is consistent with literature trends that show MBC and CO₂ have a positive, linear relationship (Anderson and Domsch, 1985).

3.4.2 CO₂: Interactions

3.4.2.1. CO₂: Rotation x Management

Microbial respiration showed a significant interaction between rotation and management. Both forage-grain no-input (1.20 mg-CO₂-C/kg-soil/hr) and forage-grain organic (1.35 mg-CO₂-C/kg-soil/hr) released significantly more CO₂ than forage-grain conventional (1.00 mg-CO₂-C/kg-soil/hr) and all other treatments from the annual rotation (Fig. 5.1). Within the annual rotation, the conventional soil (0.73 mg-CO₂-C/kg-soil/hr) released significantly more CO₂ than both annual organic (0.62 mg-CO₂-C/kg-soil/hr) and annual no-input (0.62 mg-CO₂-C/kg-soil/hr). CO₂ trends for management were opposite for the forage-grain compared to the annual rotation. Results from CO₂ echo results from MBC, which was predicted by the literature demonstrating a positive linear relationship between MBC and respiration (Anderson and Domsch, 1985). Therefore as MBC increases so does CO₂ emission from soil.

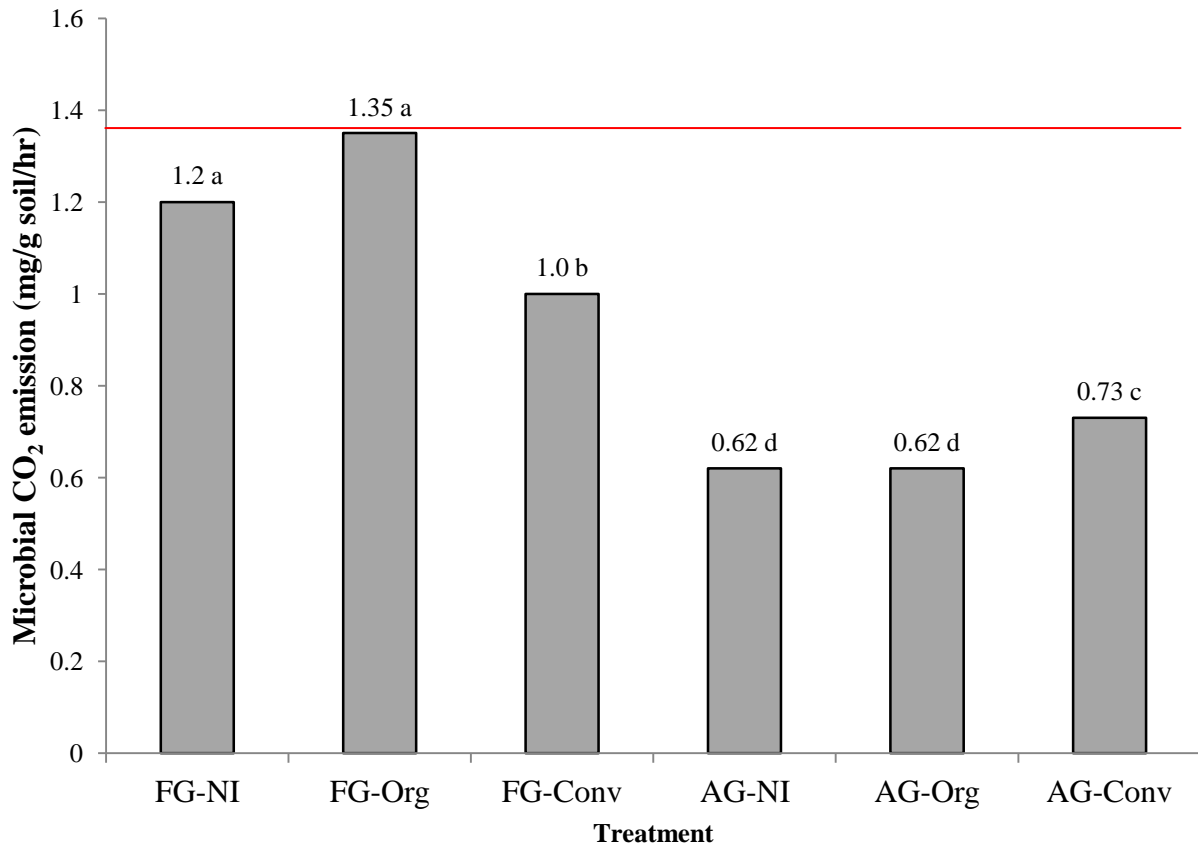


Figure 5.1 Microbial respiration measurements ($\mu\text{g MBC/g soil}$) under organic (Org), no-input (NI), and conventional (Conv) management treatments for forage-grain (FG) and annual-grain (AG) rotations across all sampling dates. Red line is the prairie seasonal average MBC value.

3.4.2.2 CO₂: Rotation x Date

Across all treatments, total CO₂ emissions were significantly higher in the forage-grain than the annual grain on all sample dates (Fig. 5.1). Both rotations significantly reduced CO₂ emissions from date 2 to date 3, and from date 3 to date 4 when soil moisture also significantly declined. From date 4 to date 5, all treatments significantly increased microbial CO₂ as soil was rewetted by rainfall. Overall, results show that the higher CO₂ emission potential with forage-grain was observed more when moisture conditions improved compared with the annual rotation.

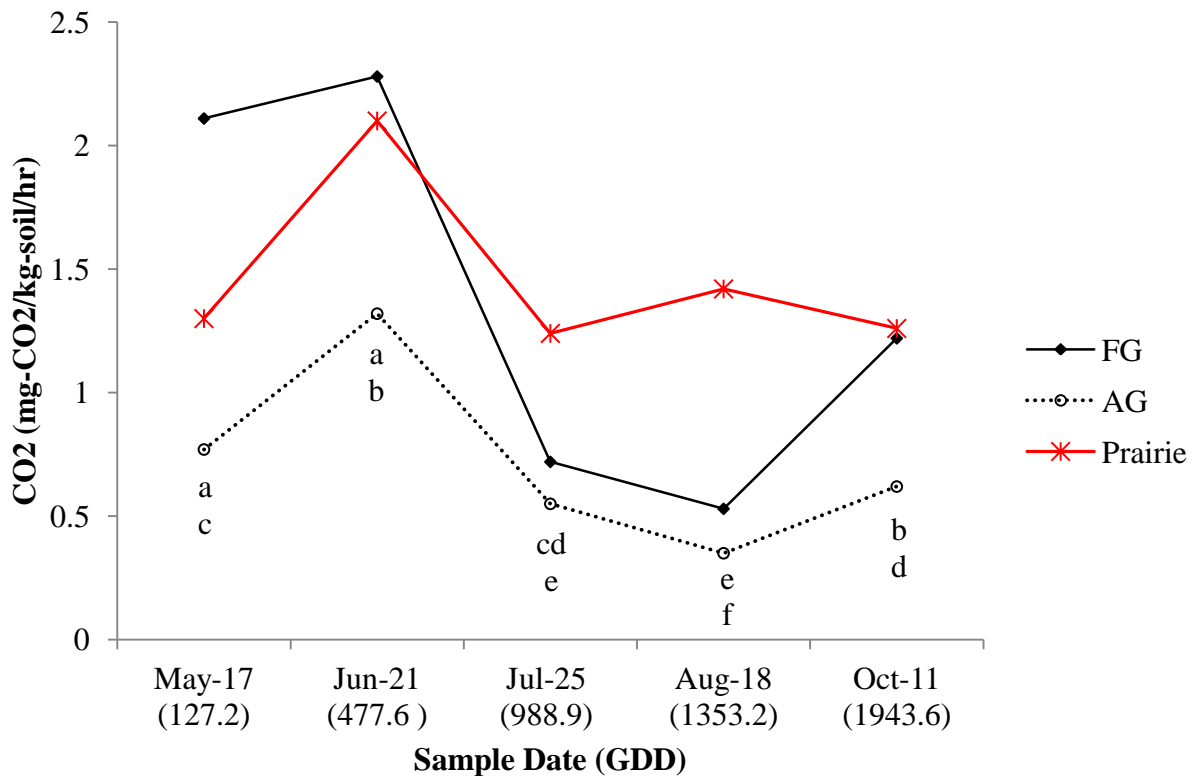


Figure 6.1 Seasonal microbial respiration measurements (mg-CO₂/kg-soil/hr) under forage-grain (FG), and annual-grain (AG), and prairie grassland across five sampling dates. Letters represent significant differences ($\alpha = 0.05$) within and across FG and AG data series only and *do not* apply to prairie grassland values.

3.5 Microbial Metabolic Quotient (qCO₂)

3.5.1 qCO₂: Main Effects

The microbial metabolic quotient showed significant differences for rotation but not management (Table 6.1). The qCO₂ was higher in the forage-grain (0.75 mg-CO₂-C/g-MBC/hr) than the annual (0.60 mg-CO₂-C/g-MBC/hr) rotation. Both soils fall within the acceptable range of qCO₂ (0.5-2.0 mg-CO₂-C/g-MBC/hr) for agricultural soils (Anderson and Domsch, 2003).

Trends for qCO₂ under forage-grain rotations are not recorded in the literature.

3.5.2 qCO₂: Interactions

3.5.2.1 qCO₂: Rotation x Management

Statistical interactions between rotation and management were significant (Fig. 7.1) indicating that management systems responded differently in the two cropping systems. The interaction occurred because the conventional system was lower than the organic and no-input systems in the forage-grain rotation but higher in the annual rotation. Still, within the forage-grain rotation and within the annual rotation there were not any differences in qCO₂ and the same result occurred within the annual rotation. MBC and CO₂ were significantly higher for organic and no-input treatments compared to the conventional in the forage-grain rotation and qCO₂ was equivalent, which may be a consequence of mature soils reaching equilibrium between active and passive fractions of the microbial population. The same explanation could apply to the annual rotation where significantly higher MBC and CO₂ was observed for the conventional treatment but qCO₂ values were not different.

Soils in the annual rotation were significantly lower than organic and no-input soils from the forage-grain rotation. Conventional forage-grain and conventional annual soils were not significantly different, but annual organic and annual no-input soils had significantly lower qCO₂ than the forage-grain conventional treatment. This observation illustrates that management and rotation together have a larger effect on organic systems than conventional systems. Organic and no-input treatments demonstrated significantly different results for qCO₂ while conventional treatments performed the same.

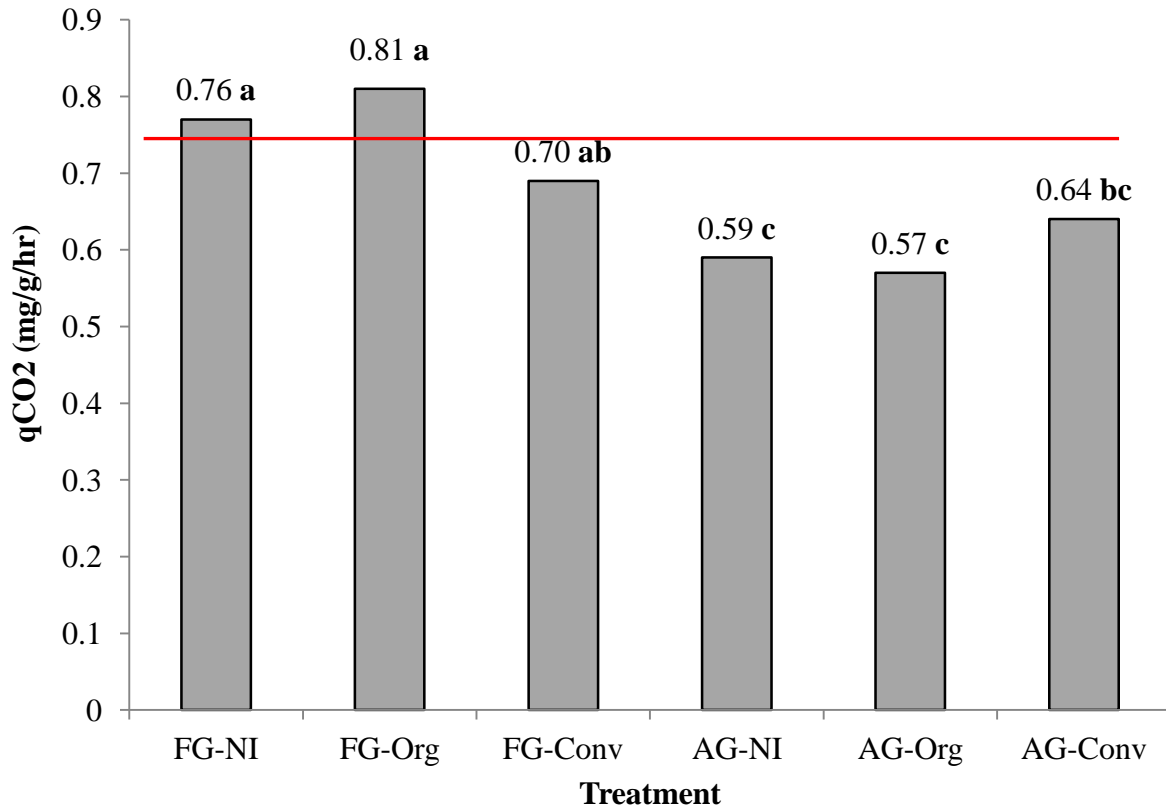


Figure 7.1 Microbial metabolic quotient measurements ($\mu\text{g MBC/g soil}$) under organic (Org), no-input (NI), and conventional (Conv) management treatments for forage-grain (FG) and annual-grain (AG) rotations across all sampling dates. Red line represents prairie grassland average.

Microbial metabolic quotients for agricultural soils ranges between 0.5-2.0 mg CO₂-C/g-MBC/hr, and values outside of this range should be considered critical (Anderson, 2003).

Decreasing the microbial biomass, reduced respiration, and metabolic quotients less than 0.5 mg CO₂-C/g-MBC/hr represent microbial populations acting in a state of self-induced dormancy.

Quotient values above 2.0 mg CO₂-C/g-MBC/hr reflect unhealthy rates of carbon dioxide emissions from the microbial biomass.

Both rotations and all management practices were within the range of healthy soil metabolic activity when all dates were combined. Since all soils can be considered healthy, we

should be interested in the performance of individual treatments and what that means within the context of particular system requirements.

In the forage-grain rotation, organic and no-input soils are releasing significantly more CO₂ per unit of microbial biomass carbon compared to their annual-grain counterparts, which may be a result of increased substrate availability from perennial roots. In the forage-grain rotation, the microbial population is significantly more active per unit of microbial biomass carbon evident by increased qCO₂, implying increased substrate source compared with annual soils (Anderson and Domsch, 1985). This comparison illustrates that not only does the annual rotation support less MBC in organic and no-input soils, the relative active fraction of these populations are significantly lower than these soils in the forage-grain rotation. The lower values for qCO₂ for the AG-no-input and organic systems are approaching 0.50 mg CO₂-C/g-MBC/hr and therefore may be somewhat static and insufficiently recycling nutrients for plant up-take. Dormancy values tend to occur when carbon substrates are limiting (Anderson and Domsch, 1985) and may explain statistical differences between treatments.

Organic and no-input soils in the forage-grain rotation closely resembled qCO₂ values in the restored grassland prairie. However, lack of statistical analysis limits the interpretation of these values. Still, one can speculate that organic and no-input soils in the forage-grain rotation mimic natural systems.

3.5.2.2 qCO₂: Rotation x Date

Seasonal fluctuations were also found to have a significant interaction with rotation (Fig. 8.1). qCO₂ values fell within the range of healthy values for the forage-grain rotation on dates 1, 2, and 5, but fell below the lower limit (0.5 mg CO₂-C/g-MBC/hr) on date 3 and date 4 when

moisture declined. Values for qCO_2 in the annual-grain rotation fell below the healthy limit on date 4. However, forage-grain plots were significantly higher than annual-grain plots on date 1, date 2, and date 5. On date 3 and date 4, qCO_2 did not differ statistically between rotations.

These observations clearly show that metabolic quotients in both rotations decreased as soil moisture decreased. Date 3 and date 4 coincided with long periods without precipitation, and on date 5 as soil moisture increased so did qCO_2 . On date 5 this result illustrates that the forage-grain rotation has a greater capacity to recover from a self-induced dormancy and re-build an active microbial population in soil.

These results indicate that microbial metabolic quotients change significantly over time, and to different degrees depending on the cropping system. Seasonal data for qCO_2 in long-term studies has not been reported in the literature. Seasonal variation in metabolic quotients exemplifies the importance of repeated sampling. Repeated sampling also offers opportunities to observe system resilience to changing environmental conditions.

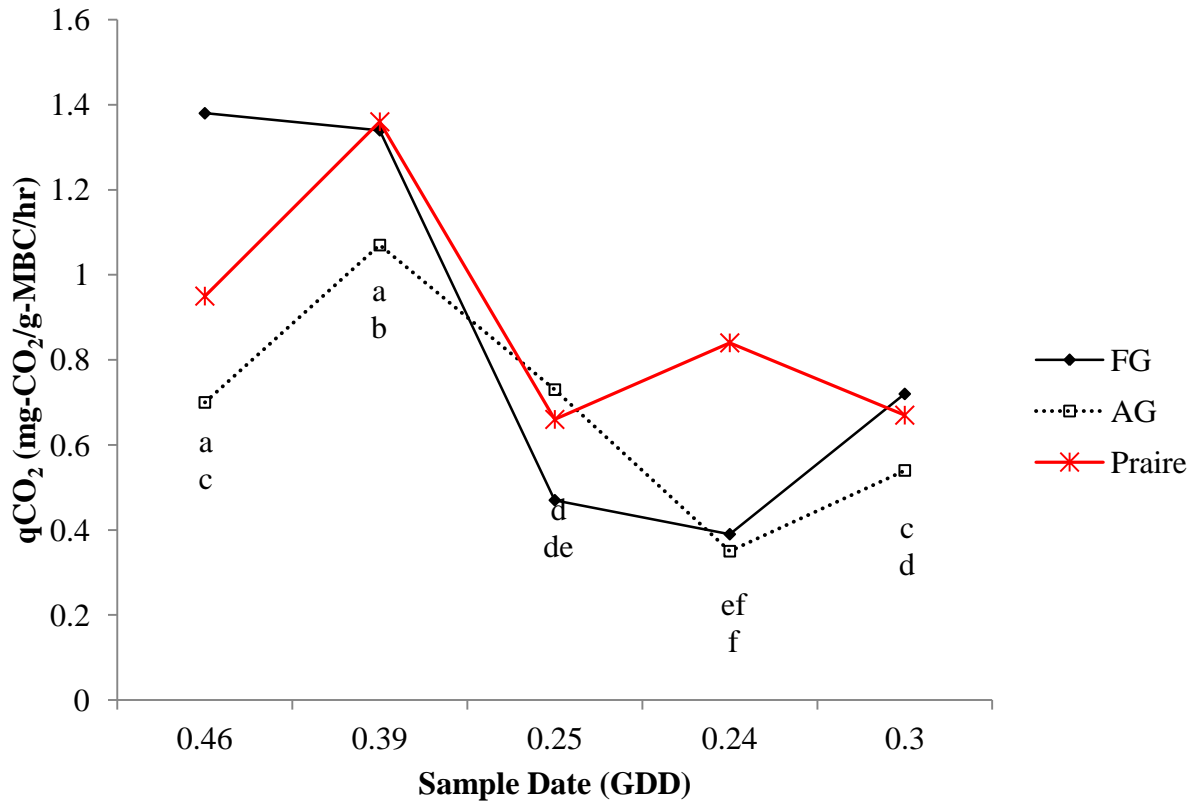


Figure 8.1 Seasonal microbial metabolic quotient measurements (mg CO₂-C/g-MBC/hr) under forage-grain (FG), and annual-grain (AG), and prairie grassland across five sampling dates. Letters represent significant differences ($\alpha = 0.05$) within and across FG and AG data series only and **do not** apply to prairie grassland values.

Over the course of the growing season, soils from all treatments underwent a state of self-induced dormancy at the same time moisture was statistically the lowest. In both forage-grain and annual-grain rotations, microbial biomass carbon, respiration, and subsequently qCO₂ significantly decreased on date 3 and date 4. Values for forage-grain and annual-grain on date 3 and date 4 were less than 0.50 mg CO₂-C/g-MBC/hr. Therefore, both rotations entered into a state of self-induced dormancy (Anderson and Domsch, 1985). Significant decreases in moisture also occurred on date 3 and date 4 are an indirect cause for these decreases MBC, CO₂ and qCO₂. Considering the grassland prairie never decreased its metabolic quotient below 0.50 mg CO₂-

C/g-MBC/hr, microbes did not experience moisture stress, an effect of the system. Therefore it was agricultural management itself that explains the period of self-induced dormancy for both forage-grain and annual-grain rotations. Dormancy could be attributed to tillage and the absence of perennial plants at any time in rotation. Possible effects of management that could account for the state of self-induced dormancy include moisture retention and access to nutrients in the soil environment.

Since an interaction between management and date was not significant, it can be assumed that all management systems had the same metabolic quotients on date 5. If we consider this in terms of MBC increases on date 5, it becomes clear that the organic and no-input systems have a significantly greater rate and capacity to re-build and sequester carbon into the microbial biomass. While conventional and organic soils are emitting the same amount of CO₂ at the same rate, the organic soil is building significantly more microbial biomass carbon on date 5. The inability of the conventional system to rebuild its MBC embodies the stress this soil is experiencing. This observation can teach us that sequential sampling regimes capture a unique perspective that represents the dynamic, breathing nature of soil.

3.6 Microbial Biomass Phosphorus (MBP)

3.6.1 MBP: Main Effects

Main effects for MBP were significant for rotation and date. MBP was significantly higher in the forage-grain (53 µg-P/g soil) compared to annual-grain (36 µg-P/g soil) rotation. MBP fluctuated over the course of the growing season and decreased significantly as soil moisture declined. When soil was rewetted on date 5, MBP significantly increased to 129 µg-P/g soil demonstrating that large amounts of P can be stored in the microbial biomass.

3.6.2 MBP: Interactions

3.6.2.1 MBP: Rotation x Date

Microbial biomass phosphorus was significantly affected by agricultural rotation over time. During the spring (date 1) and fall (date 5), when conditions favored microbial activity, the forage-grain rotation responded with a greater increase than the annual-grain rotation. On all other dates (2-4) there were no significant differences between rotations (Fig. 9.1).

While MBP increased significantly over time for both FG and AG rotations, forage-grain MBP was significantly higher than annual-grain values on date1 and date5. These results illustrate that rotations with legume perennials have a higher capacity for building phosphorus in the microbial biomass under favorable moisture conditions compared with annual rotations.

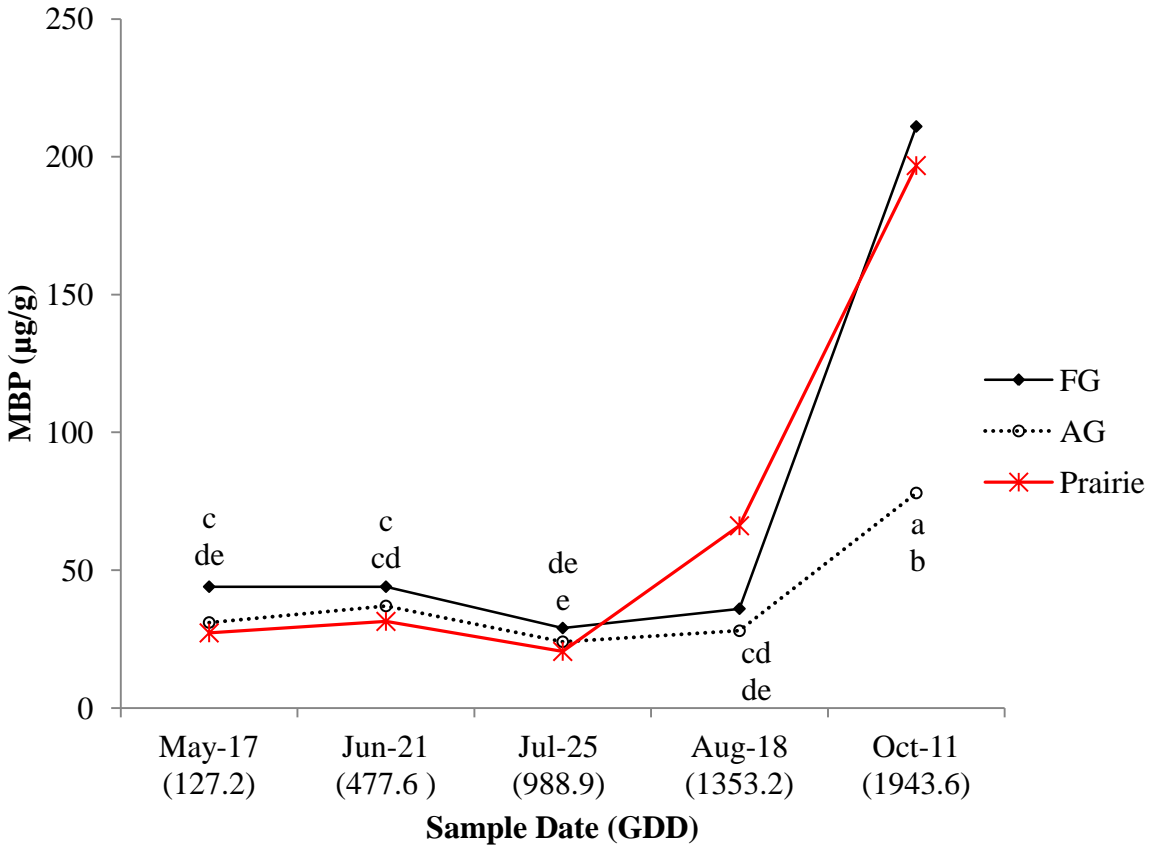


Figure 9.1 Seasonal microbial biomass phosphorus measurements ($\text{mg-CO}_2/\text{kg-soil/hr}$) under forage-grain (FG), and annual-grain (AG), and prairie grassland across five sampling dates. Letters represent significant differences ($\alpha = 0.05$) within and across FG and AG data series only and **do not** apply to prairie grassland values.

3.6.2.2 MBP: Management x Date

Across all treatments microbial biomass phosphorus demonstrated the ability to increase significantly (Fig. 9.1) over the course of one growing season. Organically managed soils had significantly higher MBP on the first sample date compared to no-input and conventional systems. On sample dates 2-4 MBP showed no differences between management systems. However, on date 5 no-input soils supported significantly more microbial biomass phosphorus compared to the conventional system. Organically managed soils were not different from no-input and conventional soils on date 5. These results indicate that soils without nutrient inputs

and soils receiving organic nutrient inputs have the greatest ability to build phosphorus in the microbial biomass.

When conditions are optimal for building P in microbes (date 5), no-input soils showed greater potential compared with conventional soils. Under unfavorable conditions (date 4) the restored prairie began to increase MBP. Maybe a reason for this observation is prairie soils increase P availability to microbes. Phosphorus dynamics in the microbial biomass suggests that large amounts of P are present in the soil environment with the potential to move into and through the microbial biomass.

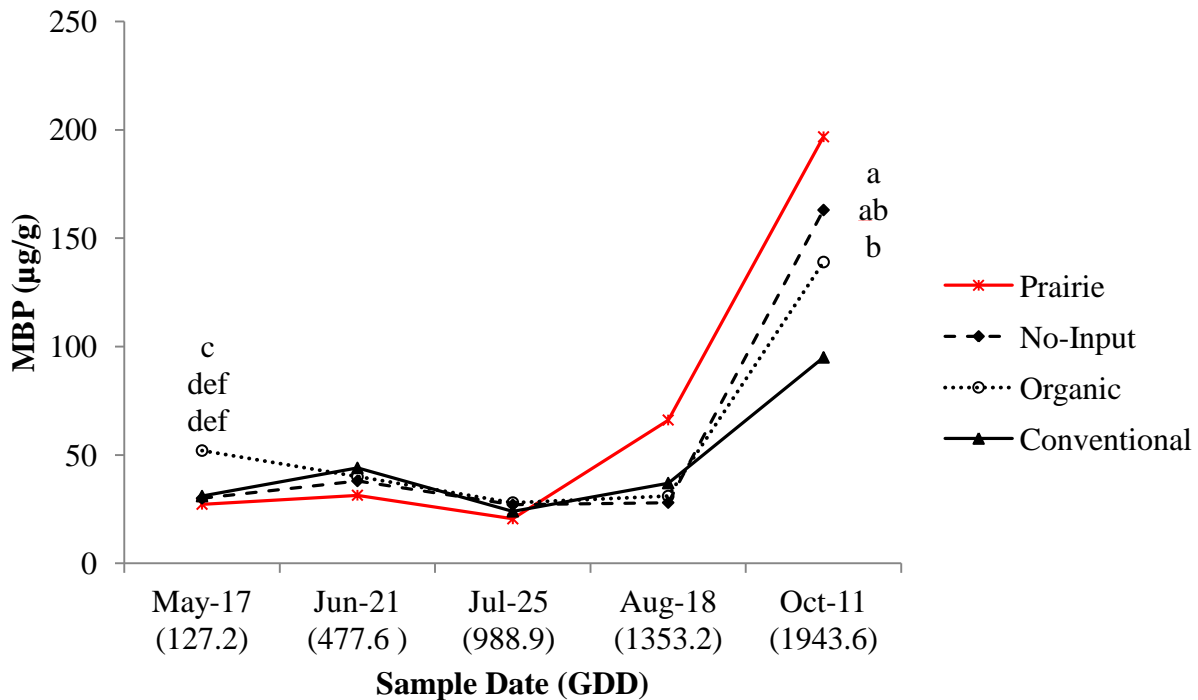


Figure 10.1 Seasonal microbial biomass phosphorus measurements ($\mu\text{g MBP/g soil}$) under organic, no-input, and conventional management, and prairie grassland across five sampling dates. Letters signify differences ($\alpha = 0.05$ and $P < 0.0533$) between and across management and date, but **do not** apply to prairie grassland (red line).

Microbial biomass phosphorus values in the present study are within the range of literature values for MBP in both annual and perennial rotations (7.0-193 $\mu\text{g-P/g soil}$). On date 5,

MBP values are somewhat higher than these literature values. However, previous studies comparing long-term organic and conventional rotations did not evaluate MBP over time.

Results from microbial phosphorus measurements demonstrate that rotation significantly affected the ability of microbial populations to build phosphorus in their biomass. Forage-grain soils had significantly more microbial biomass phosphorus on date 1 and date 5. One reason for this could be fall termination in the second year of mixed forage. Since nitrogen fixation nodules of legumes are a natural sink for inorganic phosphorus, fall termination of perennial forages might provide a greater opportunity for microbes to build phosphorus in the biomass without competition from plants. Conversely, the system with annual legumes may not have the same capacity for building nodules in soil over time. Increased carbon from perennial roots may also facilitate P uptake compared to the annual rotation. Therefore, differences in rotation may have played a part in the different MBP results.

Significant differences between no-input and conventional MBP on date 5 could be related to microbial biomass carbon and carbon substrate availability. Since MBC and MBP tend to increase and decrease together (Brookes et al., 1982), the low microbial P in the conventional system is likely due to low microbial biomass carbon. Conventional systems that use synthetic phosphorus fertilizers are often not deficient in plant available P because synthetic fertilizers are synthesized in plant available forms. However, the presence of synthetic P might have negative impacts on microbial populations. If conventional microbial populations do not depend entirely on environmental P, they may not accumulate quantities of poly-phosphates comparable to microbial communities in organic systems. If microbes in organic systems accumulate more P, then they can also accumulate more C.

One implication of these findings is that bacteria and fungi in conventional systems could be more susceptible to environmental conditions such as drought and flood. Since drought and flooded conditions affect osmotic and electrostatic potential, microbes without accumulated polyphosphates would not be resilient under severe stress. If microbes do not depend entirely on environmental P for survival and reproduction, and do not acquire the trait to accumulate polyphosphates, they could lose the ability to adapt to extreme environmental conditions.

All soils significantly increased microbial biomass phosphorus on date 5 compared to all other dates. Restore grassland prairie also increased MBP on date 5, which eliminates rotation and management as causes of this increase. Hypothetically, microbes in cold climates may sense declining temperatures and begin to accumulate poly-phosphates that can be utilized in metabolism over winter months.

Since polyphosphates can substitute for ATP (Kulaev et al., 1985), microbes in soils that freeze for part of the year may be able to utilize these endogenous polymers for metabolism once inorganic P has frozen and become unavailable in the soil environment. This could increase microbial growth and development in organic rotations over conventional microbial populations since physiological activity has more potential energy from P reserves. More endogenous P would allow organic microbes to continue metabolism when environmental conditions are stressful or limiting, prolonging growth and development. Therefore, microbial populations in organic systems may have an increased capacity to store large amounts of phosphorus in the microbial biomass.

3.6.3 Microbial Biomass Carbon: Microbial Biomass Phosphorus Ratios

Microbial biomass C:P ratios are an important indication of microbial physiology in soil. In addition, changes in C:P over time can help agricultural systems understand microbial P cycling, which has the potential to enhance nutrient management. This aspect of microbial behavior in soil is particularly important for organic systems that depend on microbial activity for P availability to crops.

Microbial biomass carbon to phosphorus ratios were not significantly affected by agricultural management or rotation (Table 6.1). This result illustrates that while MBC and MBP can be statistically different across management and rotation, the stoichiometric ratio of C:P in microbes is consistent. While C:P values were consistent across treatments, ratios changed significantly over time. Significant increase in C:P occurred between date 2 and date 3, while significant decrease in C:P occurred on date 5 compared to all other dates when soil moisture increased. The decrease in C:P on date 5 indicates that microbial populations under different agricultural management and rotation are not different in their stoichiometric adaptations to environmental conditions over time.

The lower ratio on date 5 was mitigated by the large accumulation of phosphorus while microbes also increased MBC. This result helps exemplify the need to build and manage soil carbon and phosphorus together. Microbial life is the medium for storing these nutrients and more information about seasonal changes will aid our understanding of best management practices.

3.7 Microbial Nitrous Oxide Emissions (N₂O)

Microbial N₂O emissions were measured on date 1 only, before fertilizer was applied to the conventional treatments. Soil moisture was highest on date 1 (46% GMC) and was under

anaerobic conditions as a result. Since anaerobic conditions in the spring favor the microbial process of denitrification and releases N₂O, only date 1 was chosen for comparisons.

3.7.1 N₂O: Main Effects

Nitrous oxide emissions were significantly affected by rotation and management. N₂O emissions under the FG (4.98E-04 mg N₂O-N/g-MBC/hr) rotation were significantly higher than the AG (1.11E-04 mg N₂O-N/g-MBC/hr) rotation (Table 6.1). Emissions for N₂O were statistically lower under organic (1.41E-04 mg N₂O-N/g-MBC/hr) compared to no-input (3.48E-04 mg N₂O-N/g-MBC/hr) and conventional (2.65E-04 mg N₂O-N/g-MBC/hr) cropping systems. Organic management systems therefore resulted in less noxious N₂O green house gas emissions.

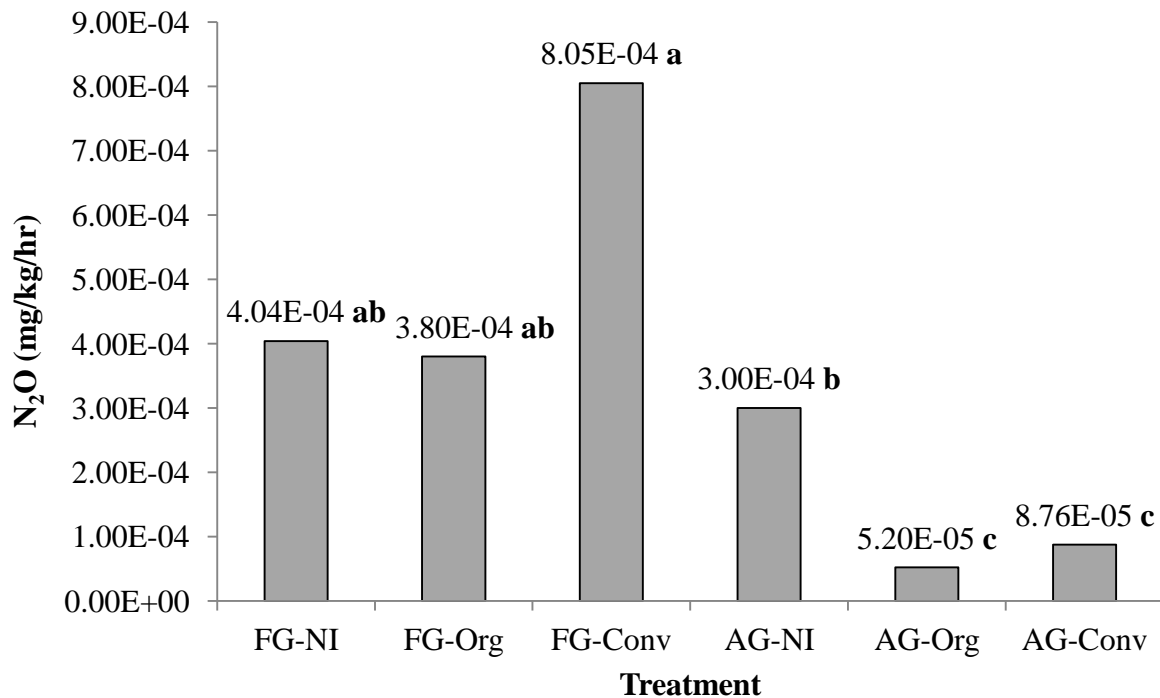


Figure 11.1 Microbial nitrous oxide emissions (mg-N₂O-N/g-MBC/hr) under organic (Org), no-input (NI), and conventional (Conv) management treatments for forage-grain (FG) and annual-

grain (AG) date1 before fertilization. Letters represent significant difference within each main effect ($\alpha = 0.05$).

3.7.2 N₂O: Interaction

3.7.2.1 N₂O: Rotation x Management

Significant interaction between rotation and management represents variable tendencies of cropping systems to release N₂O under anaerobic spring conditions. The forage grain rotation did not demonstrate significant differences within the rotation even though numerical differences appear quite large (Fig. 11.1). The conventional treatment in the forage grain rotation was statistically higher than all treatments in the annual-grain rotation. N₂O emissions for the forage-grain organic and forage-grain no-input systems were statistically the same as the annual-grain no-input, but significantly higher than annual-grain organic and annual-grain conventional rotations. Within the annual-grain rotation, the no-input system released significantly higher quantities of N₂O compared to organic and conventional systems, which were not statistically different (Fig. 11.1). These results demonstrate that perennial legume cropping systems emit more N₂O greenhouse gases compared to annual legume rotations. In addition, organic management can reduce the negative impact of this greenhouse gas under anaerobic spring conditions.

3.8 Microbial Nitrous Oxide Quotient (qN₂O)

3.8.1 qN₂O: Main Effects

Nitrous oxide quotients were found to be statistically significant different for rotation and management main effects (Figure 3.11). Interaction between rotation and management was not

significant indicating that management system responded similarly in both crop rotations. This result demonstrates that while there were significant interactions for N₂O gas emissions, when this variable is considered per unit of MBC, management systems behave in the same way.

The qN₂O was significantly higher in the forage-grain than annual grain rotation. Values were almost five times higher for the forage-grain (6.33E-04 mg N₂O-N/g-MBC/hr) compared to the annual-grain (1.09E-04 mg N₂O-N/g-MBC/hr) system. Nitrous oxide quotients for management treatments were greatest under conventional systems. Organic management (2.34E-04 mg N₂O-N/g-MBC/hr) was significantly lower than conventional (6.22E-04 mg N₂O-N/g-MBC/hr) soils but statistically higher than no-input (1.24E-04 mg N₂O-N/g-MBC/hr) systems.

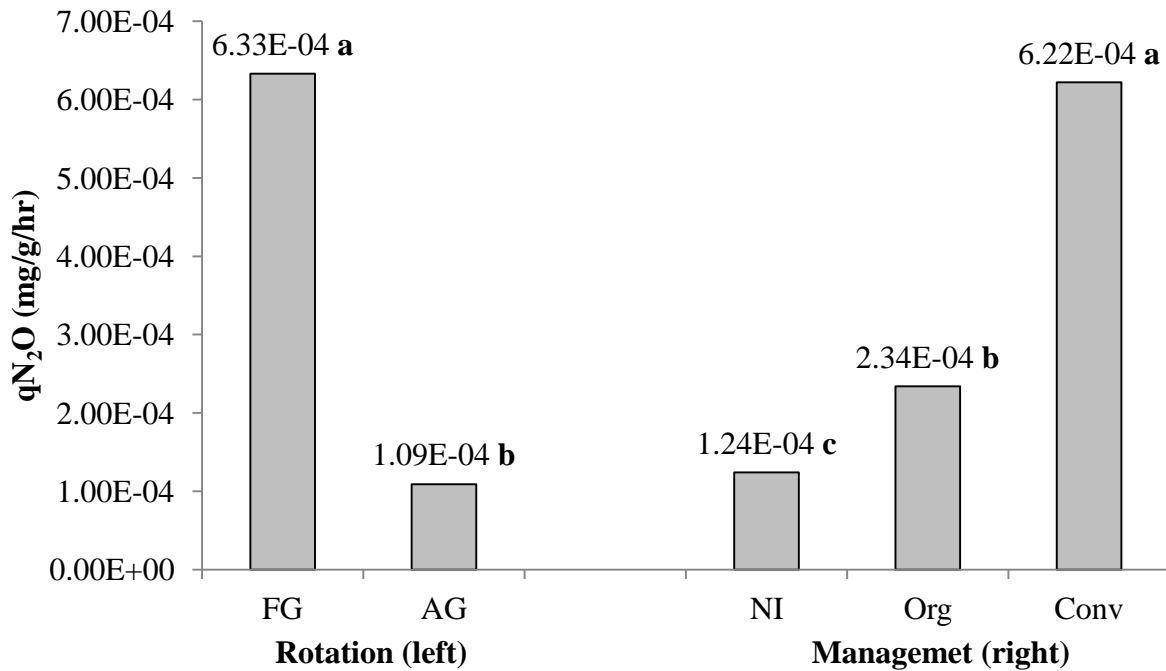


Figure 12.1 Microbial nitrous oxide quotients (mg-N₂O-N/g-MBC/hr) for main effects of rotation (FG: forage-grain; AG: annual-grain) and management (NI: no-input; Org: organic; Conv: conventional) on sample date 1 before fertilization. Letters represent significant difference within each main effect ($\alpha = 0.05$).

These results demonstrate that conventional soils have emitted the most N₂O *and* had significantly highest qN₂O. If we consider this in terms of the microbial biomass carbon, (and remember that MBC was significantly lower for conventional soils compared to the potential carrying capacity demonstrated by the forage-grain organic and no-input MBC), then we can make the observation that significantly smaller MBC populations have emitted significantly more N₂O than their organic and no-input counterparts. Finally, consider qCO₂ and qN₂O together. On date 1, forage-grain soils emitted significantly more N₂O and CO₂ green house gasses per unit of microbial biomass carbon.

Increased microbial qN₂O in the forage-grain system compared to the annual-grain system is likely due to higher amounts of nitrogen in the soil from perennial legumes. Increased values for qN₂O in the conventional system could be due to microbial community structures. The microbial community in the conventional system that receives synthetic fertilizers may have built a microbial population with a competitive advantage for this readily available nutrient source. Since synthetic fertilizers are inorganic, the availability of inorganic nitrogen as an electron acceptor for anaerobic respiration is higher compared to the organic forms of nitrogen in the no-input and organic cropping systems. Anaerobic microbes in the no-input and organic systems would therefore not have large communities of denitrifiers compared to conventional management systems with inorganic fertilizers.

3.9 Prairie vs Agricultural Soils

When MBC, CO₂, and qCO₂ values for rotation and management treatments were contrasted with Prairie soils, results indicated only the no-input and organic systems under perennial rotation did not differ statistically from the Prairie (Table 7.1). All other treatments

were significantly different from Prairie soils. This result indicates that microbial populations in perennial soils under no-input and organic management closely resemble the natural system of prairie grasslands.

Table 7.1 Statistical ANOVA for rotation and management treatments contrasted with Prairie soils. Averaged across all dates. Asterisks represent significant differences from Prairie soils where $\alpha = 0.05$.

	MBC	CO ₂	qCO ₂
AG-Conventional	*	*	*
AG-No-input	*	*	*
AG-Organic	*	*	*
FG-Conventional	*	*	*
FG-No-input	ns	ns	ns
FG-Organic	ns	ns	ns

Organic and no-input soils most closely resembled restored grassland prairie soils. One likely explanation for this is that synthetic fertilizers deplete soil carbon (Muvaney et al., 2009) which reduces MBC. Subsequently, microbial populations are less healthy due to limited carbon, a nutrient which causes stress on the soil system and decreases soil health. Inputs to the organic system are in organic forms when composted manure is applied and this form of nutrients reflects the organic nutrients in prairie soils. Still, the no-input system that does not receive soil carbon amendments was also statistically the same as the prairie for MBC, CO₂, and qCO₂. This result suggests that withholding carbon amendments does not reduce microbial efficacy in soils, or soil health. Therefore, it appears that the addition of synthetic fertilizers that is reducing soil health in conventional agriculture management.

4.1 Summary and Conclusions

T.H. Anderson (Anderson and Domsch, 2010) and has extensively investigated the nature of soil health. He concludes that it is the range and degree of activity in the soil that indicates its

health. A body alone is not enough to describe health unless we know to what degree it is living and functioning in the universe.

Overall, the objectives of this study were met through the various measurements used to assess soil health. Hypotheses for each measurement were not shown to be accurate to results displayed in the data and statistical analysis. Instead, new information was established regarding the nature of rotation and management on seasonal dynamics of soils health.

Results from the present study indicate that soil health is dependent on building nutrients like carbon and phosphorus together. Perennial organic systems and no-input systems closely resemble natural restored prairie and have demonstrated greater capacities to sustain biological life over time. Extended periods of drought have been shown to have deleterious effects on MBC (Hueso et al., 2012) but forage-grain organic and no-input systems showed resilience to extended periods of drought (Fig 3.2). Forage-grain rotations under organic management may have benefits for soil health compared to conventionally managed soils. It is unclear if the benefit is due to ecological properties of species diversity, or the exclusion of synthetic fertilizer and pesticides. Laboratory incubation studies would increase our understanding of these variables on soil biological properties.

Microbial metabolic quotients in previous literature have demonstrated significant differences between management systems for $q\text{CO}_2$ values (Fleissbach et al., 2007). The present study did not show significant differences between management to be the major contributing factor to differences in metabolic quotients. Results from the present study suggest that rotation played a more important role in soil health, exemplifying the beneficial aspects of perennial rotations, since they were living and breathing to a greater extent than the annual rotation.

Microbial metabolic quotients and MBC together indicate the strong interaction of rotation and management in terms of soil health. While $q\text{CO}_2$ was not different within rotations, MBC was significantly highest in the perennial organic and no-input treatments. This result shows that organic systems can support a larger microbial population that is as energy efficient as the smaller conventional MBC population. Similarities in $q\text{CO}_2$ among treatments could be attributed to the age of soils (19 years) and subsequent stability as a result of long-term management.

Finally, the resistance of prairie soils to dry conditions provides a biological example of a healthy soil. While the organic system was resilient to dry conditions, demonstrated by maintaining MBC values, it was not able to increase its biomass. The conventional soil decreased MBC over the season indicating stress on the microbial biomass and consequently, stress on soil health.

Forage-grain organic and no-input systems also demonstrated the greatest capacity to build MBP. Currently, the literature has not reported seasonal MBP dynamics. Whether or not it is typical for microbes to build large amounts of P in the biomass in the fall should be investigated using repeated sampling over multiple years and environmental conditions.

Seasonal dynamics of microbial biomass in agricultural soils offers a critical assessment of soil health over time, in response to environmental conditions. Isolated sampling does not incorporate the changing, dynamic nature of soils. Overall, the prairie soil illustrated the most potential for supporting and increasing its population and activity in spite of unfavorable moisture conditions. The organic soil behaved most similarly to the prairie soil but did not demonstrate the same capacity for resistance to environmental stress. The conventional soil was

negatively affected by unfavorable moisture conditions and was unable to sustain its biomass over the growing season. This result suggests that agriculture systems that reflect natural ecosystems may be healthier and more resilient under stress.

The results of the present study have implications under the specific management and rotation practices controlled over the last 19 years. Future studies should consider a wide case of management practices and rotations under current on-farm practices as recorded by the farmer as far back as possible. Regional studies will be the most useful for the future of agriculture, soil health, and crop productivity. This type of research will as a result, not only provide important information to actual farmers about how they have managed their soil, it can also help increase communication between farmers-farmers, farmers-researchers, and researchers-researchers. Too many scientific studies have become preoccupied with methodology, limiting the potential knowledge there is to gain about how-things-are-in-agriculture. While the controlled field experiment has its uses, it has also lost both the benefit of the controlled environment and the practical application of the results. To render soil health data useful, this type of information should be collected on actual farms that can account for their management history at least 15 years.

To supplement soil health data, soil microbial DNA analysis and other soil ecology measurements should be collected. This data would put into perspective what types of soil ecological communities develop under different practices. The future of research does not fall into many isolated and reduced experiments but by interconnected and collaborative fact building.

Appendix

Table 8.1 Anova table for microbial properties.

	MBC	MBP	FUM-NH ₄ ⁺	CO ₂	qCO ₂	qN ₂ O
Rotation (R)	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Management (M)	0.0355	0.2279	<.0001	0.4621	0.9379	<.0001
Date (D)	<.0001	<.0001	<.0001	<.0001	<.0001	na
RxM	<.0001	0.1163	<.0001	0.0002	0.0513	0.1787
RxD	0.2247	0.0533	0.9549	<.0001	<.0001	na
MxD	0.0169	0.0043	0.2033	0.1236	0.1845	na
RxMxD	0.0913	0.3302	0.8861	0.2675	0.3762	na

Table 9.1 Anova table for soil properties

	Volumetric Soil Moisture	Soil N	Soil P	Soil Extractable C	Bulk Density	Wheat Yield
Rotation (R)	0.0405	0.1119	<.0001	<.0001	0.0900	0.8366
Management (M)	0.0415	<.0001	<.0001	<.0001	0.0116	<.0001
Date (D)	<.0001	<.0001	0.7203	0.5812	na	na
RxM	0.4307	0.1243	<.0001	0.0032	0.6077	0.5403
RxD	0.9943	0.0622	0.9828	0.9699	na	na
MxD	0.7611	0.0027	0.7750	0.9699	na	na
RxMxD	0.9630	0.1330	0.7750	0.9993	na	na

Table 10.1 Average dry biomass yield for 2011 second year mixed forage and wheat dry biomass and grain yield.

Roatation	Management	Forage Biomass (kg/ha)	Wheat Yield (kg/ha)	Wheat Final Biomass (lbs/ac)
Forage	No-Input	8673.4	574.7	2559.1
Forage	Organic	12251.6	1071.1	2343.4
Forage	Conventional	10569.9	2507.9	5514.2
Annual	No-Input	na	888.2	1864.8
Annual	Organic	na	905.6	2404.8
Annual	Conventional	na	2473.0	4649.3

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