Long-term impacts of tillage, crop rotation and cover crop systems on soil bacteria, archaea and their respective ammonia oxidizing communities in an Ontario agricultural soil

by

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ABSTRACT

LONG-TERM IMPACTS OF TILLAGE, CROP ROTATION AND COVER CROP SYSTEMS ON SOIL BACTERIA, ARCHAEA AND THEIR RESPECTIVE AMMONIA OXIDIZING COMMUNITIES IN AN ONTARIO AGRICULTURAL SOIL

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This research assessed the seasonal effects of contrasting tillage and crop rotation systems on soil ammonia oxidizing bacteria (AOB) and archaea (AOA). Four different cropping systems under till and no-till were analyzed in a 30 year-old agricultural field trial. Samples were collected during the 2010 growing season at times corresponding with agronomic events. Nucleic acids were preserved in the field and subsequently analyzed by quantitative real-time polymerase chain reactions (qPCR). Tillage decreased AOB activity and abundance in the plow layer (0-15 cm) immediately after fall moldboard plow events, but observed AOB dynamics at other times suggest tillage had a long-term distribution effect across depth (0-30 cm). AOA abundance was significantly greater in no-till plots at all times indicating tillage had longer-term effects on these communities. Crop rotation had minimal effect on AOB and AOA, but there was a noted yield advantage for corn following wheat, regardless of tillage treatment.
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Chapter 1: Introduction

The need for Best Management Practices (BMPs) in agriculture is becoming increasingly important due to rising global food demand and ecological degradation associated with many agronomic practices. Evaluating the economic performance of BMPs is straightforward, but ecological evaluation of BMPs is more complex. Our ability to identify and implement effective BMPs is enhanced through the study of soil microbial communities in agricultural systems. Soil microbial communities are a good proxy measure for overall ecosystem health as their processes are tightly linked to overall soil functioning (Kowalchuk and Stephen, 2001, Philippot and Hallin, 2005) and because they modulate key cycles, such as carbon and nitrogen cycles, among others. Both carbon and nitrogen are important for crop production via plant uptake, but can also be emitted in gaseous form by various means. In Canada, agricultural soils account for 8% of total national greenhouse gas (GHG) emissions, of which about 50% is N\textsubscript{2}O (Environment Canada, 2010). Other soil nitrogen losses through leaching and runoff can cause economic loss to farmers and major contamination of waterways. Thus, microbial communities involved in the nitrogen cycle are particularly important for study in agricultural systems and the development of BMPs should include analyses of these N-cycle related communities.

1.1 Goals and Objectives

The goal of this thesis was to evaluate different agronomic practices and their effects on soil microbial communities, especially ammonia oxidizers, in a long-term field trial established in 1980 at the Elora Research Station, Elora, Ontario, Canada. Treatments
included till and no-till, and different crop rotations commonly employed by farmers in southern Ontario. The objectives were to evaluate how different variations on these management systems affected soil properties and soil ammonia oxidizing community dynamics. This was achieved by evaluating the following under different tillage and crop rotation treatments at times relating to key agronomic events during a growing season:

1) Soil N and C dynamics at specific soil depths to 30 cm.
2) Water stable aggregates in surface soils to 5 cm depths.
3) Abundance and activity of soil microbial communities; specifically ammonia oxidizing archaea and bacteria, at specific soil depths of 0-5 cm, 5-15 cm and 15-30 cm.

1.2 Format of Thesis

This thesis is divided into four chapters. The first chapter is an introduction and the second a literature review. The following two chapters are stand alone manuscripts. Chapter 3 reports on a field study assessing different tillage and crop rotation effects on soil nutrients, soil microbial communities, ammonia oxidizing bacteria, and ammonia oxidizing archaea across depths and over an entire growing season. Chapter 4 reports on a field study assessing tillage and crop rotation effects on soil aggregation, soil microbial communities, ammonia oxidizing bacteria, and ammonia oxidizing archaea during a growing season. Chapter 4 expands on analysis from Chapter 3 to include additional treatments analyzed only in the 0-5 cm layer. In addition, Chapter 4 compares treatment effects on soil aggregation and microbial dynamics. The final concluding chapter summarizes and relates findings from both studies and provides suggestions for future research.
Chapter 2: Literature Review

A healthy soil is defined as a stable system with high levels of biodiversity, activity, internal nutrient cycling and resiliency to disturbance (Govaerts et al., 2007). Agricultural crop production requires many soil disturbances that can stress soil health. Determining best management practices (BMPs) that minimize these disturbances without compromising crop yields is essential. Soil microbial communities (SMC) are a good proxy to determine overall soil health as they modulate many key soil functions (Wessen and Hallin, 2011b). Functional communities involved in the nitrogen cycle are ideal proxies, as they have already been extensively studied and have become a model system in microbial ecology (Kowalchuk and Stephen, 2001, Philippot and Hallin, 2005). Communities involved in the nitrogen cycle are particularly important in agricultural systems due to the typically high amounts of annual N input to these systems. Recently it was estimated that worldwide annual fertilizer input is equivalent to 100 Tg N (Zhang et al., 2010, Gruber and Galloway, 2008). Since N loss from agricultural soils has the potential for ecological damage, it is critical that BMPs strive to maximize current N-use efficiency.

2.1 Soil Ammonia Oxidation

Nitrification results in the conversion of ammonia to nitrate. Nitrate is the most plant-available form of nitrogen, but it is also prone to leaching loss due to its high mobility in soil. Leached nitrate can cause pollution of groundwater leading to eutrophication and/or contamination of drinking water. The nitrification process itself is not 100% efficient and its rate-limiting step of ammonia oxidation can lead to emissions of nitrous oxide, a potent
greenhouse gas (Phillips et al., 2000, Capone, 1991). Ammonia oxidation is a two-step process whereby 1) ammonia is oxidized by ammonia monoxygenase to hydroxylamine that is then 2) oxidized by hydroxylamine oxido-reductase to nitrite (Prosser, 1989). The first step of this process is rate-limiting (Kowalchuk and Stephen, 2001), thus molecular studies focus on the ammonia monoxygenase gene (*amoA*) to infer microbial ammonia oxidation dynamics.

Microbial ammonia oxidation is performed by both bacterial and archaeal communities. Ammonia oxidizing bacteria (AOB) were long thought to be the only ammonia oxidizers in soil (Lettl, 1985, Lang and Jagnow, 1986). The recent discovery of archaeal ammonia oxidizers (AOA) (Konneke et al., 2005, Treusch et al., 2005, Venter et al., 2004) and subsequent findings of their ubiquitous nature in soils (Leininger et al., 2006) have complicated measurements of soil microbial ammonia oxidation. Studies have since attempted to determine whether AOB or AOA were the primary driver of ammonia oxidation under different conditions. Many of these studies have catalogued the diversity of AOB and AOA communities (Smith et al., 2010, Phillips et al., 2000). However, Phillips et al. (2000) outlined inherent limitations of diversity measures when they found significant differences in measured soil nitrification rates without any significant differences in soil ammonia oxidizing community diversity. Population size is postulated as a more important variable, due to the observed correlation of abundance and ammonia oxidizing activity seen in some soils (Wessen et al., 2011, Hallin et al., 2009). Still, there is contention over the value of gene abundance as a proxy without gene transcript data as well (RNA) (Nicol et al., 2008). Yet half-lives of RNA in soil are not known, and likely vary depending on gene fragment length and soil conditions (Nicol et al., 2008). Despite these limitations, *amoA* gene abundance and activity measurements have correlated with
measured soil ammonia oxidation rates in various studies (Wessen and Hallin, 2011a, Wessen et al., 2011, Jia and Conrad, 2009a, Di et al., 2010, Offre et al., 2009, Gubry-Rangin et al., 2010, He et al., 2007, Zhang et al., 2010)

2.2 Ammonia Oxidizing Archaea and Bacterial Communities

AOB and AOA community dynamics are very different. AOB are considered k-strategists (Bothe et al., 2000) and are generally found in aerobic environments where ammonia and carbon dioxide are readily available, whereas AOA tend to prefer low-nutrient environments. There is further evidence to suggest AOA thrive under chronic energy shortages (Valentine, 2007). Regardless of soil type and treatment, AOA ammonia monoxygenase (crenamoA) DNA gene counts consistently outnumber AOB ammonia monoxygenase (amoA) DNA gene counts (Shen et al., 2008, Prosser and Nicol, 2008, He et al., 2007, Leininger et al., 2006, Di et al., 2010, Wessen et al., 2010c). Using only gene abundance parameters, AOB were still suggested as the primary ammonia oxidizer in some soils - where AOA abundance was greater - based on the following observations: a) AOA abundance was negatively correlated with ammonia oxidation rates (Wessen and Hallin, 2011b), b) relative ratios of AOA:AOB decreased when NH$_3$ availability increased (Zhang et al., 2010, Di et al., 2010).

2.3 Fertilization Effects

Fertilization input of N to soils has differing effects on AOB and AOA communities. Much depends on the type of fertilizer applied (Gomez and Garland, 2012). AOB abundance is noted to increase following NH$_4$ input (Glaser et al., 2010, Chu et al., 2007,
Enwall et al., 2007, Gubry-Rangin et al., 2010, Cavagnaro et al., 2008, Herrmann and Witter, 2002, Mendum and Hirsch, 2002, Gomez and Garland, 2012, Okano et al., 2004, Shen et al., 2008, Di et al., 2010, Jia and Conrad, 2009b). In these high nutrient environments it was also suggested that AOB were the primary driver of ammonia oxidation. AOA do not seem to be as influenced by nitrogen fertilizer (Glaser et al., 2010, Shen et al., 2008). Interestingly, AOA and AOB activity responses do not follow similar trends as their abundances (Di et al., 2010, Jia and Conrad, 2009a).

The availability of NH$_3$ affects the activity of both AOA and AOB. AOA activity (assessed by RNA transcript counts) was higher than AOB activity in unfertilized soils, but the reverse was found in fertilized soils (Di et al., 2010, Jia and Conrad, 2009a), suggesting that AOB are the primary driver of ammonia oxidation in fertilized and agricultural soils (Zeglin et al., 2011, Di et al., 2010, Jia and Conrad, 2009a). In unfertilized soils, literature suggests AOA are the primary driver of ammonia oxidation, because gene abundances were positively correlated with measured nitrification rates (Offre et al., 2009, Wessen et al., 2010a, Zhang et al., 2010) or soil NO$_3$ concentrations (Gubry-Rangin et al., 2010). Furthermore, Mendum and Hirsch (2002) found no correlation between ammonia oxidation rates and AOB abundance in an unfertilized soil. This suggests that in low-nutrient environments the role of AOB is limited. Literature confirms that both AOA and AOB can drive ammonia oxidation in soils. Soil properties, are particularly important to consider and should be included in studies of AOA and AOB to completely elucidate soil ammonia oxidation dynamics.
2.4 Soil Property Effects

Edaphic factors often drive soil microbial community dynamics. Specific soil variables postulated as the primary influence on different soil microbial dynamics include pH (Shen et al., 2008, Wessen et al., 2010b, De Boer and Kowalchuk, 2001, Nicol et al., 2008, Lehtovirta-Morley et al., 2011, Gubry-Rangin et al., 2010), soil type (Philippot et al., 2009, Dias et al., 2012), soil organic carbon input (Govaerts et al., 2007), temperature (Avrahami and Conrad, 2005, Tourna et al., 2008), aggregation (Lupwayi et al., 2001), and NH$_4$ concentration (Prosser and Nicol, 2008, Di et al., 2010, Hoefferle et al., 2010). The influence of pH on AOA and AOB is highlighted amongst studies as particularly important. In neutral and slightly alkaline soils, AOB tend to have higher abundances and control ammonia oxidation (Hafeez et al., 2012, Shen et al., 2008). However, it may still be nutrient availability that is the primary factor controlling AOB abundance and that pH effects are actually indirectly affecting the stoichiometry of nutrients thereby reducing availability in acidic soils. Nicol et al. (2008) specifically observed that AOB populations fail to grow below neutral pH, but postulated that this was because NH$_3$ was ionized to NH$_4$ under acidic conditions. NH$_4$ is less available for ammonia oxidation due to increased energy requirements for transport compared to NH$_3$ (Frijlink et al., 1992, Nicol et al., 2008). In contrast, AOA have been demonstrated to thrive under such energy shortages (Valentine, 2007) and indeed are often found in greater abundance in acidic oligotrophic soils (Wessen et al., 2011, Nicol et al., 2008, Gubry-Rangin et al., 2010). Moreover, AOA may not be solely dependent on NH$_3$ for energy needs and have been postulated as being either mixotrophic or heterotrophic (Hallam et al., 2006, Tourna et al., 2008, Jia and Conrad, 2009a, Wessen et al., 2010a).
2.5 Tillage Effects

Tillage disturbs the soil through mechanical inversion, which can increase spring soil temperatures and aeration with a concomitant decrease in soil water content (Kladivko, 2001). No-till soils are generally less aerobic (Jangid et al., 2008, Wardle et al., 1999), and have better aggregate stability (Six et al., 2000, Hernanz et al., 2002). This greater aggregate stability is contingent on retained crop residues (Kravchenko and Thelen, 2009). The action of tillage has been directly linked with reduction in aggregate sizes and depletion of soil organic matter (Lupwayi et al., 2001, Beare et al., 1994, Carter, 1992). Tillage effects are well known – i.e. redistribution of nutrients across depths, physical destruction of large soil aggregates exposing more surface area for microbial action, and creation of compaction zones beneath plow layers (Paustian et al., 2000, Oades, 1984) - yet a mechanistic description of exactly how tillage increases decomposition of organic matter and reduces aggregate size is highly soil dependent and not well elucidated (Six et al., 2000).

Differences in nutrient contents between till and no-till soils are highly dependent on depth. No-till soils consistently exhibit higher soil organic carbon levels in surface soils (Hernanz et al., 2002, Buchanan and King, 1992). Specifically, higher surface (0-5cm) soil organic carbon levels up to 19.2% greater were observed in Elora, Ontario (Deen and Kataki, 2003b). However, soil organic carbon tends to be highly concentrated in surface no-till soils and decrease significantly with depth (Hernanz et al., 2002). When soil organic carbon levels are considered across a deeper depth (i.e. 30 cm) it is not clear if no-till results in significant sequestration of carbon. Angers et al. (1993) determined that total organic carbon was negatively affected by tillage treatment, but this study only sampled to
15 cm, a typical plough depth. Studies sampling below plough depths have noted significantly higher soil organic carbon at depth in tilled soils compared to no-till soils (Deen and Kataki, 2003b, Hernanz et al., 2002, Kladivko, 2001). Soil organic nitrogen has also been found to follow similar trends as carbon with up to 40% higher levels in surface no-till soils to 10 cm depth (Spedding et al., 2004). This stratification of soil organic nitrogen is particularly important due to its significance as a plant nutrient. Research by Spargo et al. (2008) demonstrated that no-till soils, relative to tilled soils, could contain up to 22 kg kg N ha\(^{-1}\) more in surface soils to 15 cm.

Tilled soils exhibit key traits suggesting overall soil microbial functioning is accelerated. Past research has shown that tillage is associated with higher CO\(_2\) respiration rates (Kladivko, 2001, Lal, 2008), nitrification rates (Phillips et al., 2000) and N\(_2\)O emissions (Smith et al., 2010). Accordingly, soil organic matter (SOM) inputs, such as crop residues, are more effectively metabolized after tillage events (Kladivko, 2001) and tilled soils can effectively decompose 48% more SOM in a given time frame compared to no-till soils (Lupwayi et al., 2004). Excessive tillage, without sufficient inputs of organic matter, will eventually result in a system where nutrients are limited due to SOM depletion by oxidation (Spedding et al., 2004).

Tillage effects on soil microbial communities are unclear (Smith et al., 2010, Calderon et al., 2000). Although long-term tillage effects have been shown to increase soil microbial activity and functionality (Gomez and Garland, 2012). Lupwayi et al. (2001) found higher bacterial diversity in no-till soils and attributed this directly to tillage reducing soil aggregate size and thus decreasing potential ecological niches. Likewise, Kladivko (2001) postulated that soil microbial communities are more abundant under no-till systems because of better aggregation as well as more uniform moisture and
temperature conditions across depths. Short-term effects on soil microbial communities are not often apparent in agricultural soils (Calderon et al., 2000). Unpronounced short-term responses to tillage may be because soil microbial communities in environments with a long-term history of frequent cultivation events may be more resilient (Calderon et al., 2000), and may possibly have “genetic memory” of previous adaptation responses, thereby “inoculating” them against tillage-related stresses (Calderon et al., 2000).

Tillage effects on AOA and AOB have not been extensively studied. Studies indicate tillage increases AOB population size (Cavagnaro et al., 2008, Patra et al., 2005, Bruns et al., 1999) and decreases diversity (Phillips et al., 2000). To date we know of no study that has quantified AOA abundance and activity responses to tillage.

2.6 Crop Rotation Effects

Crop rotations maximize nutrient utilization and crop rhizosphere effects can enhance soil conditions for subsequent crops. Miller and Dick (1995) found rhizosphere effects from legumes increased key soil enzymatic activity relating to carbon (\(\beta\)-glucosidase) and nitrogen (amidase) mineralization; increasing the availability of nutrients for crop uptake. Legumes can also increase total nitrogen (TN) levels in soils via symbiotic relationships with diazotrophs (nitrogen fixing bacteria). Generally crop rotations that include a greater variety of crop types over a longer time span are more efficient (Moore et al., 2000). Hence in addition to legumes, cereal crops that leave root biomass in the soil for easy microbial consumption generate benefits for subsequent crops (Govaerts et al., 2007). However, wheat crop residues have been noted to negatively affect subsequent no-till corn due to residual alleopathic effects (Kravchenko and Thelen, 2009).
Studies posit that rhizosphere effects from crops can influence soil microbial community composition (Wardle and Lavelle, 1997) well into subsequent years (Guo et al., 2011). Diverse crop residue inputs can enhance functional niches for microbial communities thereby increasing soil biodiversity (Acosta-Martínez et al., 2007). More biodiverse microbial communities under diverse crop rotations are linked with soils that resist acidification, have lower C:N ratios and have higher SOC, and TN (Moore et al., 2000), but also higher denitrification rates (Guo et al., 2011).

Various cover crops are included within crop rotations to increase SOM, retain nitrogen in the soil over the winter, and for their fertilizer value (Blanco-Canqui and Lal, 2009). Continuously covering the soil with a crop is also advantageous as a preventative measure against soil erosion, runoff losses, and salinity buildup due to evaporation (Blanco-Canqui and Lal, 2009). Use of cover crops generally results in a positive soil microbial community response that is dependent on the type of crop used. As in crop rotations, different types of crops provide different benefits. Legume cover crops such as red clover have been shown to increase subsequent crop yields by as much as 130% (Govaerts et al., 2007). Legumes feed diazotrophs with a greater amount of high quality residue that has a low C:N, is low in lignin and polyphenols, and has less recalcitrant material (Fageria, 2007). Moreover, equivalent fertilizer-N credit from previous plowed down red clover cover crops was recently estimated to be 96.7 kg N ha⁻¹ at Elora, Ontario (Gaudin et al. 2013).

Conflicting research has demonstrated that other variables such as tillage practices and climate must be accounted for in determining best management practices BMPs relating to cover crop use (Baggs et al., 2000, Elfstrand et al., 2007, Lupwayi et al., 2004). In humid climates, tilled leguminous cover crops result in higher fungal and bacterial
populations sustained throughout entire growing seasons (Elfstrand et al., 2007, Baggs et al., 2000). This suggests that incorporation of leguminous cover crops in humid climates may be a viable method to alleviate the adverse effects of tillage on soil microbial communities.

2.7 Spatial and Temporal Variability

Soil depth effects and varying seasonal conditions are often not adequately addressed in studies evaluating AOA/AOB and other soil microbial communities. Thus, interpreting literature results can be confounding, as different studies quantify qualitative measures, such as “surface soils” differently (i.e. 5 cm or 10 cm depth). Moreover, in agricultural systems, there are several agronomic events throughout the growing season, the effects of which can change both short-term and long-term soil microbial dynamics greatly.

Studies evaluating AOA and AOB dynamics sampled a variety of soil depths. Some did not evaluate any depth effects and sampled from 0-5 cm (Phillips et al., 2000), 0-10 cm (Jangid et al., 2008, Offré et al., 2009, Di et al., 2009) or 0-20 cm (Wessen et al., 2010a, Philippot et al., 2009). The differences in collected sample depths confounds comparisons of AOB and AOA, as their dynamics across depths are different; notably AOB abundance and activity tends to decrease with depth and AOA shows less variability (Hafeez et al., 2012, Di et al., 2010). Thus, a sample collected from the 0-20 cm layer is likely to overestimate the AOA:AOB ratios compared to if that same sample were only collected from the 0-5 cm layer.

It has been noted that soil microbial populations and activity cannot be adequately determined from one sampling and that at least one full growing season is required to
average the effects of environmental conditions (Buchanan and King, 1992, Nannipieri, 1984). However, most studies evaluating AOB and/or AOA populations and activities have done so with just one (Hafeez et al., 2012, Di et al., 2010, Philippot et al., 2009, Govaerts et al., 2007, Hernanz et al., 2002) or two sampling times (Jangid et al., 2008, Phillips et al., 2000). In particular, studies should consider the seasonality of tillage effects (Kladivko, 2001), freeze-thaw events in the spring (Smith et al., 2010, Herrmann and Witter, 2002) and root exudate effects (Buchanan and King, 1992). Tillage effects are particularly important and literature findings have been previously described above (See 2.5 Tillage Effects). However, other seasonal event should also be considered. In early spring, bacterial cell lysis during freeze-thaw events increases nutrient availability (Smith, 2007, Ivarson and Sowden, 1970, Hantschel et al., 1995) and can provide significant additions of labile carbon increasing microbial activity (Herrmann and Witter, 2002). Similarly, additions of soil organic carbon through root growth and residue incorporation after crop harvest has also been linked to increases in soil microbial abundances (Buchanan and King, 1992). Not accounting for these seasonal effects undermines efforts to determine the relevant effects of agricultural practices on soil ammonia oxidation.

2.8 Conclusion

Many studies have analyzed AOB and AOA microbial dynamics, but there remain areas that have not been extensively researched. Agricultural management practices have been shown to affect AOB and AOA, but often studies have found conflicting results. In general studies have focused on specific variables at the expense of ignoring others. In particular, seasonal and depth effects have been ignored. Analyzing in situ AOB and AOA
communities before and after key agronomic events such as crop plant, tillage, fertilization, and harvest could contribute to better understanding. This study will attempt to fill these knowledge gaps and determine tillage and crop rotation effects on AOB and AOA across soil depths with samples collected before and after the key agronomic events mentioned above. Through utilization of quantitative molecular techniques this research will further understanding on linking tillage and crop rotation effects on AOB and AOA community. This knowledge will help evaluate the ecological impact of different management practices and assist in the development of best management practices.
Chapter 3: Long-term effects of different tillage and crop rotation practices across soil depth on soil nutrients and ammonia oxidizers during a growing season.

3.1 Introduction

Managing soil N efficiently is critical for agricultural systems to maintain crop productivity, optimize costly fertilizer inputs and reduce associated deleterious environmental effects. Understanding how different agronomic practices affect ammonia oxidation, the rate-limiting step of nitrification, is particularly important. Ammonia oxidation provides substrate for the production of nitrate, which is the most plant-available form of nitrogen, but nitrate is also highly mobile in soil and prone to leaching causing groundwater pollution. Under specific conditions ammonia oxidation can also lead to the direct evolution of nitrous oxide, a potent greenhouse gas (Phillips et al., 2000, Capone, 1991). Agricultural best management practices must be evaluated on how they influence ammonia oxidation, as this will lead to better understanding of soil N dynamics.

The recent discovery of the functional ammonia monoxygenase gene in archaea (Treusch et al., 2005, Konneke et al., 2005, Venter et al., 2004) and its abundance in soil (Leininger et al., 2006) complicates quantification of microbial ammonia oxidation. Before this discovery, it was thought that only bacteria were capable of ammonia oxidation. Literature since then has postulated that ammonia-oxidizing bacteria (AOB) thrive in higher-nutrient environments, whereas ammonia-oxidizing archaea (AOA) are oligotrophic (Wessen et al., 2010a, Gubry-Rangin et al., 2010, Di et al., 2009, Erguder et al., 2009). Most studies however, do not fully account for temporal effects across growing
seasons and/or account for spatial heterogeneity across depth (Wessen et al., 2010a, Kladivko, 2001). Yet, AOA/AOB communities can vary across depths greatly (Eilers et al., 2012, Erguder et al., 2009). In addition, different tillage and crop rotation practices can influence nutrient distributions across depth through mechanical disturbance and rhizosphere effects, respectively. Since nitrate can be lost at various soil depths, through denitrification, leaching or runoff, it is important to fully account for depth when evaluating these agricultural practices.

Previous studies that have assessed AOA/AOB across depth have often lacked a seasonal aspect (Di et al., 2010, Hernanz et al., 2002), whereas studies that assessed AOA/AOB across a growing season often did not account for depth effects (Wessen et al., 2010a). In light of this literature gap, this study was designed to examine both seasonal and depth effects and to evaluate how soil nutrients and microbial dynamics change across these spatial and temporal scopes. Specific objectives of this study were to evaluate how different tillage and crop rotation systems affect soil N and C, microbial abundance and activity, and AOA/AOB abundance and activity. This study is unique in its application of quantitative molecular techniques to assess tillage and crop rotation effects. Previously, the effects of tillage on soil microbial and ammonia oxidizer abundances were determined through most-probable number estimates on cultivated samples (Phillips et al., 2000), but these techniques are not fully representative of in situ soil microbial communities due to inherent limitations (Smith, 2007, Alexander, 1977). More accurate observations are obtained by directly targeting in situ DNA and RNA gene fragments, which was possible in this study by recent technical advances enabling nucleic acid preservation in the field (MoBio, Carlsbad, CA, USA). These technologies enabled this study to provide in situ
microbial community “snapshots” at key times corresponding with agronomic events during a growing season.

3.2 Methods

3.2.1 Experimental Design

This study was conducted during the 2010 growing season in long-term experimental trial established in 1980 and located at the University of Guelph’s Elora Research Station (ERS) in Elora, Ontario (43°39' N, 80°25' W, elevation 376 m). For more details on the experimental design please refer to Meyer-Aurich et al. (2006). The soil is classified as an imperfectly drained Guelph silt loam with an average pH of 7.6 (Smith, 2007, Canadian Agricultural Services Coordinating Committee, 1998).

The trial was arranged as a randomized split-plot design with four replications (Figure 3.1) with seven crop rotations (main-plots) and two tillage treatments (split-plots). Data collection occurred during the growing season of 2010 on each replicate of 4 selected treatments. Main treatments were 4 different crop rotation that were split into till and no-till split treatments. Crop rotation treatments analyzed included continuous corn (CC), and a four-year rotation of corn-corn-soy-winter wheat with a red clover cover crop underseeded to the winter wheat (RC) and chemically killed the previous fall. Tillage included spring cultivation (May 6th) prior to plant operations (May 7th) and fall moldboard plowing (October 18th) to a depth of 15 cm. In 2010, all plots were planted in corn. All plots received the same fertilizer input. This consisted of 157 kg ha⁻¹ of 5-20-20 applied at planting on May 7th, and a second application of urea-ammonium-nitrate (UAN) on June 18th (420 L of 28-0-0, 150 kg N ha⁻¹) side-dressed midway between the corn rows.
Figure 3.1: Experimental design of long-term field plots. Crop rotations (main plots) were arranged in a randomized split-plot design with four replications. Till and no-till treatments (split-plots) were grouped in pairs within different crop rotations.
3.2.2 Soil Sampling

Collection of soil samples was timed around tillage and fertilization events during the 2010 growing season and occurred on May 3\textsuperscript{rd} (pre-fertilization = before cultivation on May 6\textsuperscript{th} and corn planting on May 7\textsuperscript{th}), May 25\textsuperscript{th} (pre-till = after cultivation and planting), June 30\textsuperscript{th} (pre-fertilization = after UAN fertilization on June 18\textsuperscript{th}), October 12\textsuperscript{th} (pre-plow = before corn harvest and moldboard plowing on October 18\textsuperscript{th}), and finally on November 23\textsuperscript{rd} (post-plow = after corn harvest and moldboard plowing). Samples were collected on all days to a depth of 30 cm. All samples were acquired using a soil corer with a 5 cm diameter. Four subsamples per plot were collected along a diagonal transect and then mixed in a clean bucket to obtain one composite sample. Subsamples were separated and pooled together for depths of 0-5 cm, 5-15 cm and 15-30 cm. Nucleic acids were preserved in the field by placing 2 g of fresh soil from each collected sample immediately into 5 mL of Lifeguard Soil Preservation Solution (MoBio, Carlsbad, CA, USA). Nucleic acids were extracted within 30 days of sampling for molecular analyses and the remaining bulk soil was stored at -20\textdegree C for nutrient analyses.

3.2.3 Nucleic Acid Extraction and Preparation

Following manufacturer protocols, RNA was extracted from soils using the RNA Powersoil Total RNA Isolation Kit and DNA was eluted from the remaining solution using the RNA Powersoil DNA Elution Accessory Kit (MoBio, Carlsbad, CA, USA). Extracted RNA was subjected to reverse transcription-PCR (Promega, Madison, WI, USA) according to the manufacturer’s protocol to create cDNA that allowed for further analyses. Isolated DNA and cDNA were sub-sampled into a working stock and an original
stock to minimize freeze-thaw effects. Stocks and RNA samples were stored at -80°C. Only working stocks of DNA and cDNA were used for subsequent analyses.

3.2.4 Quantitative Polymerase Chain Reactions

A quantitative polymerase chain reaction assay (qPCR) was used to enumerate starting quantities of *amoA*, *crenamoA*, *bac16s*, and *arch16s* gene fragments using the primers listed in Table 3.1. Thermocycle conditions are listed in Table 3.2 for each gene. All qPCR assays were performed in a Bio-Rad iQ5 detection system (Bio-Rad Laboratories, Mississauga, ON). Briefly, gene copy quantification was achieved by measuring fluorescent emissions from SYBR Green Dye (Bio-Rad Laboratories, Hercules, CA, USA). Fluorescence from environmental samples was measured relative to standard reference samples containing known target gene quantities, allowing for gene copy quantification. For each 1 µL template, reactions were performed in a total volume of 25 µL per sample, with 12.5 µL of 1x SYBR Green Supermix, 10 µM (10 pmol µL⁻¹) each forward and reverse primers, 1 µL T4 gene 32, and RNase and DNase free water. The SYBR Green Supermix contained 100 nM KCl, 40 mM Tris-HCl, 0.4 mM dNTP, 50 units mL⁻¹ iTaq DNA polymerase, 6mM MgCl₂, SYBR Green 20 nM fluorescein, and stabilizer (Bio-Rad Laboratories, Hercules, CA, USA). T4 gene 32 protein (New England Biolabs, Pickering, ON) was employed at a final concentration of 0.025 ng/µL in all runs, as it has been proven to significantly reduce the inhibitions effects of soil humic acids (Vahjen and Tebbe, 1994, Tebbe and Vahjen, 1993). Melt curve analyses were performed to verify amplicon specificity (Table 3.2).
3.2.5 Cloning

To facilitate quantification of targeted genes in quantitative polymerase chain reactions (qPCR) reference strains were created by cloning PCR products of *amoA*, *crenamoA*, *bac16s*, and *arch16s* gene fragments using TOPO TA cloning kits (Invitrogen, Carlsbad, USA) according to the manufacturer’s protocol. Subsamples from *E. coli* clones that grew on selective media underwent PCR with one of the four provided primers: M13F, M13R, T3, or T7 from the TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA). PCR was done in a Mastercycler Epgradient S thermocycler (Eppendorf, Hamburg, Germany). Amplification of 1 µL of extracted DNA was performed in a total volume of 25 µL (5 µL of 5x PCR buffer, 1-2 µL of 25 mM MgCl₂, 0.125-0.25 µLdNTPs, 5-10 pmol forward primer, 5-10 pmol reverse primer, 3.35 U Taq DNA polymerase (Promega, Madison, WI, USA)). DNase and RNase free water was used in negative control samples as a substitute for template. The standard curve was analyzed only if the following reaction conditions were met: efficiency= 85-105%), R² ≥ 99%), and a slope = -3.0 to -3.8. Table 3.1 outlines the specific primers used. Thermocycle conditions are outlined in Table 3.3.

Cloned PCR products were screened by visually assessing amplicon sizes via agarose gel electrophoresis. Selected PCR products of the clones were then sent to the Laboratory Services Department at the University of Guelph for DNA sequencing using an ABI Prism 3720 (Applied Biosystems, Foster City, CA, USA).

3.2.6 Nutrient Analyses

Inorganic nitrogen was extracted from frozen bulk soil samples in 2 M KCl following protocol described by Keeney and Nelson (1987). Extracted nitrate (NO₃) and ammonium (NH₄) were determined colourimetrically via segmented flow analysis (AA3, Seal
Soil carbon levels were analyzed by dry combustion techniques stipulated by Carter and Gregorich (2008). Specifically, total carbon and inorganic carbon were analyzed by placing approximately 0.300 g of dried, ground and homogenized sample into a clean, carbon-free combustion boat. This was then placed in a LECO CR-12 Carbon Analyzer (LECO CR-12, Model #781-700, Leco Instruments Ltd., St Joseph, Michigan, USA). Organic carbon was determined as the difference of total carbon and inorganic carbon.

3.2.7 Statistical Analysis

Data was analyzed using Statistical Analysis Software for Windows version 9.2 (SAS Institute, Cary NC). Statistical differences were determined amongst and between different treatments, sample times, and sample depths. When applicable, gene count data were transformed using a square-root-transformation. When necessary nutrient data were normalized using a log_{10} transformation. All data were analyzed in a generalized linear mixed model (Proc Glimmix). Gene count data were analyzed with an over dispersed Poisson distribution assumption. Independent and interactive fixed effects were analyzed for tillage, crop rotation, season, and soil depth. Random effects were associated with block and block interactions. Multiple means comparisons of gene counts were analyzed using a Tukey adjustment (\( \alpha = 0.05 \) unless otherwise stated). Significant differences among and between least-square means were evaluated statistically with p-values. Significant differences between least-square means were numerically evaluated with difference of estimates tables.
Table 3.1: Primer pairs, sequences and target genes.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’-3’)</th>
<th>Target Gene</th>
<th>Literature Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>amoA 1F</td>
<td>ggg gtt tct act ggt ggt</td>
<td>Ammonia monoxygenase</td>
<td>(Rotthauwe et al., 1997)</td>
</tr>
<tr>
<td>amoA 2R</td>
<td>ccc ctc kgs aaa gcc ttc ttc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>crenamoA 23f</td>
<td>atg gtc ggc twa gac g</td>
<td>Ammonia monoxygenase</td>
<td>(Tourna et al., 2008)</td>
</tr>
<tr>
<td>crenamoA 616r</td>
<td>gcc atc cat ctg tat gtc ca</td>
<td></td>
<td></td>
</tr>
<tr>
<td>bac16S 338f</td>
<td>act cct acg gga ggc agc ag</td>
<td>16S small ribosomal gene</td>
<td>(Fierer et al., 2005)</td>
</tr>
<tr>
<td>bac16s 518r</td>
<td>att acc ggc gct get gg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>arch16s 364f</td>
<td>cgg ggy gca sca ggc ggc aa</td>
<td>16S small ribosomal gene</td>
<td>(Kemnitz et al., 2007)</td>
</tr>
<tr>
<td>arch16s 934r</td>
<td>gtg ctc ccc cgc caa ttc ct</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gene</td>
<td>Thermocycle</td>
<td>Literature source</td>
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<td></td>
</tr>
<tr>
<td>amoA</td>
<td>Cycle 1 – 1x</td>
<td>Adapted from (Glaser et al., 2010)</td>
<td></td>
</tr>
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<td></td>
<td>95°C for 5 minutes</td>
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<td></td>
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<tr>
<td></td>
<td>Cycle 2 – 40x</td>
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<tr>
<td></td>
<td>95°C for 30 seconds</td>
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<td></td>
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<tr>
<td></td>
<td>57°C for 1 minute</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>72°C for 1 minute</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cycle 3 – 1x</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>72°C for 10 minutes</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Cycle 4 (Melt Curve) – 51x</td>
<td></td>
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<tr>
<td></td>
<td>57°C to 70°C</td>
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<tr>
<td>crenamoA</td>
<td>Cycle 1 – 1x</td>
<td>(Tourna et al., 2008)</td>
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<tr>
<td></td>
<td>95°C for 10 minutes</td>
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<tr>
<td></td>
<td>Cycle 2 – 39x</td>
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<tr>
<td></td>
<td>94°C for 45 seconds</td>
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<tr>
<td></td>
<td>55°C for 45 seconds</td>
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<tr>
<td></td>
<td>72°C for 45 seconds</td>
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<td></td>
<td>Cycle 3 – 1x</td>
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<tr>
<td></td>
<td>Cycle 4 (Melt Curve) – 81x</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>55°C to 72°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>bac16S</td>
<td>Cycle 1 – 1x</td>
<td>Adapted from (Fierer et al., 2005)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>95°C for 5 minutes,</td>
<td></td>
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<tr>
<td></td>
<td>Cycle 2 – 30x</td>
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<tr>
<td></td>
<td>55°C for 30 seconds</td>
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<td></td>
<td>72°C for 30 seconds</td>
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<tr>
<td></td>
<td>Cycle 3 – 1x</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>72°C for 10 minutes</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Cycle 4 (Melt Curve) – 41x</td>
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</tr>
<tr>
<td></td>
<td>55°C to 72°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>arch16S</td>
<td>Cycle 1 – 1x</td>
<td>(Kemnitz et al., 2007)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>94°C for 5 minutes,</td>
<td></td>
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<tr>
<td></td>
<td>Cycle 2 – 40x</td>
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<tr>
<td></td>
<td>94°C for 35 seconds</td>
<td></td>
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<tr>
<td></td>
<td>56°C for 30 seconds</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>72°C for 50 seconds</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Cycle 3 – 1x</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>72°C for 10 minutes</td>
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<td></td>
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<tr>
<td></td>
<td>Cycle 4 (Melt Curve) – 69x</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>56°C to 72°C</td>
<td></td>
<td></td>
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<tr>
<td>Gene</td>
<td>Thermocycle</td>
<td>Literature source</td>
<td></td>
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</tr>
</tbody>
</table>
| *amoA* | Cycle 1 – 1x  
94°C for 2 minutes  
Cycle 2 – 35x  
94°C for 30 seconds  
55°C for 1 minute  
72°C for 1 minute  
Cycle 3 – 1x  
72°C for 10 minutes | Adapted from  
(Wessen et al., 2010a) |
| *crenamoA* | Cycle 1 – 1x  
95°C for 5 minutes  
Cycle 2 – 10x  
94°C for 30 seconds  
55°C for 30 seconds  
72°C for 1 minute  
Cycle 3 – 25x  
92°C for 30 seconds  
55°C for 30 seconds  
72°C for 1 minute  
Cycle 4 – 1x  
72°C for 10 minutes | Adapted from  
(Wessen et al., 2010a) |
| *bac16S* | Cycle 1 – 1x  
94°C for 2 minutes  
Cycle 2 – 35x  
94°C for 30 seconds  
56°C for 1 minute  
72°C for 30 seconds  
Cycle 3 – 1x  
72°C for 2 minutes | Adapted from  
(Wessen et al., 2010a) |
| *arch16S* | Cycle 1 – 1x  
94°C for 5 minutes  
Cycle 2 – 35x  
94°C for 35 seconds  
66°C for 30 seconds  
72°C for 50 seconds  
Cycle 3 – 1x  
72°C for 10 minutes | (Kemnitz et al., 2007) |
3.3 Results

3.3.1 Soil Inorganic Nitrogen and Organic Carbon Concentrations

Soil nutrients were successfully extracted and analyzed from all sample dates, except pre-till. post-till samples collected on May 25th were not analyzed further due to sample loss. Soil inorganic nitrogen concentrations were influenced by season – specifically at post-fertilization - but not crop rotation or tillage, whereas soil organic carbon concentrations were affected by tillage, but no crop rotation (Appendix B: Table B.1-B.3). Post-fertilization samples exhibited high levels of soil nitrate and ammonium within the top 0-5 cm of soil (P<0.05) (Figure 3.2 and 3.3). Soil organic carbon concentrations remained relatively consistent in the top 0-5 cm of soil throughout the season (Figure 3.4). At post-fertilization organic carbon was distributed homogenously across depths in till plots, but not in no-till plots (P<0.05) (Figure 3.5). Lower organic carbon concentrations below 15 cm were present in no-till soils at this time relative to till plots (P<0.05) (Figure 3.5). This distribution trend was not observed on other sample days (Data not shown). Generally, organic carbon levels were highest in surface soil relative to soil levels below 5 cm, except in pre-plow samples (Figure 3.4). This was due to increased carbon levels below 15 cm at this time while levels in the 0-5 cm depth fraction remained consistent (Figure 3.4).
Figure 3.2: Mean extracted NO₃ content from soil at 0-5 cm, 5-15 cm, and 15-30 cm depths. Samples were collected during the 2010 growing season. Error bars represent calculated standard errors (n= 16, α= 0.05).
Figure 3.3: Mean extracted NH$_4$ content from soil at 0-5 cm, 5-15 cm, and 15-30 cm depths. Samples were collected during the 2010 growing season. Error bars represent calculated standard errors (n= 16, α= 0.05).
Figure 3.4: Mean seasonal soil organic carbon concentrations (%) at 0-5 cm, 5-15 cm, and 15-30 cm depths. Samples were collected during the 2010 growing season. Error bars represent calculated standard errors (n= 16, α= 0.05).
Figure 3.5: Mean soil organic carbon concentrations (%) at post-fertilization from soil in till and no-till plots at 0-5 cm, 5-15 cm, and 15-30 cm depths. Samples were collected during the 2010 growing season seven days after fertilization with urea-ammonium-nitrate (UAN 150 kg N ha\(^{-1}\)). Error bars represent calculated standard errors (n = 8, \(\alpha = 0.05\)).
3.3.2 Soil Microbial Abundance and Activity

Fragments of bac16S and arch16S DNA and cDNA (reverse-transcribed from collected RNA) were not always successfully amplified due to procedural disruptions caused by soil humic acids inhibiting gene expression during PCR amplification. In addition, post-till samples collected on May 25th were not analyzed due to sample loss.

Seasonal bac16S DNA copies were higher pre-fertilization relative to other sample days (Figure 3.6). Seasonal bac16S cDNA copies trended higher at post-fertilization (P<0.1) and were significantly lower at post-plow (P<0.05) (Figure 3.7). Seasonal bac16S DNA copies were evenly distributed across depths, except at pre-fertilization where they were significantly higher below 5 cm depths (Figure 3.8). The distribution of bac16S cDNA across depths was homogenous during the entire season (Figure 3.9).

Tillage affected arch16S genes differently than bac16S. DNA copies of arch16S were consistently higher in no-till plots, except in pre-plow samples (P<0.05) (Figure 3.10). Seasonally, arch16S DNA copies were lower at post-fertilization and pre-plow (P<0.05) (Figure 3.6), whereas activity was highest at post-fertilization, intermediate at pre-fertilization and pre-plow, and lowest at post-plow (P<0.05) (Figure 3.7). Higher abundances of arch16S DNA were apparent in the 5-30 cm depth fraction at pre-fertilization relative to 0-5 cm levels (P<0.05) (Figure 3.11).
Figure 3.6: Mean seasonal $bac16S$ and $arch16S$ DNA copies g$^{-1}$ dry soil averaged from samples collected in 2010 in the 0-30 cm layer. Data points represent mean gene copies aggregated across all treatments (two tillage systems and two different crop rotations) and soil depths; 0-5 cm, 5-15 cm, and 15-30 cm. Error bars represent calculated standard errors (n= 48, $\alpha$=0.05) and percentages indicate the relative abundance of $arch16S:bac16S$ DNA at each sampling time.
Figure 3.7: Mean seasonal \textit{bac16S} and \textit{arch16S} cDNA copies g\textsuperscript{-1} dry soil averaged from samples collected in 2010 to a 30 cm depth. Data points represent mean gene copies aggregated across all treatments (two tillage systems and two different crop rotations) and soil depths; 0-5 cm, 5-15 cm, and 15-30 cm. Error bars represent calculated standard errors (n= 48, \(\alpha= 0.05\)) and percentages indicate the relative abundance of \textit{arch16S:bac16S} cDNA at each sampling time.
Figure 3.8: Mean seasonal \( \text{bac}16\text{S DNA gene copies g}^{-1} \text{ dry soil} \) at 0-5 cm, 5-15 cm and 15-30 cm depths. Soil was sampled in 2010 in long-term treatments. Data points represent mean gene copies aggregated across all treatments (two tillage systems and two different crop rotations). Error bars represent calculated standard errors \((n=16, \alpha=0.05)\).
Figure 3.9: Mean seasonal $\text{bac}_{16}S$ cDNA gene copies g$^{-1}$ dry soil at 0-5 cm, 5-15 cm and 15-30 cm depths. Soil was sampled in 2010 in long-term treatments. Data points represent mean gene copies aggregated across all treatments (two tillage systems and two different crop rotations). Error bars represent calculated standard errors ($n=16$, $\alpha=0.05$).
Figure 3.10: Mean seasonal *arch16S* DNA gene copy g⁻¹ dry soil in till and no-till plots. Soil was sampled in 2010 in long-term treatments. Data points represent mean gene copies aggregated across two different crop rotation treatments and three soil depths; 0-5 cm, 5-15 cm, and 15-30 cm. Error bars represent calculated standard errors (n= 24, α=0.05).
Figure 3.11: Mean seasonal *arch16S* DNA gene copies g$^{-1}$ dry soil at 0-5 cm, 5-15 cm and 15-30 cm depths. Soil was sampled in 2010 in long-term treatments. Data points represent mean gene copies aggregated across all treatments (two tillage systems and two different crop rotations). Error bars represent calculated standard errors (n= 16, α= 0.05).
3.3.3 Soil Ammonia Oxidizer Abundance and Activity

Fragments of *amoA* and *crenamoA* DNA and cDNA (reverse-transcribed from collected RNA) were successfully amplified on all sample dates, although not all experimental plots were necessarily amplified within some dates. Post-till samples collected on May 25th were not analyzed further due to sample loss. DNA copies of *amoA* tended to be higher in CC plots relative to RC plots at pre-fertilization (P=0.054) and at post-plow (P<0.05) (Figure 3.12). Tilled plots had a more homogenous distribution of *amoA* DNA copies across depth at pre-plow and post-plow sample times, whereas *amoA* DNA copies tended to decrease with depth no-till plots, especially below 15 cm (Figure 3.13). Copies of *amoA* cDNA followed a similar trend at post-plow with more copies in no-till versus till soils above 5 cm (P<0.1) (Figure 3.14).

Before spring plant operations (pre-fertilization), DNA copies of *crenamoA* tended to be lower in the top 0-5 cm compared to lower depths (P<0.1) (Figure 3.15) and also higher in no-till plots vs. till plots (P<0.05) (Figure 3.16). At post-fertilization, no-till RC plots exhibited the greatest abundance of *crenamoA* DNA copies (P=0.091) (Figure 3.17). Unfortunately, observed *crenamoA* cDNA copies were too low in abundance to statistically analyze them. Methodological errors were not responsible for this, since the same cDNA stocks were utilized successfully to analyze other genes including *crenamoA* DNA.
Figure 3.12: Mean seasonal *amoA* DNA gene copies g\(^{-1}\) dry soil in continuous corn (CC) and corn-corn-soy-wheat under-seeded to red clover (RC) rotation in its first corn year. Soil was sampled in 2010 in long-term treatments. Data points represent mean gene copies aggregated across till and no-till treatments and three soil depths; 0-5 cm, 5-15 cm, and 15-30 cm. Error bars represent calculated standard errors (n= 24, \(\alpha\) = 0.05).
Figure 3.13: Mean amoA DNA gene copies g\(^{-1}\) dry soil in till and no-till plots before and after fall harvest and applicable plow events at 0-5 cm, 5-15 cm, and 15-30 cm depths. Soil was sampled in 2010 in long-term treatments. Data points represent mean gene copies across two different crop rotations. Error bars represent calculated standard errors (n= 8, α= 0.05).
Figure 3.14: Mean amoA cDNA gene copies g\(^{-1}\) dry soil in till and no-till plots before and after fall harvest and applicable plow events at 0-5 cm, 5-15 cm, and 15-30 cm depths. Soil was sampled in 2010 in long-term treatments. Data points represent mean gene copies across two different crop rotations. Error bars represent calculated standard errors (n= 8, \(\alpha= 0.05\)).
Figure 3.15: Mean seasonal *crenamos* DNA gene copies g$^{-1}$ dry soil at 0-5 cm, 5-15 cm and 15-30 cm depths. Soil was sampled in 2010 in long-term. Data points represent mean gene copies across two tillage systems and two different crop rotations. Error bars represent calculated standard errors (n= 16, α= 0.05).
Figure 3.16: Mean *crenamoA* DNA gene copies g\(^{-1}\) dry soil in till and no-till plots before and after fall harvest and applicable plow events. Soil was sampled in 2010 to a 30 cm depth in long-term treatments. Data points represent mean gene copies across two different crop rotations and three depths; 0-5 cm, 5-15 cm, and 15-30 cm. Error bars represent calculated standard errors (n= 24, α= 0.05).
Figure 3.17: Mean *crenamo*A DNA gene copies g⁻¹ dry soil in till continuous corn (CCT) and till corn-corn-soy-wheat under-seeded to red clover rotation in its first corn year (RCT), and no-till treatments of the same rotations (CCNT and RCNT respectively). Soil was sampled to a 30 cm depth in 2010 in long-term treatments. Data points represent mean gene copies across three depths; 0-5 cm, 5-15 cm, and 15-30 cm. Error bars represent calculated standard errors (n=12, α=0.05).
3.3.4 Seasonal Conditions

Ambient air temperatures dropped as the season progressed (Table 3.4). Soil moisture was highest at pre-fertilization and lowest at post-fertilization and post-plow times (Table 3.4).

Table 3.4: Soil moisture and ambient surface air temperature during sample times.

<table>
<thead>
<tr>
<th>Time</th>
<th>Soil Moisture (%)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-fertilization</td>
<td>22.1</td>
<td>18.1</td>
</tr>
<tr>
<td>Post-fertilization</td>
<td>16.4</td>
<td>15.2</td>
</tr>
<tr>
<td>Pre-plow</td>
<td>18.5</td>
<td>8.9</td>
</tr>
<tr>
<td>Post-plow</td>
<td>16.4</td>
<td>4.8</td>
</tr>
</tbody>
</table>

3.4 Discussion

3.4.1 Soil Nutrients

In this study soil nitrate (NO₃) and ammonium (NH₄) levels were affected by season – particularly at post-fertilization (Figure 3.2-3.3) - but not tillage or crop rotation. This is in contrast to previous studies that have linked tillage with increased spring soil temperatures coinciding with increased soil-N mineralization (Agbede, 2008, Licht and Al-Kaisi, 2005, Malhi and Osullivan, 1990, Nyborg and Malhi, 1989). It was also expected that soils previously cropped with winter wheat under-seeded to red clover would have higher inorganic nitrogen levels credited from leguminous nitrogen fixation. However, this study was not able to observe short-term treatment effects on soil inorganic nitrogen from any crop rotations. Difficulty analyzing post-till samples (May 25th) may have limited this study’s ability to accurately assess the short-term effects of tillage and/or crop rotation (Data not shown), as this sample time was intended to differentiate seasonal effects relating to tillage and fertilization events. Moreover, 25 years of data at this site have found results consistent with literature, suggesting both rotation and tillage significantly
affect soil inorganic nitrogen (Deen, 2010). Red clover was chemically killed the previous fall, thus the dynamics of its N-mineralization were unknown in this study.

Side-dressed urea-ammonium-nitrate (UAN 28-0-0) applied at 150 N kg ha\(^{-1}\) (Deen, 2010) theoretically could have increased soil inorganic nitrogen concentrations by about 80 mg kg\(^{-1}\) dry soil in the top 15 cm (Appendix A: Table A.1). In this study, NO\(_3\) concentrations in 0-5 cm and 5-15 cm soils did not rise to this theoretical concentration (Figure 3.2). Samples were collected 12 days after fertilization so it is likely that soil fixation and microbial immobilization combined could have represented a large sink. In addition, crops were likely very competitive for inorganic-N at this time, as corn was in the 6-leaf stage and these soils were low in N for corn production purposes according to “OMAFRA Fertilizer Recommendations” (2010).

Seasonal soil organic carbon concentrations (SOC) correlated well with belowground carbon inputs associated with corn root development (Figure 3.4). For example, pre-plow samples exhibited SOC that converged to similar values in all depth fractions (Figure 3.4). Corn plants at pre-plow had grown to their maximum biomass above and below ground, therefore, root growth and accumulation of root exudates in the soil were maximized at this time. However, based on observed crop yields (refer to Chapter 4: Figure 4.7) this increase could not fully account for the observed SOC. More likely differences in bulk density affected the observed concentrations.

SOC was seasonally lowest at post-fertilization (Figure 3.4). Previous studies have suggested that SOC may initially decrease with nitrogen fertilization due to increased decomposition activity (Moran et al., 2005), however, in this study the decrease in SOC at post-fertilization was mainly due to significantly lower SOC concentrations at depths below 15 cm (Figure 3.4), where inorganic N levels were minimal (Figure 3.2 and 3.3).
Thus, it is unlikely that decomposition was responsible for lower SOC after UAN application. Rather, it is plausible that SOC additions at post-fertilization were minimal as corn plants were at the 6-leaf stage and had only minimally developed roots. Therefore, any decomposition occurring before this time would have decreased SOC concentrations, which were later replenished by belowground additions via root growth as described above. Interestingly, tilled soils had higher SOC below 15 cm in post-fertilization samples (Figure 3.5). Tillage homogenizes SOC across depths and can act as a carbon input to soils at depth by incorporating residues (Alvarez, 2005, Deen and Kataki, 2003a, Angers et al., 1997).

3.4.2 Soil Microbial Abundance and Activity

Our results indicate seasonal copies of soil bacterial 16S DNA (bac16S DNA) were about 5-8x more abundant than copies of soil archaeal 16S DNA (arch16S DNA) (Figure 3.6). However, these results should be interpreted with caution. Copy numbers can vary per cellular genome (Fogel et al., 1999), thus gene copies do not indicate absolute cellular abundance. In addition this study was completed in one location and on one soil type. Edaphic factors have been noted as the primary factor effecting soil microbial community composition, often negating any in situ treatment effects (Kuramae et al., 2012, Smith et al., 2010). Although, previous studies suggest that bacteria are more ecologically versatile and thrive especially well in nutrient rich environments like those found in agricultural soils, while archaea adapt best in oligotrophic environments with minimal disturbances (Gubry-Rangin et al., 2010, Di et al., 2010, Wessen et al., 2010a, Erguder et al., 2009, Valentine, 2007).
Bac16S DNA copy abundance was significantly higher at pre-fertilization and then remained at consistent levels the rest of the season (Figure 3.6). Cell lysis resulting from spring freeze-thaw events can release significant amounts of nutrients and increase microbial abundances (Herrmann and Witter, 2002). Smith et al. (2010) observed higher microbial diversity at spring thaw at the same location as this study, but did not quantify abundances. Interestingly, in this study, higher abundances at pre-fertilization did not lead to increased bacterial activity (bac16S cDNA), which actually increased only at post-fertilization during the season (Figure 3.7). It is possible that the half-life of bac16S DNA is much longer than RNA. If true, this would imply that qPCR could have detected DNA copies of dead cell’s bac16S, but not cDNA. This would imply qPCR in the early spring overestimated bacterial abundance. Alternatively, bac16S DNA copies may have declined independently of bacteria cellular abundance as the season progressed. Individual bacterial genomes can contain between 1-12 copies of the bac16S gene (Fogel et al., 1999). Thus, data from this study does not ascertain whether there was indeed a drop in bacterial abundance or if agronomic events influenced bacterial evolution by selecting cells with lower transcriptional capabilities (i.e. less bac16S DNA copies per cell). To determine which is occurring, future studies should focus on analyzing molecular abundance and activity in conjunction with microbial diversity and non-molecular measures of total bacterial abundance.

The distribution of bac16S DNA and cDNA copies also differed seasonally across depth (Figure 3.8 and 3.9). Significantly greater abundances of bac16S DNA copies in deeper soils at pre-fertilization could be a relic of preserved DNA genes from lysed cells (Figure 3.8). As mentioned above, half-lives for bac16S DNA are not well known (Nicol et al., 2008), thus qPCR may not differentiate DNA from living and dead sources.
Samples at depth were more likely to preserve dead cell DNA due to cooler temperatures caused by warming lags inherent to deeper soils. Data from this study is insufficient to explain this satisfactorily. Furthermore it is also possible that bare surface soil (before crops were planted) presented a harsh environment that limited microbial abundances, because of frequent disturbances. Conversely, soils below 5 cm at pre-fertilization were sheltered, which may have provided conditions favourable for increased bacterial abundance.

Interestingly, neither \textit{bac16S} DNA nor cDNA copies increased at post-fertilization (Figure 3.8 and 3.9). This conflicts with previous work that found increases in \textit{bac16S} DNA and cDNA after fertilization events (Yevdokimov et al., 2012, Girvan et al., 2004). Girvan et al. (2004) noted these peaks in copy numbers 4 days after fertilization, whereas this study sampled 12 days after UAN applications. Thus, it is possible that a peak in \textit{bac16S} cDNA copies was missed in this study.

Archaeal populations were variable throughout the season and their abundance and activity were affected differently by nutrient availability at different times. At post-fertilization, abundance (\textit{arch16S} DNA copies) was relatively lower than before fertilization (Figure 3.6), whereas activity (\textit{arch16S} cDNA copies) was significantly greater than before (Figure 3.7). This drop in abundance is supported by literature citing archaea’s oligotrophic nature (Gubry-Rangin et al., 2010, Di et al., 2010, Erguder et al., 2009), however, it is not clear why activity would have increased at this time.

This study found that archaea were much more sensitive to soil disturbances than bacteria and this is consistent with past research suggesting AOB are better suited to cultivated soils than AOA (Zeglin et al., 2011). In this study, this was shown by consistently higher \textit{arch16S} DNA copy numbers in no-till soils across the season, except
at pre-plow (Figure 3.10). Pre-plow samples were undisturbed for the longest time compared to other sample times, also rhizosphere effects would have been greatest at this pre-plow. In addition, throughout the season arch16S DNA copies tended to be highest in 5-15 cm depths (Figure 3.11). Interestingly, one study noted a continual increase in archaeal populations with increasing soil depth to 50 cm (Eilers et al., 2012). However, this study was not in an agricultural system where soil carbon and nitrogen concentrations are much more concentrated at the surface due to fertilization and cultivation of annual crops with relatively shallow root development. In our study, there was minimal difference in SOC between 0-5 cm and 5-15 cm depths in till or no-till plots, but there was a noted decrease in SOC below 15 cm (Figure 3.4). Therefore, our results suggest archaea balanced their nutrient needs while avoiding disturbance and adapted best at 5-15 cm depths that were relatively nutrient rich – especially in labile carbon - but also where the effects of agronomic activities would be lessened.

3.4.3 Soil Ammonia Oxidizer Populations and Activity

Copies of amoA DNA were significantly greater in CC vs. RC plots at pre-fertilization (Figure 3.12). This was likely due to the different rhizosphere effects from preceding corn versus winter wheat under-seeded to red clover. Ammonia oxidizing bacteria (AOB) are well adapted to conditions found in cultivated soils (Zeglin et al., 2011) and previous studies have postulated that AOB communities are stable in relation to plant community composition; suggesting that AOB may be well adapted to agricultural soils where conditions are controlled to minimize variation (Mintie et al., 2003, Phillips et al., 2000). Continuous monocultures represent the extreme in terms of controlled cultivated soils and possibly create conditions optimal for AOB by limiting any temporal variation resulting
from crop rotation. In addition, cereal and legume mixes are known to alter AOB community structure at season-end (Song et al., 2007, Alvey et al., 2003) and these rhizosphere effects can extend into subsequent growing seasons (Guo et al., 2011). In this study, immediate effects from post-harvest residues were apparent with higher *amoA* DNA copies in CC after harvest (Figure 3.12). This supports the theory that AOB do best under minimal temporal biodiversity, as found in corn monocultures. Results from this study reaffirm literature findings and suggest AOB thrive under typical agricultural soils, particularly corn monocultures with high fertilizer N-inputs.

Interestingly, AOB population and activity were homogenous across depths in till plots at pre-plow and post-plow (Figure 3.13 and 3.14). Before plow events, till plots had significantly higher AOB abundance (DNA) relative to no-till plots at depths below 15 cm (Figure 3.13). This is likely due to regular nutrient addition to this layer through mechanical soil inversion and residue incorporation caused by moldboard plowing that distributed nutrients from surface layers to depths below. This trend was apparent after fall plow events, but above 15 cm the reverse trend was apparent with reduced AOB populations in till plots after moldboard plowing within the plow layer (0-15 cm) (Figure 3.13). Activity of AOB (cDNA) was not affected by moldboard plowing, but responding positively in no-till soils in the 0-5 cm layer (Figure 3.14). This study did not test residue effects, but it is thought that residues left unincorporated on the surface of no-till plots may have provided conditions conducive to AOB activity and abundance.

Populations of ammonia oxidizing archaea (AOA), measured via copies of *crenamoA* DNA, followed similar trends to the archaeal domain in general (*arch16S* DNA copies) (Fig 3.15-3.17). Unfortunately, differences in AOA activity were not discernible due to
low copy numbers of extracted *crenamoA* cDNA that were below detection limits (range of 0-100 copies g\(^{-1}\) dry soil, Data not shown).

AOA were more abundant where disturbances were minimized, specifically at depth (Figure 3.15) and in no-till plots (Figure 3.16). In general, AOA populations have been noted to increase with depth (Erguder et al., 2009), and this was consistent with general trends observed in this study- although only significant at pre-fertilization (Figure 3.15). No-till soils in this study also tended to harbor more abundant AOA populations (Figure 3.16). Interestingly, there was no significant difference in AOA populations between till and no-till at post-plow. This suggests that either corn harvest events influence populations more than tillage, masking plow effects temporarily (until early next season possibly), or alternatively that plow effects are delayed. Post-plow samples were collected late in the season under ambient temperatures approaching 0\(^{\circ}\) C (Table 3.4) so it is possible that communities reacted slowly with the full effects not apparent until the subsequent spring thaw – as suggested by pre-fertilization observations (Figure 3.16). Slower responses by AOA to treatment effects may be a result of a diverse community structure more adapted for natural (non-agricultural) conditions than AOB (Zeglin et al., 2011, Wessen et al., 2010a). For example, AOA populations were significantly greater in no-till RC plots at post-fertilization (Figure 3.17). This treatment mimics natural conditions best, as it included the most temporal biodiversity in its crop rotation and was never mechanically disturbed by any tillage events.

3.5 Conclusion
This study was unique in its comprehensive account of soil nutrients, soil microbial communities, ammonia oxidizers, and for taking into account both seasonal and depth factors. Spatial and temporal heterogeneity is often neglected in studies and rarely are both simultaneously accounted. Findings from this study indicate that fertilization and tillage affect soil microbial communities, whereas crop rotations have minimal effects, although this may be due to the relatively greater stresses from fertilization and tillage dictating soil microbial dynamics regardless of rotation. Interestingly, it seems that high soil inorganic nitrogen concentrations observed post-fertilization coincided with higher total bacterial and AOB activity, but not abundance. Fertilization was homogenously applied to all treatments in this study and thus a specific fertilization effect cannot be confirmed, as its effects were confounded with seasonal effects in this study. Regardless, it seems that bacterial and AOB abundance and activity were independent of each other under conditions of relatively higher soil inorganic nitrogen concentrations. Future studies, should more clearly address the effect of fertilization and experiments should be designed to evaluate it as a separate treatment. Generally, total archaeal and their respective ammonia oxidizing populations were more abundant in no-till soils. Tillage had both short and long-term effects on AOB abundance and activity, and particularly affected its total and active population distributions across depth. AOB total populations were less abundant in the plow layer (0-15 cm) immediately after tillage, but samples taken at other times suggest that tillage homogenized AOB population sizes across depths to 30 cm. The activity of AOB reacted similarly. AOA populations were more abundant at depth and results suggest this community did not adapt as well to agricultural conditions as AOB. Moreover, since no active AOA (cDNA copies) were detected, it seems likely that AOB were the primary ammonia oxidizing community in these soils.
Chapter 4: Long-term effects of different tillage and crop rotation practices on soil aggregation and microbial ammonia oxidizers during a growing season.

4.1 Introduction

Efficient agricultural best management practices (BMPs) maximize soil N-use efficiency while minimizing ecological damage and economic loss. It is important to understand how different management practices influence nitrogen transformations from immobile to mobile forms, especially in agricultural systems where high inputs of fertilizer nitrogen are required for optimum yields. Ammonia oxidation is a particularly important process, as it is the rate-limiting step of nitrification, producing nitrate as the end product. Nitrate is the most plant-available form of nitrogen, but it is also highly mobile in soil and can pollute groundwater via leaching and/or act as a precursor to denitrification; this in turn produces nitrous oxide, a greenhouse gas that is 310 times more potent than carbon dioxide (Environment Canada, 2010).

Ammonia oxidation is a microbial process performed by both soil bacteria and archaeal communities. Ammonia oxidizing bacteria (AOB), first discovered by Winogradsky (1890), were long thought to be the only ammonia oxidizing microbial community (Lang and Jagnow, 1986, Lettl, 1985). More recently the ammonia monooxygenase gene was discovered in archaea (Treusch et al., 2005, Venter et al., 2004,
Konneke et al., 2005). Early after this discovery it was observed that ammonia-oxidizing archaea (AOA) outnumbered AOB in a broad range of soils (Leininger et al., 2006). However, literature since then has been unable to determine whether the primary driver of ammonia oxidation is indeed the AOB (Di et al., 2010, Jia and Conrad, 2009a, Shen et al., 2008) or AOA (Wessen et al., 2011, Wessen et al., 2010a, Zhang et al., 2010, Offre et al., 2009, Prosser and Nicol, 2008). Ratios of AOA:AOB abundance seem to largely be dictated by pH (Gubry-Rangin et al., 2010), with AOA predominance especially high in low pH soils (Wessen et al., 2010a, Nicol et al., 2008, He et al., 2007). Lower ammonia concentrations in soils are also thought to favour AOA abundance (Wessen et al., 2011, Di et al., 2009, Erguder et al., 2009). Nicol et al. (2008) proposed that low soil ammonia levels may be the primary effect dictating AOA and AOB relative abundances, and that a low pH merely limits ammonia availability by increasing ionization to ammonium (NH$_4^+$). NH$_4^+$ requires more energy to oxidize than NH$_3$ due to its larger mass and reduced ability to diffuse across cell membranes (Nicol et al., 2008, Frijlink et al., 1992). This effect is not apparent in soils with more neutral pH, as NH$_3$/NH$_4$ exist in relative equilibrium. In these soils AOA:AOB abundance varies, but AOB activity is higher wherever there is N-input via fertilization (Di et al., 2010, Jia and Conrad, 2009a, Erguder et al., 2009, Shen et al., 2008).

Although microbial dynamics are constantly in flux, studies evaluating AOA and AOB have often focused more on spatial rather than temporal heterogeneity (Wessen et al., 2010a, Gubry-Rangin et al., 2010, Di et al., 2010, Offre et al., 2009) In agricultural systems this temporal focus is particularly important, because crop nutrient needs change throughout the growing season. Effective BMPs must ensure that times of higher crop need coincide with times of increased nutrient availability. Split applications of fertilizer
accomplish this to an extent, but other agronomic practices, such as tillage and crop rotation, may also have residual long-term effects throughout a growing season. In addition, while the effects of fertilization on AOA and AOB dynamics have been extensively studied (Wessen et al., 2011, Wessen et al., 2010a, Di et al., 2010, Jia and Conrad, 2009a, Erguder et al., 2009, Shen et al., 2008), the long-term effects of tillage and crop rotation on these communities have not. One of the few studies to assess the effects of tillage on AOB utilized most-probable number (MPN) estimates to quantify AOB activity (Phillips et al., 2000). However, MPN and other cultivation techniques capture less than 1% of bacteria (Alexander, 1977) and recent advances in quantitative molecular techniques, particularly quantitative and reverse-transcription polymerase chain reactions (qPCR and RT-PCR respectively) enable much more accurate analyses. To our knowledge, no study has utilized these techniques to analyze the seasonal effects of different tillage and crop rotation systems on AOA and AOB abundance and activities.

In this study, four crop rotations and two tillage treatments were studied in Elora, Ontario, throughout a corn-growing season. Microbial samples were collected before and after both spring tillage/fertilization and fall harvest/plow events. In addition, samples for water-stable aggregate analysis were collected in August. The objectives of this study were to identify the effects of tillage and crop rotation on soil aggregation, and on AOB and AOA abundances and activities over a growing season.

4.2 Methods

4.2.1 Experimental Design

This study was conducted in long-term experimental trial established in 1980 and located at the University of Guelph’s Elora Research Station (ERS) in Elora, Ontario (43°39' N,
80°25′ W, elevation 376 m). For more details on the experimental design please refer to Meyer-Aurich et al. (2006). The soil is classified as an imperfectly drained Guelph silt loam with an average pH of 7.6 (Smith, 2007, Canadian Agricultural Services Coordinating Committee, 1998).

The trial was arranged as a randomized split-plot design with four replications (Figure 3.1) with seven crop rotations (main-plots) and two tillage treatments (split-plots). Data collection occurred during the growing season of 2010 on each replicate of 4 selected treatments with different crop and tillage systems. Crop rotations analyzed included continuous corn (CC), and four-year rotations of corn-corn-soy-soy (CS), corn-corn-soy-winter wheat (CSW), and corn-corn-soy-winter wheat with a red clover cover crop under-seeded to the winter wheat (RC). Each rotation was split into till and no-till. Tillage included spring cultivation prior to planting operations and fall moldboard plowing. In 2010, all rotations were planted in their first year of corn. All plots received the same fertilizer input. This consisted of 157 kg N ha\(^{-1}\) of 5-20-20 applied at planting on May 7\(^{th}\), and a second application of urea-ammonium-nitrate (UAN) on June 18\(^{th}\) (420 L of 28-0-0, 150 kg N ha\(^{-1}\)) side-dressed in a band between the corn rows.
Figure 4.1: Experimental design of long-term field plots. Crop rotations (main plots) were arranged in a randomized split-plot design with four replications. Till and no-till treatments (split-plots) were grouped in pairs within different crop rotations.
4.2.2 Water Stable Aggregates

Soil samples for water stable aggregate analysis were only collected on August 13th, 2010 in all experimental plots to a depth of 5 cm with a 5 cm diameter soil corer. Water stable aggregates were determined by methods outlined by Nimmo and Perkins (1986) and Kemper et al. (1985). Briefly, 10 replicates of 4 g air-dried soil samples were sieved to a 2-4 mm size fraction and placed in a 250 µm sieve that oscillated up and down into a water filled vat for 3 minutes. Particles remaining after the process were weighed and the % of water stable aggregates was determined on a dry-weight basis.

4.2.3 Soil Sampling for Microbial Analyses

Refer to Chapter 3: 3.2.2. Samples were collected at the same times, but only in the 0-5 cm layer.

4.2.4 Nucleic Acid Extraction and Preparation

Refer to Chapter 3: 3.2.3

4.2.5 Quantitative Polymerase Chain Reactions

Refer to Chapter 3: 3.2.4

4.2.6 Cloning

Refer to Chapter 3: 3.2.5

4.2.7 Statistical Analysis

Refer to Chapter 3: 3.2.6
4.3 Results

4.3.1 Water-Stable Aggregates

The percentage of water-stable aggregates (WSA %) in the 2-4 mm size fraction was greater in no-till plots (P<0.05) (Figure 4.2). No significant difference in WSA % was observed between different crop rotations.
Figure 4.2: Mean percentages of water stable aggregates (WSA) of the 2-4 mm size fraction from soil sampled in 2010 from the following crop rotations; continuous corn (CC), corn-corn-soy-soy (CS), corn-corn-soy-wheat (CSW), and corn-corn-soy-wheat under-seeded to red clover (RC). Data points represent mean gene copies aggregated across two tillage treatments. Error bars represent calculated standard errors (n= 8, α= 0.05).
4.3.2 Soil Microbial Abundance and Activity

Fragments of \textit{bac16S} and \textit{arch16S} DNA and cDNA (reverse-transcribed from collected RNA) were successfully amplified and quantified by qPCR on all sample dates, although not all experimental plots were necessarily amplified within some dates; this related to procedural disruptions caused by soil humic acids inhibiting gene expression during PCR amplification. Copy numbers of \textit{bac16S} and \textit{arch16S} DNA and cDNA followed similar seasonal trends (Figure 4.3 and 4.4, respectively). DNA copies tended to decrease from pre-fertilization to pre-plow, but after fall harvest and plow events copy numbers increased and reached seasonal highs for \textit{arch16S} (Figure 4.3). \textit{Arch16S} DNA copy numbers were significantly lower than \textit{bac16S} DNA copy numbers at all sample dates and varied between 7.5-14.2\% in relative abundance. Seasonal \textit{bac16S} and \textit{arch16S} cDNA copies were highest at post-fertilization, although only significantly for \textit{bac16S} (P<0.05) (Figure 4.4). \textit{Bac16S} cDNA copies were higher in no-till plots before (P=0.0573) and after fall (P<0.05) moldboard plow events (Figure 4.4). \textit{Arch16S} cDNA copies did not vary between tillage treatments. The relative abundance of \textit{arch16S:bac16S} cDNA copy numbers was highest at pre-fertilization (30.7\%), but then decreased and remained relatively constant throughout the rest of the season (Figure 4.4).
Figure 4.3: Mean seasonal *bac16S* and *arch16S* DNA copies g$^{-1}$ dry soil sampled in 2010. Data points represent mean gene copies aggregated across two tillage treatments and four crop rotations. Error bars represent calculated standard errors (n= 32, α= 0.05) and percentages indicate the relative abundance of *arch16S:bac16S* DNA at each sampling time.
Figure 4.4: Mean seasonal *bac16S* and *arch16S* cDNA copies g$^{-1}$ dry soil sampled in 2010. Data points represent mean gene copies in two tillage treatments and four crop rotations. Error bars represent calculated standard errors (n= 32, $\alpha= 0.05$) and percentages indicate the relative abundance of *arch16S:* *bac16S* cDNA at each sampling time.
4.3.3  *Soil Ammonia Oxidizer Abundance and Activity*

DNA copies of the ammonia monoxygenase gene subunit A (*amoA*) were higher at pre-fertilization and post-fertilization compared to later in the season (P<0.05) (Figure 4.5a). Copies of *amoA* cDNA were significantly higher at post-fertilization (Figure 4.5b). Both *amoA* DNA and cDNA copies were higher in no-till relative to till plots after fall moldboard plowing (P<0.05) (Figure 4.6). Seasonal abundance of AOA were consistently higher than AOB, which had DNA copy numbers that ranged from 2.98-7.56% relative to AOA DNA copies. DNA copies of the crenarchaeal ammonia monoxygenase gene subunit A (*crenamoA*) tended to decrease as the season progressed, but then increased after fall plow and harvest operations reaching seasonal highs (P<0.05) (Figure 4.5a). Copies of *crenamoA* cDNA were below detection limits, precluding statistical analyses.
Figure 4.5a: Mean seasonal amoA and crenamoA DNA copies g\(^{-1}\) dry soil sampled in 2010. Data points represent mean gene copies aggregated across two tillage treatments and four crop rotations. Error bars represent calculated standard errors (n= 32, α= 0.05) and percentages indicate the relative abundance of amoA:crenamoA DNA at each sampling time.
Figure 4.5b: Mean seasonal amoA cDNA copies g\(^{-1}\) dry soil sampled in 2010 in long-term treatments. Data points represent mean gene copies aggregated across two tillage treatments and four crop rotations. Error bars represent calculated standard errors (n= 32, α=0.05).
Figure 4.6: Mean DNA and cDNA copies of *amoA* in till and no-till plots before and after fall harvest and applicable plow events. Data points represent mean gene copies aggregated across four crop rotations. Error bars represent calculated standard errors (n=16, α=0.05).
4.3.4  *Corn Grain Yields*

The CC plots yielded significantly less grain than other rotations (P<0.05) (Figure 4.7). Positive tillage effects on yields were apparent in CC and CS, but not in RC and CSW plots (P<0.05) (Figure 4.7).
Figure 4.7: Mean corn grain yields in 2010 in the following crop rotations; continuous corn (CC), corn-corn-soy-soy (CS), corn-corn-soy-wheat (CSW), and corn-corn-soy-wheat under-seeded to red clover (RC). Error bars represent calculated standard errors (n=4, α=0.05).
4.3.5 Seasonal Soil Moisture and Ambient Temperature

Refer to Chapter 3: 3.3.4 (Table 3.4).

4.4 Discussion

4.4.1 Water Stable Aggregates

Tillage has long been associated as a mechanism reducing soil aggregate stability (Boone et al., 1976). Exactly how tillage reduces aggregate stability is less clear (Andruschkewitsch et al., 2013). Results from this study indicate that the % of water stable aggregates (WSA %) within the 2-4 mm fraction were significantly greater in no-till plots regardless of crop rotation (Figure 4.2). This supports previous literature findings on the destructive impact of tillage (Jacobs et al., 2011, Boone et al., 1976). However, other research has theorized that biomass input may be the primary predictor of WSA % (Andruschkewitsch et al., 2013). In other studies, tillage acted as a vertical distributive agent and reduced the relative biomass input to surface soils by distributing it across depths (Deen and Kataki, 2003a, Hernanz et al., 2002, Buchanan and King, 1992, Oades, 1984). Jacobs et al. (2011) found that increased WSA % in surface soils was directly correlated with higher concentrations of organic-carbon containing residues in surface soils. This implies that residue placement, quality, and quantity are all important factors determining WSA %. In this study, there was no difference in WSA % between no-till and till plots that were previously cropped in wheat or wheat and red clover, despite their significantly different C:N ratios (Appendix A: Table A.2). No-till plots that were previously cropped in corn or soy did tend to have slightly higher WSA % compared to tilled plots (P<0.05) (Figure 4.2). Since these plots were fertilized (150 kg N ha\(^{-1}\)), the
total carbon input from crop residues, rather than residue C:N ratio, may be a more important determinant of residue decomposition dynamics and resulting aggregate formation. Even though wheat residue has a much higher C:N ratio than corn, corn generally yields about twice as much residue as wheat and after harvest events adds significantly more labile organic carbon to soil for decomposition and aggregate formation (Kludze et al., 2010). Total labile carbon additions in this study were calculated based on previous year’s crop yields, harvest indices (grain:residue ratios), and C:N ratios (Table A.2). The slight increase in WSA % found in no-till corn plots is likely because of the significantly higher amount of surface residue input in that rotation. However, as reported above crop residues had much less of an impact on WSA % than tillage.

4.4.2 Soil Microbial Abundance and Activity

In this study qPCR targeted the 16S rRNA subunit gene for bacteria and archaea, and the observed DNA copy numbers were used as a proxy for total abundance of each domain. Similar seasonal trends were observed for both domains, with higher copy numbers at the beginning and end of the growing season. Observed copy numbers were in accordance with previous literature findings (Pereira et al., 2012, Wessen et al., 2010b). Interestingly, abundance for either domain did not correlate with WSA % and no differences were observed between no-till and till plots (Data not shown). Evidence from this study does indicate that 16S DNA copies were negatively affected by inorganic N addition (Figure 4.3). Both bac16S and arch16S DNA copy numbers decreased in abundance from pre-fertilization to post-fertilization and again to pre-plow sample dates, but then increased significantly at post-plow events, but only significantly for arch16S DNA (Figure 4.3). High copy numbers at the beginning and end of the growing season correlate with the
availability of labile organic carbon. At the end of the season crop residues after fall harvest events would provide significant organic carbon input. High copy numbers at the beginning of the season were likely due to leftover carbon in frozen soils becoming available after spring thaw. Cell lysis resulting from spring thaw could have also have increased nutrient availability and microbial abundance at this time (Herrmann and Witter, 2002). Smith et al. (2010) posited a similar scenario at the same location, specifically that a nutrient flush during spring thaw lead to increased microbial diversity of denitrifiers.

The activities of archaea and bacteria in this study were also measured with qPCR that targeted the 16S rRNA subunit gene, but rather than DNA, RNA copies were measured after being reverse-transcribed via PCR to complementary DNA (cDNA). Activities for bacteria and archaea followed similar trends, but were different from the trends observed for their respective total population abundances. Specifically, activity of both domains reached seasonal highs at post-fertilization and lows at post-plow (Figure 4.4). This aligns with previous research showing increased bacterial activity after nitrogen input (Yevdokimov et al., 2012), but conflicts with research suggesting archaea are not affected by soil nutrient additions (Wessen et al., 2011) or are actually oligotrophic (Wessen et al., 2010a, Gubry-Rangin et al., 2010, Erguder et al., 2009).

Interestingly, bacterial activity was lower in tilled plots before and after fall harvest and plow events as compared to no-till plots (Figure 4.4). This suggests that tillage effects could last longer than one year, but are masked by other agronomic events, as tillage effects were not observed earlier in the season; no difference in bacterial activity in till and no-till plots were observed at pre-fertilization or at post-fertilization (Figure 4.4). This implies that deleterious tillage effects on bacterial activity were negligible compared to the positive effects from spring thaw and fertilization events. In addition, moisture did not
significantly vary between till and no-till plots at any sample time (Data not shown), but WSA % was higher in no-till plots (Figure 4.2). Thus, it seems likely that better soil aggregation in no-till plots were indicative of better conditions for bacterial activity. However, in this study WSA % were determined from samples collected in August about 2 months after post-fertilization samples (June 25th) and 2 months before pre-plow samples were collected (October 12th). Previous work has shown that WSA % can be seasonally variable (Cosentino et al., 2006), therefore, it is possible that soil aggregation was different earlier in the season contributing to different conditions for bacterial activity.

4.4.3 Soil Ammonia Oxidizer Abundance and Activity

Utilizing qPCR, the ammonia monoxygenase gene in bacteria (amoA) and archaea (crenamoA) were targeted as proxies to enable quantification of ammonia oxidizing bacterial (AOB) and archaeal (AOA) communities. Observed DNA copy numbers indicated total abundance and reverse-transcribed RNA copies (cDNA) indicated activity. AOA populations (DNA copies) were on average 20-30 fold more abundant than AOB (Figure 4.5a) and were in range of reports from previous studies (Wessen et al., 2010a, Jia and Conrad, 2009a, Hallin et al., 2009, Shen et al., 2008, Chen et al., 2008, He et al., 2007, Leininger et al., 2006). AOB were most abundant early in the season at pre and post-fertilization, whereas AOA were highest later in the season at pre and post-plow (Figure 4.5a).

Despite the relatively high populations of AOA, no activity was detectable. This suggests that AOA communities were not driving soil ammonia oxidation (Data not shown). Previous studies conflict with this study’s findings and determined that AOA were the primary drivers of soil ammonia oxidation (Wessen et al., 2010a, Hallin et al., Leininger et al., 2006).
However, these studies did not measure the transcriptional activity of AOA (crenAMO cDNA) or AOB (amoA cDNA) and instead correlated the relative abundances of AOA to AOB with potential ammonia oxidation rates to determine which community was the primary driver of soil ammonia oxidation. Yet even with this approach, AOA would not have been considered the primary ammonia oxidizer in this study, as the ratio of AOA:AOB abundance actually decreased at post-fertilization when seasonal inorganic nitrogen levels were highest (Figure 4.5a). Although potential ammonia oxidation was not measured directly in this study, AOB activity was significantly higher at post-fertilization (P<0.05) (Figure 4.5b). This higher transcriptional rate is indicative of increased soil ammonia oxidation by AOB and suggests it was the primary community driving soil ammonia oxidation when seasonal NH₄ levels were highest.

AOB abundance remained at similar levels pre and post-fertilization, unlike observed AOB activity (Figure 4.5ab). This indicates that AOB can increase transcription rates in response to increased nutrient availability without necessarily increasing total abundance. If AOB activity and abundance are independent, it is also possible that each is limited by different parameters.

Soil moisture was significantly higher in pre-fertilization vs. post-fertilization samples (Table 3.4), but previous work has attributed minimal effects of soil moisture on AOB (Szukics et al., 2012). It may be that the higher water content in the spring was relatively higher in nutrients from previous crop residues; dissolved nutrients and insoluble organic materials carried in soil water would contain a variety of nutrients essential for cell function (Herrmann and Witter, 2002). Innerbner et al. (2006) observed significantly greater increases in soil AOB populations after nutrient input from composted material compared to inorganic N addition from mineral fertilizers. Thus, non-nitrogen nutrient
limitations at post-fertilization may have limited AOB population size while activity was simultaneously increased by inorganic N addition. This is further supported by observations of higher AOB abundance and activity after fall harvest compared to pre-plow in no-till plots only (Figure 4.6). After harvest, crop residues would have been spread on the soil surface. In till plots these residues would have been incorporated across depths. In no-till plots the nutrient input from the residue was concentrated in the surface soils and AOB were likely able to better utilize these nutrients to increase in population size and activity. The significantly lower AOB abundance observed at post-plow compared to pre-fertilization was likely a temperature effect (Figure 4.5, Table 3.4).

4.4.4 Corn Grain Yields

Corn grain yields in 2010 followed expected patterns for southern Ontario with continuous corn monocultures yielding less than other rotations, regardless of tillage treatment (Figure 4.7) (Katsvairo and Cox, 2000). No-till yields are usually less in southern Ontario because of excessive soil moisture in the spring resulting in delayed crop emergence (Deen, 2010). Interestingly, no negative effect from no-till was observed in the corn-corn-soy-wheat or corn-corn-soy-wheat under-seeded with red clover rotations (Figure 4.7). These rotations also yielded significantly more grain compared to tilled continuous corn, no-till continuous corn and no-till corn-corn-soy-soy rotations. In 2010 of this study, there seems to be a clear benefit of corn following wheat in no-till operations. This conflicts with previous research in the region detailing negative effects on yields in no-till corn following wheat (Kravchenko and Thelen, 2009, Opoku and Vyn, 1997). However, the 2010 growing season was particularly optimal with an early start and record yields reported across the region (Deen, 2010).
Under-seeded red clover had minimal effect on yields. Any nitrogen credit from previous red clover was probably moot since these systems were fertilized at times corresponding to crop need; specifically, at planting and at the 6-leaf stage of corn (Deen, 2010). Thus, soil-conditioning benefits from preceding wheat could have had a larger impact on subsequent yields. Previous research indicates that belowground biomass from wheat can have a positive effect on subsequent crop yields (Legere et al., 2011, Katsvairo and Cox, 2000). In contrast, yield losses in crops following wheat have been attributed to negative effects from residue, however appropriate fertilization has also been proven to alleviate these effects (Kravchenko and Thelen, 2009).

4.5 Conclusion

This study was unique in its approach to quantify the abundances and activities of soil bacteria and archaea and their respective ammonia oxidizing communities in an agricultural soil across an entire growing season. In addition, the experimental fields have been managed under similar practices for 30 years. Thus, this study was able to identify long-term treatment effects. Crop rotations had minimal effect on soil microbial communities and specifically ammonia oxidizers, although there were significant yield advantages to corn following wheat versus corn or soy. Tillage effects were more pronounced and reduced aggregate sizes. Interestingly, fall plow did not directly affect AOB, but AOB abundance and activity were higher in no-till plots at post-plow; likely this was due to residue effects, but this study was unable to confirm this. Future studies should include measures of ammonia oxidation rates to determine how well community abundances and activity correlate with observed microbial dynamics.
Chapter 5: Conclusions and Recommendations

5.1 Conclusions

This thesis aimed to evaluate the seasonal effects of different tillage and crop rotation practices on soil bacteria and archaea, and their respective ammonia oxidizing communities. Targeting both total and active populations during a growing season and across soil depth enabled analysis of both short and long-term effects. In addition, this field study was unique, as the experimental plots have been managed under identical practices for 30 years.

Season and tillage had greater effects on microbial dynamics compared to crop rotations. At post-fertilization, increased total bacteria and AOB activities were evident, but interestingly their total populations were not similarly affected. This suggests AOB were the primary driver of ammonia oxidizing in these soils and that bacterial activity is independent from its abundance.

Tillage reduced aggregate stability and affected archaea and bacteria differently. Long-term effects from tillage on total archaea and AOA decreased populations consistently throughout the season. AOB populations were negatively affected by tillage, but only right after fall plow events and not at other times during the season. Tillage did tend to homogenize AOB populations across depths and this was observable throughout the season. Data on the activity of AOB from this study suggest that they were the primary ammonia oxidizer in these soils, even though AOA were consistently more abundant.

Crop rotations seemed to have minimal effect on soil microbial dynamics, although this may be due to the relatively greater effects from fertilization and tillage dictating soil microbial dynamics regardless of rotation. Crop rotations did result in significant yield
advantages to corn following wheat versus corn or soy. In addition, no-till corn following wheat (with or without under-seeded red clover) had similar yields to till corn of the same rotation. Tilled corn following soy or corn was significantly higher than the same rotations in no-till. This indicates that previous wheat crops ameliorate crop yield losses common to no-till corn plantings in southern Ontario.

5.2 Future Research

Abundances and activities measured in this thesis provided valuable information, however there are limitations to using molecular quantitative techniques. Future studies should correlate targeted DNA and RNA gene copy numbers with process rates. For AOA and AOB, process rates that should be determined are potential nitrification rates, ammonia oxidation rates, and NO₃ leaching. Regression analyses between and amongst these variables will determine the relative contributions of AOA and AOB to these processes and enable quantification of treatment effects. Finally, in this study it was determined that AOB were likely the primary microbial community involved in ammonia oxidation based on observed activity dynamics. In addition, their activity was almost exclusively in the 0-5 cm layer. Future studies should prioritize analyzing this depth and this community.
Bibliography


Deen, B., 2010. personal communication.


Appendix A

Table A.1: Theoretical determination of soil inorganic nitrogen concentration across a hectare furrow slice (haf = 0-15 cm) after fertilization with Urea-Ammonium Nitrate (28-0-0) at 150 kg N ha\(^{-1}\). Assumes applied nitrogen is concentrated above 15 cm soil depth.

<table>
<thead>
<tr>
<th>Fertilizer</th>
<th>Urea-Ammonium-Nitrate (28-0-0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N concentration</td>
<td>28%</td>
</tr>
<tr>
<td>Application rate</td>
<td>150 kg N ha(^{-1})</td>
</tr>
<tr>
<td>Mass of soil</td>
<td>2,000,000 kg ha(^{-1})</td>
</tr>
<tr>
<td>Theoretical inorganic N increase</td>
<td>80 mg kg(^{-1})</td>
</tr>
</tbody>
</table>
Table A.2: Mean 2009 crop yields in experimental plots with calculated aboveground residue inputs. Harvest indices and C:N ratios are based on data obtained from Kludze et al. 2010.

<table>
<thead>
<tr>
<th>Rotation</th>
<th>Continuous Corn</th>
<th>Corn-Corn-Soy-Soy</th>
<th>Corn-Corn-Soy-Wheat</th>
<th>Corn-Corn-Soy-Wheat (RC*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009 Crop</td>
<td>Corn</td>
<td>Soy</td>
<td>Wheat</td>
<td>Wheat (RC*)</td>
</tr>
<tr>
<td>Yield (kg ha⁻¹)</td>
<td>7933</td>
<td>2456</td>
<td>4952</td>
<td>5384</td>
</tr>
<tr>
<td>Harvest Index</td>
<td>1:1</td>
<td>1:1</td>
<td>1:1</td>
<td>1:1</td>
</tr>
<tr>
<td>Residue Input (kg ha⁻¹)</td>
<td>7933</td>
<td>2456</td>
<td>4952</td>
<td>5384</td>
</tr>
<tr>
<td>Residue C:N</td>
<td>60:1</td>
<td>12:1</td>
<td>80:1</td>
<td>80:1</td>
</tr>
</tbody>
</table>

*RC = under-seeded red clover cover crop.