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EFFECTS OF MANAGEMENT ON SELECTED SOIL PROPERTIES AND NITROUS OXIDE FLUXES IN DAIRY CROPPING SYSTEMS

A Thesis in

Soil Science

by

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ABSTRACT

This thesis investigates selected soil properties and management decisions and their effect on nitrous oxide (N_2O) emissions from agricultural soils. Nitrate, an inorganic form of N, is extremely mobile in soils, making it susceptible to loss through processes like denitrification. Denitrification is an anaerobic microbial process that reduces nitrate to N_2 or incompletely to N_2O, a potent greenhouse gas. The experimental site for this research was the Sustainable Dairy Cropping System (SDCS) located at Penn State's Agronomy Farm. Chapter one is a review of the literature on nitrogen (N) cycling in agriculture, N loss pathways and the management and environmental factors affecting denitrification. This process is driven by soil properties, nitrate availability, and other factors. A prior study in this experiment in 2015 and 2016 found that the driving factors for N_2O emissions in some of the same treatments were explained by days after manure application, growing degree days (GDD), and manure rate.

Research on the effects of prior crop and management on N_2O emissions in a typical PA dairy cropping system is described in chapter two. Labile carbon, total carbon, inorganic N species, and other environmental data were measured to determine their impact on measured N_2O fluxes in 2017 and 2018. However, the measured soil and environmental properties in this experiment were not able to explain the observed patterns in N_2O emissions through a regression analysis. The highest N_2O fluxes were measured in 2018 in Corn after two years of Alfalfa (*Medicago sativa)* + Orchardgrass (*Dactylis glomerata*). Cumulative emissions were more than six times higher than those measured in treatments without a winter cover in the same year.

Based on findings in 2017, chapter three investigates the impact of termination timing of Alfalfa+Orchardgrass on spring N_2O fluxes and soil properties in 2018. This management decision is becoming more popular in the Northeast as spring conditions become wetter, making the proper timing of spring management events difficult. The findings from this experiment are

promising for farmers interested in adopting this management practice as yields did not significantly differ from the subsequent corn crop and although they did not significantly differ, spring cumulative emissions from the spring terminated treatment were more than three times those from the fall terminated treatment. Because N_2O emissions were not measured in the fall, however, the comparison of the two treatments in this study was not comprehensive.

Chapter four described an investigative study on redox potentials in unsaturated agricultural soils. Equipment constraints and spatial variability made understanding and interpreting these results difficult. There were diurnal trends exhibited in some treatments, reflecting diurnal changes in soil moisture but not others. There also seemed to be stratification in depth, although this trend also differed across treatments. Overall, there is evidence that different crops can facilitate different redox environments and in turn, different microbial processes. However, more research and equipment advances need to take place before redox potential could be considered a useful indicator of microbial processes in unsaturated soils.

Finally, the conclusions summarized the major findings of each of these experiments and discussed the impact of sustainable management practices on improving soil resiliency. Implementing sustainable practices like cover cropping and no-till can improve soil, although trade-offs of higher N_2O emissions may result. Further research on these practices and their impact on soil properties is necessary as the effects of climate change are becoming more apparent.

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Chapter 1

Introduction and Literature Review

With the development of synthetic fertilizers and their widespread commercialization after WWII, farmers were given the opportunity to specialize in the aspect of agricultural production they were best suited based on their regional location. For example, farmers in the Midwest no longer had to raise livestock to produce manure to meet their crop needs. Instead, many have been able to specialize in crop production, leaving a larger portion of livestock husbandry to other parts of the country (Lanyon, 1995). This disconnect still exists and has led to an excess of manure in animal operations and the general rise of factory farming, as improved transportation has also lessened the need for on-site feed production. At these farms, because of the large number of animals in comparison to site acreage, an excess of manure exists and general shortage of land/crops to apply it to.

Nutrient Management

The mobility of nitrogen as a component of manure, as well as site over application, has led to a nitrogen imbalance issue in parts of the US, especially in the eastern states (Driscoll et al., 2003). In maintaining a healthy agricultural soil, a large management concern is maintaining the proper nutrient balance to meet crop needs but avoiding excess application. Nutrient over application leads to leaching of nitrogen into drinking water and other sources, runoff, as well as potential intervention from government organizations as failure to follow established nutrient management plans are subject to fines and other penalties in most states. Their importance has increased in nutrient “problem areas” like the Chesapeake Bay watershed. Contamination of the bay has led to eutrophication and inherent loss of seagrasses, as well as other ecologically damaging issues (Boesch et al., 2001). To inhibit nutrient losses, farmers have been implementing different manure application methods, utilize nitrification inhibitors, and optimize proper timing of fertilizer applications, all with varied levels of success.

By incorporating more sustainable agricultural practices, farmers can minimize losses of nutrients, like nitrogen, to the environment while also improving soil quality, or soil health. This chapter describes the nitrogen cycle, agricultural conservation practices, and how nitrogen cycling processes, especially denitrification, are affected by management practices.

Nitrogen in Agriculture

The primary component in our atmosphere is nitrogen gas (N_2), making up 79% of dry air. The oxide of nitrogen, N_2O, is a greenhouse gas with almost 300 times the ability of carbon dioxide (CO_2) to trap heat in the atmosphere (USA EPA, 2016). Agriculture is the largest contributor of atmospheric N_2O due to losses in multiple aspects of farming operations, including amended agricultural fields, animal production, as well as transported nitrous oxide into ground or surface water through runoff (Mosier et al., 1998). The addition of nitrogen through soil amendments or fertilizer applications is critical for increasing, or even maintaining crop yields, because sufficient amounts of biologically available N to sustain crop growth is not present in most soils. New nitrogen enters cropping systems primarily through organic amendments (management inputs), N fertilizers (inorganic), or biological N_2 fixation. Organic and inorganic forms of N differ based on their availability to the crop. Organic N, contained within decaying crop residues and soil organic matter, cannot be taken up directly by plants, while inorganic N is readily available. By incorporating a leguminous cover crop in a cash crop rotation, atmospheric nitrogen (N_2) can undergo biological fixation and be made available to the subsequent crop through symbiotic relationships as well as free-living microorganisms (Robertson and Vitousek, 2009). The option more popular with conventional farmers is applying synthetic fertilizers (inorganic) because of the low cost involved with purchasing, transporting, and applying the product relative to the subsequent increase in yields, as well as the costs associated with hauling manure (organic) if it is not already on-site. The application of N in a readily available form with synthetic fertilizers is also more desirable than a delay in N availability associated with the microbial process of converting organic N into a plant available form (mineralization). The development of synthetic fertilizers has replaced the need for biological N_2-fixing crops, while also contributing nutrients like Phosphorous, Potassium, as well as secondary nutrients. (Robertson and Vitousek, 2009).

Nitrogen Cycling

Forms of N

As previously stated, not all forms of N are available for plant uptake. Inorganic forms are readily available while organic forms must undergo mineralization to be made available. Sources of organic N include animal manures and crop residues. The two main forms of inorganic N, nitrate and ammonium, are applied through synthetic fertilizers. Manures also provide ammonium N and usually very little nitrate. Nitrogen can also enter the soil through biological nitrogen fixation by legumes. These crops contain rhizobia, a symbiotic bacterium, within their root systems and fix atmospheric nitrogen (N_2) into ammonia (NH_3) (Kuypers et al., 2018).

Many processes within the nitrogen cycle are microbially mediated so it is important for the right soil conditions to be present to encourage N retaining processes. However, even with proper application rates, placement, and timing, applied N is still susceptible to loss through leaching, runoff, microbially-mediated processes, or chemical processes like volatilization.

Volatilization

Ammonium (NH_4^+), one form of plant-available N, is susceptible to gaseous loss to the atmosphere through the process of volatilization. The conversion of ammonium to ammonia gas (NH_3) through volatilization tends to occur after fertilization in conditions where both pH and ammonium concentrations are high. This commonly occurs when organic N is applied in the form of animal manures or urea (Killpack and Buchholz, 1993). Higher losses can also occur when liquid manure or synthetic fertilizers are surface applied in dry soil conditions (Robertson and Vitousek, 2009). The release of this gas to the atmosphere decreases the available nitrogen fraction from manure, or synthetic fertilizer, to be utilized by crops.

Mineralization and Nitrification

Mineralization is the microbially mediated conversion of organic N from soil organic matter (plant residues, manure, etc.) to an inorganic form available to plants. The first step is ammonification, where organic N is converted to ammonium, one of the two main forms of inorganic N. Ammonium can either be taken up by crops, retained on soil cation exchange sites, or oxidized to nitrate, another form of inorganic N, through nitrification (Paul, 2014). Microbes can also immobilize nitrogen to satisfy their own demand, reducing the supply available to crops. Because nitrate is negatively charged (NO_3^-), it is not attracted to negatively charged clay or organic matter and is extremely mobile (Lamb et al., 2014). Although the nitrification process increases the mobility of nitrogen in the soil and movement to plant roots, this also increases the potential of leaching and loss of nitrate if not immediately taken up by the crop as nitrate is water soluble.

Denitrification

Denitrification is a microbial process that reduces nitrate to N_2 or incompletely to N_2O, a known greenhouse gas. This process is known to occur under saturated, or anaerobic conditions (Tiedje, 1988). Although agricultural soils are typically unsaturated, denitrification can occur within saturated microsites, such as decomposing litter, soil aggregates, and rhizospheres.

The denitrification process generates ATP, or energy, for heterotrophic bacteria, and can be inhibited by low water content in the soil, nitrate supply, or low availability of electron donors, namely reduced carbon. Many species of bacteria are known to carry out complete denitrification and they can represent up to 20% of the microbial biomass within soil. Carbon availability, in addition to nitrate, determines denitrification potential in unsaturated soils. Carbon is a source of donor electrons for denitrifiers and promotes the development of anaerobic conditions by stimulating oxygen consumption by heterotrophs (Paul, 2014).

Complete denitrification is the reduction of nitrate to N_2 gas and requires denitrifying organisms to have all enzymes involved in each reduction step (Figure 1-1). "Incomplete denitrifiers" can reduce nitrate to nitrite but may not carry out further stepwise reductions of nitrite to NO, N_2O, or N_2. Since denitrification enzymes are induced by anaerobic conditions, a lag time can occur before the emission of gaseous denitrification products, N_2O being the most concern for global warming, because N_2 gas is inert (Paul, 2014).

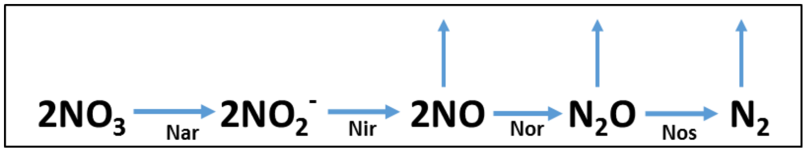

Figure **1-1**. The denitrification process and gaseous intermediates or products (represented by vertical arrows). Corresponding enzymes are denoted below the horizontal arrows. Adapted from Paul, 2014.

There is also the potential for N_2O emissions from soils under oxygenated conditions through nitrification. Ammonia oxidizing organisms oxidize ammonia (NH3) to nitrite (NO_2) as their primary energy metabolism. These organisms involved in the nitrification process are also able to produce NO via NO_2 reduction, resulting in the production of N_2O gas. This is a result of NO_2 being used as an electron acceptor in limited oxygen conditions (Paul, 2014). Coupled nitrification-denitrification is also known to occur under specific soil conditions. This is when nitrate or nitrite produced by nitrification is used by denitrifying organisms (Wrage et al., 2001).

Ravishankara et al. (2009) referred to N_2O as “The Dominant Ozone-Depleting Substance Emitted in the 21st Century”. This study refers to the similarities between N_2O and Chlorofluorocarbons (CFCs), as they both are very stable in the troposphere and are transported to the stratosphere where they destroy the ozone. However, N_2O is not as strictly regulated as a CFC, although there are both anthropogenic and natural sources of concern. This justifies the study for further understanding the conditions that encourage N retention and N-conserving processes, like Dissimilatory Reduction to Ammonium (DNRA).

DNRA

Dissimilatory reduction of nitrate to ammonium (DNRA) is a newly discovered process in the nitrogen cycle, relative to its counterparts, and is vastly understudied. This process is highly dependent on soil conditions, as denitrification and DNRA are competing processes for available nitrate in the soil. Unlike denitrification, the emissions of N_2O associated with this process are negligible. This makes DNRA, or Nitrate Ammonification (NA) the more favorable fate of nitrate when considering the impact of agriculture on the accumulation of greenhouse gases in the atmosphere (Rütting et al., 2011). Nitrate taken up by microbes involved in this process is synthesized and returned to the soil as ammonium, another biologically available source of nitrogen for plants (Figure 1-2).

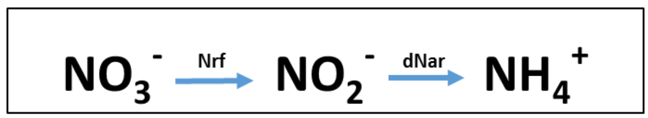

Figure **1-2**. The process of DNRA and the corresponding enzymes (denoted above the horizontal arrows).

NA encourages the retention of nitrate in the soil, a favorable outcome, however, establishing advantageous conditions for this process is ambiguous due to the minimal research done on this process in the context of agricultural soils. Table 1-1 is a summary of studies adapted from Rutting et al. (2011) where rates of Nitrate Ammonification are reported and the ecosystems in which the studies took place.

Table **1-1**. A summary of studies including rates of NA, NA:NO_3 consumption, and number of studies in selected ecosystems. Adapted from Rutting et al., 2011.

Ecosystem	**NA Rate (µg N g^{-1} soil day^{-1})**	**NA: NO_3 consumption**	**Number of Studies**
Riparian Environment	0.83	2.8	2
Arable Field	0.0-0.3	0.0-6.3	1
Temperate Forest	0.26	37.66	8
Subtropical Forest	0.02	6.93	4
Tropical Forest	0.70	40.34	9
Temperate Grassland	0.126	42.8	6

Few studies have quantified rates of DNRA in unsaturated soils, as it was once thought that this process only occurred in saturated conditions. However, recent reports have shown significant rates of DNRA in terrestrial ecosystems and in one case, found rates of DNRA 70 times greater than denitrification in humid tropical forest soils (Pett-Ridge et al., 2006). Rates of DNRA can be measured by identifying populations of organisms capable of DNRA in the soil, or utilizing ^{15}N tracing methods (Rutting et al., 2011). Rates of DNRA may be increased with the addition of manure, which can contain nitrate ammonifying bacteria such as *E.coli* (Sharma et al., 2016, Tiedje, 1988). However, more research needs to be done on characterizing bacteria in animal manures and how the addition of these bacteria may influence N cycling processes, like DNRA.

There is no agreement on what soil conditions stimulate, or even foster DNRA in agricultural soils (Rutting, 2011). Some common factors considered in studies include water filled pore space (WFPS), soil redox potential, and the ratio of carbon (total or labile) to NO_3. Very little research has been done on rates of DNRA in agricultural soils, where the retention of nitrate would have not only a positive environmental impact, but a positive financial impact as well, as nitrogen fertilizers are a large cost for farmers. DNRA increases the residence time of nitrate and has the potential to reduce denitrification, and in turn, the loss of applied nitrate as N_2O. Because of the cost of N inputs and the environmental impact of nutrient pollution, it is important to increase N retention in agricultural systems. One effective strategy is implementing conservation-based practices.

Agricultural Conservation Practices and Soil Health

Within the last 30 years, a shift to more conservation-based, or sustainable, agricultural practices has occurred as an effort to combat the negative environmental impacts of farming, as well as improve overall soil quality. Farmers are adopting reduced tillage and cover cropping, which are practices intended to increase soil carbon and water-holding capacities, farmers are also becoming more aware of timing and placement of manure. Through these practices, farmers are promoting higher soil carbon content and microbial activity to release nutrients for plant growth. Such practices are expected to create soil conditions that are more like those observed in less disturbed, native soils, where nutrient losses from leaching and denitrification are much less than they are in disturbed environments, like cropping systems (Vitousek et al., 1979). The management practices being studied in this research are; nitrogen source, cover crop selection, and reduced-tillage.

Nitrogen Source and Placement

The application of manure to agricultural fields increases soil organic matter, improves soil structure, and supplies nitrogen that can substitute for all or some synthetic fertilizers (Gollehon et al., 2001). Broadcasting, or the surface application of manure, is a common practice often followed by tillage to incorporate the manure into the soil, reducing odor and potential losses (Cornell University Cooperative Extension, 2015). In most reduced-tillage systems, manure is not incorporated into the soil. Broadcasting manure without incorporation increases the amount of ammonia-N lost to the atmosphere through volatilization (Dell et al., 2011). Without incorporation, 30 to 70% of ammonia-N in broadcasted manure typically volatilizes within one week of application, compared to negligible losses with incorporation (Wulf et al., 2002). A strategy to combat these losses, without introducing tillage, is to apply manure using injection. This method places manure below the soil surface without excessive disturbance, increasing plant-available N and reducing the potential for surface runoff (Cornell University Cooperative Extension, 2005). Although manure injection decreases N losses associated with ammonia volatilization compared to broadcasting, it is estimated that denitrification losses could be as almost 300% greater than those associated with broadcasted dairy manure (Dell et al., 2011). N_2O losses associated with broadcasted manure are typically lower than those with injection (Duncan et al., 2017), however they are not negligible and important to understand in the wake of a changing climate.

Cover Crops

Cover cropping is the strategy of planting of crops that are not harvested as a cash crop, but planted for soil/environmental benefits. This management strategy increases soil organic matter by serving as an additional source of carbon and limiting the period of time a field is in fallow. Root turnover, exudates, and crop residues provide a continuous source of carbon throughout the season. The three main types of cover crops are grasses, legumes, and broadleaves. Grasses, such as Cereal Rye, are capable of scavenging residual nitrogen in the soil while legumes, like Crimson Clover, can fix atmospheric nitrogen and provide it to a subsequent nitrogen-demanding cash crop. Winter legumes, when soil N levels are high, will also scavenge inorganic N from the soil, like non-leguminous cover crops. By forgoing a period of fallow and incorporating a legume cover crop, an increase in soil nitrate and organic matter has been shown, as well as other positive benefits to the soil. However, it is important that the period of N mineralization is appropriately timed with cash crop N demand, or a surplus of soil nitrate could be susceptible to loss. Broadleaved plants can provide diverse services, ranging from Forage Radish, which can break up compaction with a long taproot, to Canola, an early-blooming crop attractive to native pollinators, which can also be harvested as a cash crop (Penn State Extension, 2017, Blanco-Canqui et al., 2015). When integrating cover crops into a cropping rotation, it is important to consider if the selected crop is winter-hardy, and what impact it would have on the cropping rotation and subsequent termination and nutrient management strategies. In no-till systems, herbicide termination is the preferred termination strategy, rather than a tillage event. This is also the case in terminating perennials, like Alfalfa or Orchardgrass. The nitrogen provided from a cover crop should also be considered when estimating nutrient needs for the subsequent cash crop.

Tillage

The three main types of tillage in Pennsylvania are conventional, minimum tillage, and no-till, although conventional tillage is becoming less common. Conventional tillage typically involves primary and secondary tillage operations, often including plowing, inversion of the upper soil layer (~6"), and smoothing out the soil to prepare a seedbed for planting (Magdoff and van Es, 2000). Reduced or minimum tillage, which includes chisel disking, or just disking, leaves more than 15-30% residue cover at planting whereas conventional tillage leaves less than 15% cover (CTIC, 2008). Other types of reduced tillage are zone, strip, and ridge tillage, where only a narrow strip of the soil is disturbed, where seed will be placed. No-till agriculture entails planting into the residue of a prior crop with a no-till planter, rather than homogenizing the soil through tillage and planting into a prepared seed bed. By limiting disturbance, crop and plant residues are not incorporated into the soil and carbon in soil organic matter is protected from rapid oxidation (Lal and Kimble, 1997). A study in Buenos Aires on cultivated soils measured differences in carbon between conventional tillage and reduced tillage methods. After 12 years, soil organic carbon (SOC) was 42-50% greater in no-till soils than in conventionally managed soils (Alvarez et al., 1995). Limited disturbance agriculture leads to greater accumulation of labile organic matter at the soil surface while slowing residue decomposition and carbon oxidation (Spargo et al., 2008). Because there is no tillage event to incorporate crop residues, the surface concentration, or stratification, of soil carbon is characteristic of no-till systems. This stratification, as well as stratification of phosphorous and acidic surface conditions, drives many farmers to incorporate occasional tillage events in no-till systems. A strategic tillage event in these systems could re-incorporate organic matter and other nutrients, making carbon content, nitrogen, and phosphorous more uniform throughout the upper profile. However, there are anecdotal reports from PA farmers who claim even a single tillage can negatively impact beneficial soil properties (NESARE Advisory Panel, personal communication, N.D.).

A meta-analysis by Zuber et al. (2016) determined that microbial biomass and enzymatic activities were lower in tilled soils than in no-till soils. Their analysis also found no significant differences in microbial biomass carbon between no-till soils and soils receiving reduced-tillage or chisel disking. However, significantly lower microbial biomass carbon was observed between reduced or no-till soils and soils that were moldboard plowed. Additional benefits associated with reduced tillage include conserved soil moisture, as well as reduced fuel and labor costs from fewer field operations. There are both agronomic and economic motivations for a farmer to periodically cultivate no-till soils, including; manure incorporation, interrupt pest cycles, or temporarily reduce costs attributed to increased herbicide use (Grandy et al., 2006b). Despite these motivations for occasional tillage in no-till systems, a study by Grandy et al. (2006b) shows that tillage can decrease aggregation and accelerate C and N losses so that years of restoration with no-till are almost “undone”. In the long-term, plant N availability is increased and environmental losses of N are decreased through continuous no-till. Additional research needs to be done on both social and environmental barriers for no-till agriculture because of the important soil health benefits it can impart.

Soil Health

Soil health is the capacity of a soil to deliver all requirements available for stress-free plant growth while minimizing off-site losses of soil, water, and nutrients to the environment (Penn State Extension, 2012). When increased yields are necessary to feed a growing population, building more resilient soils is critical, especially in the face of climate change. Many universities and organizations offer soil health testing, which include a number of metrics that serve to evaluate different properties that they believe to be indicators of soil quality. These metrics differ from test to test but some common ones are Permanganate Oxidizable Carbon (POXC), aggregate stability, pH, organic matter, earthworm counts, etc.

One unique indicator offered by the Cornell Soil Health Testing Laboratory is Autoclaved Citrate Extractable (ACE) protein. This protein serves as an indicator of nitrogen bound in soil organic matter, which can be mineralized and made available for plant uptake. Soil protein is of interest to those studying the N cycle because plant material makes up a large fraction of soil organic matter, and protein represents the plant compound with the largest fraction of N. Protein content plays a role in N storage and mineralization, as well as soil aggregation (Hurisso et al., 2018). Inorganic N is not typically included in general soil fertility tests because of its dynamic nature and high variability day to day. Instead, there is data to support that the ACE protein indicator may better reflect changes in management and available organically-bound N than other existing metrics (Hurisso et al., 2018).

Soil health metrics can be used to evaluate changes in soil quality associated with changes in management practices. Denitrification is a microbially mediated process and sensitive to changes in soil conditions. It is important to understand what soil properties affect denitrification and how management decisions can play a role.

Soil Factors Affecting Denitrification

Soil properties affecting denitrification include nitrate availability, redox potential and moisture, labile carbon content, and temperature. Because denitrification is an anaerobic process, oxygen availability is the limiting factor on rates of denitrification (Paul, 2014).

Nitrate Availability

Up to 80% of applied N can be lost from the plant-soil system (Peoples et al., 1995). By limiting over application and timing application with the period of plant uptake, some of these losses can be mitigated. Excess soil nitrate not taken up by plants is susceptible to leaching and denitrification, especially in moist conditions.

Redox Potential and Moisture

Another factor affecting denitrification is oxidation reduction, or redox, potential, which is a measure of the capability of atoms to be reduced (gain electrons) or oxidized (lose electrons). Redox potential is an important driver of the rates and types of microbial processes that can occur in soils, as it greatly depends on water content. Denitrification typically starts to occur at 60% water filled pore space (WFPS) (Ruser et al., 2006). Redox potential is most often used in characterizing hydric soils, and has been studied much less in unsaturated environments. One of the few studies on redox potential in unsaturated soils was conducted by Clay et al. (1990). This demonstrated that redox potential in no-till soils was lower at depths of 0-7 cm and 15-22 cm than in the same depths in plowed soils. Linking redox potential to agricultural soil management is challenging, because unsaturated soils can have both aerobic and anaerobic microsites and Eh values can be extremely variable over time.

Redox potentials ranging from 300mV to 500mV have been associated with cultivated soils in a study by Husson et al. (2013). Under oxidizing conditions (greater than ~350mV), the primary electron acceptor is oxygen (Figure 1-3). Soils with less oxygen (more reduced, ~350 mV to 100mV), lead to the use of nitrate as electron acceptor by microbes. Agricultural disturbance, or tillage, introduces oxygen to the soil, changing the redox environment and shifting the primary electron acceptor back to oxygen. When microbes are utilizing nitrate rather than oxygen, there may be a higher potential for N conserving processes, like DNRA, to occur, especially when paired with high labile carbon content (Rutting et al., 2011). Therefore, reduced tillage conditions (less oxygen), may promote DNRA.

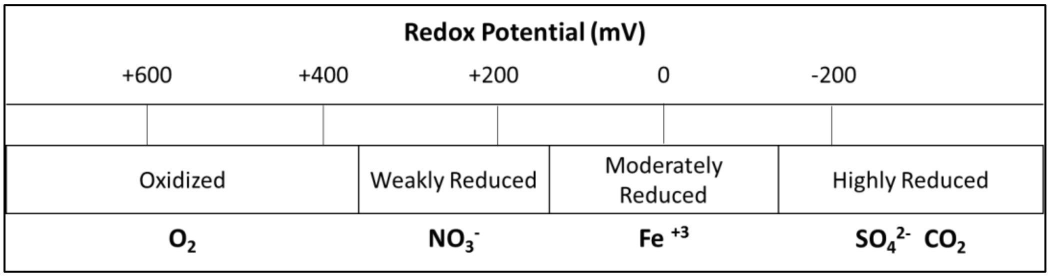

Figure **1-3**. Redox environments associated with their corresponding Eh ranges, as well as the alternative electron acceptors (adapted from Fiedler et al., 2007).

Under field conditions, O_2 is the most important control on rates of denitrification (Paul, 2014). O_2 is the preferred electron acceptor for microbes because this reaction results in the highest energy yields. Therefore, O_2 must be depleted before nitrate is used and denitrification occurs. In soils, higher C and lower redox potential (reducing conditions) provide conditions of high electron donor availability relative to electron acceptors (i.e., more labile C to nitrate-N). These conditions can facilitate microbial respiration processes such as nitrate ammonification and complete denitrification to N_2, instead of incompletely to N_2O. One study notes significantly lower redox potentials in soils receiving plant residues than soils without carbon amendments (Flessa and Besse, 1995). This supports further study of the impact of crop residues, manure, and tillage on soil redox potentials.

Many important nutrient-cycling processes within agriculture are dependent upon microorganisms involved in the nitrogen cycle, including nitrifiers, denitrifiers, and nitrate ammonifiers. All these groups are thought to be highly sensitive to the redox potential of their environment (Pett-Ridge et al., 2006). Characterizing the redox environment of agricultural soils allows for microbial processes and more efficient nutrient cycling to be better understood. Microbial activity is also mediated by soil labile carbon, a readily available source of energy.

Labile Carbon Content

Soils are the world's largest carbon (C) sink, containing more than twice the C in the atmosphere or in land biota (Lal and Kimble, 1997). Agricultural practices that involve disturbance (tillage, deforestation, etc.) introduce oxygen into the soil and encourage the mineralization of carbon and the subsequent release of CO_2 to the atmosphere (Beare et al., 1994). Rates of carbon mineralization are also dependent on soil properties, climate, management practices, and soil biota. Soil carbon can be classified into different pools based on turn-over rates, or how readily it is mineralized. Both active and slow carbon pools are impacted by management decisions and the proportions of these pools in the soil provide information about soil health and general fertility (Chan et al., 2002). Total Organic Carbon (TOC) does not reflect changes associated with short-term management so a more labile fraction, represented as Permanganate Oxidizable Carbon (POXC), is also measured when studying changes in soils over time (Weil et al., 2003). POXC represents a small fraction, usually less than 5% of TOC, but it is the most active carbon fraction in soil and a readily available food source for microbes (Paul et al., 2015). Many studies have found higher carbon in no-till soils compared to those receiving tillage (Lucas and Weil, 2012, Alvarez et al., 1995).

Because labile carbon is a readily available source of energy for microbes, labile content can also be used as a proxy to compare potential microbial activity. Shifts in C fractions resulting from changes in management practices can greatly influence microbial processes and the soil microbial community (Berthrong et al., 2013, de Graaff et al., 2010).

Temperature

Temperature plays a major role in microbial metabolisms. Although effects of temperature can be different depending on soil types, most studies show a positive correlation between denitrification activity and temperature (Braker et al., 2010). This is important when considering the timing of manure application and losses of applied N as N_2O. Rates of denitrification are also dependent on agricultural management decisions.

Management Factors Affecting Denitrification

Management decisions that can influence losses of gaseous products of denitrification include N source and rate, cover crops, tillage, and the timing of nutrient amendments.

N Source and Rates

Nitrogen source is another factor that influences rates of denitrification. Studies have shown higher rates of denitrification in soils receiving manure rather than synthetic fertilizers. This can be attributed to the addition of inorganic N, a carbon source for denitrifying organisms, and, if applied as a liquid slurry, anaerobic conditions (Loro et al., 1997). This is important to consider when considering the environmental impacts of sustainable agricultural management practices.

The proper application rate for N is dependent upon soil N and crop requirements. Ideally, the target N rate is the difference between nitrogen present in the soil and crop N requirement. However, potentially mineralizable N in the soil is difficult to estimate and inorganic N is very mobile and highly dependent on when samples are obtained (Cassman and Munns, 1980). Because N deficiencies can dramatically impact crop yields, many farmers tend to over apply N as insurance (National Research Council, 1993). Applying N at a proper rate can decrease the soil N available for denitrification.

Perennials and Cover Crops

Although there is no consensus on the impact of cover crops on N_2O emissions, a recent meta-analysis found that in 60% of the 106 studies considered, a cover crop treatment was associated with increased N_2O emissions (Basche et al., 2014). The same analysis also studied the difference between living and decomposing cover crops, finding increased emissions in soils with decomposing cover crops compared to living crops.

Pennsylvania farmers have been adjusting their crop management practices in an effort to adapt to the changing climate. One decision some farmers are making is the choice to terminate perennial crops, like Alfalfa, in the fall, with a translocated herbicide rather than in the spring prior to planting. This is being done to combat increasingly wet spring conditions attributed to climate change, which can delay field operations like terminating the prior crop and subsequent planting. This also ensures proper termination of perennials before the summer crop is planted. The decision to delay perennial termination also imparts soil health benefits by maintaining continuous living cover throughout the winter. However, the presence of a readily available C source, and nitrate under moist conditions may encourage denitrification. This relationship will be studied in Chapter 3 of thesis work.

Also, rather than terminating the prior crop before planting, “planting green” is an increasingly popular cover crop management decision. For this strategy, cash crops are planted into living cover crops to enable longer growth periods and encourage greater overall biomass accumulation, additional atmospheric fixed nitrogen in the case of legumes, and increased soil carbon (Penn State Extension, 2015). In selecting cover crops, options include winter-hardy or winter-killed cover crops in the Northeast. If cover crops are not frost killed, farmers need to terminate the cover crop prior to planting. This can be done with an herbicide, roller-crimper, or tillage (Anderson et al., 2016). When terminating with herbicides, either translocated or contact herbicides could be used. This decision depends on weather conditions, cover crop species, the following cash crop, and other factors (Legleiter et al., 2012). It is suggested that farmers wait up to two weeks after spraying to plant the cash crop to ensure proper termination and protect yields (Anderson et al., 2016). The roller/crimpers used, especially in the Northeast, flatten the living cover crop and crimp or crush the stems of the cover crop to kill it. This creates a mat-like residue to plant into, reducing weed pressure, retaining moisture, and improving seed-soil contact for the cash crop (Curran et al., 2010). Most of the interest in this termination method comes from organic farmers interested in reducing tillage. These findings support further study of emissions associated with cover crop termination.

Tillage

It is important to note that some studies report higher N_2O production is associated with no-till soils. Baggs et al. (2003) reported large fluxes of N_2O in no-till soils with Rye residue after synthetic fertilizer. These losses are attributed to the prior crop residue being a readily available (labile) source of C as well as the presence of anaerobic conditions within the residue. Moisture retention and residue accumulation in no-till soils can encourage high carbon anaerobic conditions and therefore, denitrification. However, because less oxygen is introduced in no-till soils, emission of CO_2 is also less, thus contributing to a lower overall global warming potential (GWP). Grandy et al. (2006a) discusses the trade-off between mitigated CO_2 emissions and increased N_2O flux in no-till agriculture. N_2O emissions from no-till soils are extremely variable and dependent upon soil conditions and cropping systems. There was no significant difference in

N_2O emissions between tillage treatments averaged across years, but it was estimated that C storage between 1989 and 2001 reduced soil CO_2 fluxes by 95 g CO_2 equivalents m^{-2} yr^{-1}. However, 56 to 61% of that mitigation was offset by N_2O emissions of 53 to 58 g CO_2 equivalents m^{-2} yr^{-1}. This dynamic is important because N_2O has a global warming potential 298 times that of CO_2 over a 100-year period, making it a GHG of concern (US EPA, 2016). This also reflects that although no-till may protect soil organic carbon from rapid mineralization (release of CO_2), some of that benefit is offset by the increased potential for denitrification (release of N_2O).

Nutrient Amendment Timing

Manure can be an important source of inorganic nitrogen and carbon for crops. The timing of these amendments is critical to their effectiveness. There are times in the growth stages of plants where N is readily taken up to meet crop requirements, but it may not always be feasible to apply N at these times. Denitrification occurs when soils are wetter, so proper timing of manure application and avoidance of wet and high-nitrate soil conditions are critical to reducing these losses.

Manure application, cover cropping, and reduced tillage are all conservation-based management practices encouraged to improve soil health. However, they are all practices thought to increase N_2O emissions as well. My research takes place within a cropping systems project where sustainable agricultural management practices are used and nitrous oxide emissions associated with these practices can be evaluated.

Sustainable Dairy Cropping Systems Project

The overarching goal of this thesis research is to evaluate differences in soil properties associated with N_2O emissions in reduced-tillage soils amended with manures and cover crops in the Sustainable Dairy Cropping Systems (SDCS) experiment at the Pennsylvania State University Agronomy Farm at Rock Springs. Pennsylvania is ranked fourth in the United States for dairy production, making it a large component of the state's economy (National Agriculture Statistics Service, 2018). To study the dynamics of these operations, the SDCS experiment was established in 2010 to represent a dairy farm at 1/20th of the scale necessary to sustain a herd of 65 milking cows, while also producing the required fuel from oil seed crops for equipment (Malcolm et al., 2015). This experiment consists of three separate management rotations that provide comparisons for pest management strategies, nutrient management strategies, as well as a control rotation. The first two rotations are six-year rotations with each crop entry point being represented in each of four blocks. The control is a two-year Corn Grain-Soybean rotation. The diverse rotations at the SDCS, incorporating various cover crops and manure, allow for the study of the impact of sustainable management decisions on important soil properties and changes in soil health on a long-term scale.

Prior Studies

One study within the SDCS was the comparison of two types green manure between Winter Wheat and Corn silage crops from 2011 to 2013 (Snyder et al., 2016a). One treatment was Red Clover interseeded into Winter Wheat compared to Hairy Vetch and Triticale planted after cereal harvest. Researchers found that Red Clover proved to be a better green manure than Hairy Vetch and Triticale. Red Clover better controlled weed pressure with less herbicide, was more profitable than Hairy Vetch, and produced better cover, forage, and increased Corn yield. This study evaluated the role of a cover crop on weed pressure and the influence it has on subsequent crops. My study also seeks to understand the impact of cover crops on cash crop soil properties and N_2O fluxes.

A further study of weed management in the SDCS took place in the Pest rotation in 2010, 2011, and 2012. Snyder et al. (2016b) compared weed control, crop yields, potential soil loss, and net returns to management between an integrated weed management system (Reduced Herbicide) and an herbicide-based strategy (Standard Herbicide). The standard herbicide (SH) system utilizes an herbicide-based weed management program similar to those of other farmers in the region. The reduced herbicide (RH) system employs inter-row cultivation and banded herbicide to control weeds. Both treatments had a Rye cover crop but termination time with an herbicide differed between treatments. Rye in the SH system was terminated when it was less than 30 cm tall on 19 May 2010, 06 May 2011, and 21 April 2012. In 2010 in the RH system, Rye was also terminated on 19 May but in 2011 and 2012, termination was delayed until the late boot or early head stage to allow for additional biomass and better weed suppression, 12 May in both years. Their findings showed greater weed density and biomass in the RH management although still remaining below the threshold for economic loss. Corn yields and populations did not differ between treatments, although net returns to management were higher in RH Corn than in SH Corn due to reduced herbicide costs. Net returns were higher in SH Soybean than in RH Soybean and RH yields were lower than SH yields in 2 out of 3 years. Important to note is that potential soil loss was 100% greater on a 10% slope under RH management than with SH due to cultivation in an attempt to reduce herbicide application and weed pressure. This is relevant because my research focuses on the differences in soil properties across early or late termination of perennials in the SDCS.

Malcolm et al. (2015) provided an energy and greenhouse gas analysis of northeast U.S. dairy cropping systems, using the SDCS as a model. They found that by producing feed grain on-farm, energy inputs could be reduced by 15% and be 27% more energy efficient while emitting similar GHGs to smaller dairy farms that import feed and fuel. Increasing acreage or decreasing the size of the herd would be required to increase on-farm feed production relative to herd size, which may not be feasible for a lot of farmers. N_2O emissions associated with manure injection were significantly higher compared to broadcasted manure. However, N volatilization and P runoff were likely reduced with injection. Something to note is that weather conditions were modeled for this study, meaning that true emissions associated with either injected or broadcasted manure are unclear. N_2O losses associated with injection compared to broadcasting are uncertain and dependent on soil properties and environmental conditions. Overall, growing more grain crops on the farm, rather than forage, was suggested as a strategy to reduce energy use and maintain similar GHG emissions.

N_2O Emissions in the SDCS

In relation to my research, a prior study (Ponce de Leon, 2017) analyzed measured N_2O emissions from soils the SDCS. The treatments of interest were (referring to the crop prior to Corn); Soy with broadcast manure (S-BM), Crimson Clover with broadcast manure (CC-BM), Alfalfa+Orchardgrass with broadcast manure (AO-BM), Soy with injected manure (S-IM) and Soy with synthetic fertilizer (S-UAN) in both 2015 and 2016 growing seasons (Table 1-2). Cumulative corn growing season N_2O emissions were overall highest in S-IM in 2016 (2.5 g N/ha) and the lowest in S-UAN in 2015 and 2016 (0.4 g N/ha) compared to the other selected treatments. However, when comparing N_2O emissions per unit of N, S-IM in 2015 had the highest emissions (1.1%), although S-BM had the largest amount of total N applied in 2016 (356

Mg/ha) compared to the treatments. N_2O emissions per grain yield were also highest in the 2015 S-IM treatment (322.8 g N/Mg/grain) compared to the other treatments for which values were calculated. Of all the broadcast manure treatments, AO-BM produced the highest emissions in 2016 (1.9 g N/ha) compared to the treatments with no spring residue (S-BM) or the Crimson Clover residue.

Table **1-2**. Summary of major findings from 2015-2016 study (Ponce de Leon, 2017) on N_2O Emissions at the SDCS.

	Year	**Treatment**	**Value**
Highest Cumulative Corn growing season N_2O emissions	2016	S-IM	2.5g N/ha
Lowest Cumulative Corn growing season N_2O emissions	2015/2016	S-UAN	0.4 g N/ha
N_2O emissions per unit of N	2015	S-IM	1.1%
N_2O emissions per grain yield	2015	S-IM	322.8 g N/Mg/grain
Highest emissions for BM Treatment	2016	AO-BM	1.9 g N/ha

It was also explained that the most important variables affecting denitrification in the treatments receiving broadcast manure (S-BM, AO-BM, and CC-BM) were 1) days after manure application; 2) growing degree days (GDD); and 3) manure rate. Looking at the Soy treatments separately (S-BM, S-IM, and S-UAN), the top three important predictor variables were 1) days after manure application, 2) GDD, and 3) manure placement. These factors are important to consider when looking to reduce N_2O emissions by properly timing manure amendments.

Another study conducted at Rock Springs (Adviento-Borbe et al., 2010) found that N_2O fluxes were higher in Corn after Alfalfa than in Corn with no winter cover, receiving synthetic fertilizer. These findings support further study of soil properties and environmental conditions affecting N_2O emissions at this site, especially in soils receiving Alfalfa residue.

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Chapter 2

Effects of Prior Crop on Soil Properties and N_2O Emissions in Corn

Introduction

Management decisions such as crop selection, N fertilization, and timing of operations greatly influence soil properties and rates of denitrification in agricultural soils. In response to environmental concerns associated with crop production, conservation-based farm management is increasing throughout the Northeastern U.S. Practices such as reduced tillage, cover cropping and organic amendments are thought to increase soil carbon and improve water retention, which both play a large role in determining the denitrification potential of unsaturated soils.

Legumes provide nitrogen through biological nitrogen fixation, so that the inclusion of legume cover crops reduces the need for N added as manure or synthetic fertilizer (Fox et al., 1998). However, any N that is not taken up by crops increases the probability of N loss through denitrification. In a recent meta-analysis of the literature, 60% of 106 studies reported increased N_2O emissions from systems employing cover crops compared to systems without cover crops (Basche et al., 2014). The use of non-legume species was one of the management practices associated with lower N_2O emissions from cover cropped systems, as was residue incorporation (Basche et al., 2014).

The use of manures instead of synthetic fertilizers has also been associated with higher rates of denitrification from soils, which can be attributed to manure's organic carbon, which is a carbon and electron source for denitrifying organisms. If manure is applied as a liquid slurry, additional water can promote anoxic conditions (Loro et al., 1997) as heterotrophic microorganisms consume available oxygen (Paul, 2014). Because denitrification is an anaerobic process, oxygen availability is an important factor driving rates of denitrification in unsaturated soils. Manure or fertilizer application during wet soil conditions, or prior to a rainfall event, makes abundant N available for denitrification to occur when soils become anoxic. This makes the timing of application and choice of amendments important to consider in mitigating N losses to the environment.

A previous experiment conducted at Rock Springs, PA, reported higher N_2O emissions from plots planted to Corn (*Zea mays*) after Alfalfa (*Medicago sativa*), following spring manure applications, than from continuous Corn plots receiving synthetic fertilizer (Adviento-Borbe et al., 2010). This was attributed either to alteration in the microbial community with the addition of manure or a change in soil structure and aggregation resulting from the alfalfa's perennial root system. In that experiment, Alfalfa residues were incorporated with tillage prior to Corn planting.

The Sustainable Dairy Cropping System (SDCS) experiment was the site of the present research study. In contrast to the study by Adviento-Borbe et al. (2010), the SDCS employs no-till. The objective of this study was to evaluate relationships between N_2O emissions and soil properties in no-till plots during the 2017 and 2018 growing seasons. The selected treatments differed in fertilizer management and winter cover crop prior to Corn. N_2O fluxes, inorganic nitrogen species, and carbon fractions were the focus of this research. We hypothesized that after eight years of a given management treatment, no-till soils receiving both manure and prior crop residue would result in contrasting inorganic N speciation, higher total and labile carbon, and higher N_2O flux than soils receiving synthetic fertilizer instead of manure or prior crop residue. It was also expected that soils with a legume crop prior to corn would have higher N_2O emissions than soils with no winter cover or a grass winter cover.

Materials and Methods

Site Description

This study was conducted within the Sustainable Dairy Cropping Systems Experiment (SDCS) at the Pennsylvania State University Agronomy Farm at Rock Springs (Centre County), PA. The SDCS experiment was established in 2010 to represent a dairy farm at 1/20th of the scale necessary to sustain a herd of 65 milking cows, while also producing the required fuel from oil seed crops for equipment (Malcolm et al., 2015). The soils at this site included well-drained Hagerstown series (fine, mixed, semiactive, mesic Typic Hapludalf) and Opequon series (clayey, mixed, active, mesic Lithic Hapludalf). This experiment consists of three separate management rotations that provide comparisons for pest management strategies, nutrient management strategies, as well as a control rotation. The first two rotations are six-year rotations with each crop entry point being represented in each of four blocks. The control is a two-year Corn Grain-Soybean rotation receiving manure or synthetic fertilizers. Measurements in this study focused on properties within the Manure and Control rotations (Figure 2-1a, b). The diverse rotations at the SDCS incorporate various cover crops and manure, which enables study of the impact of sustainable management decisions on important soil properties and changes in soil health over a longer time period.

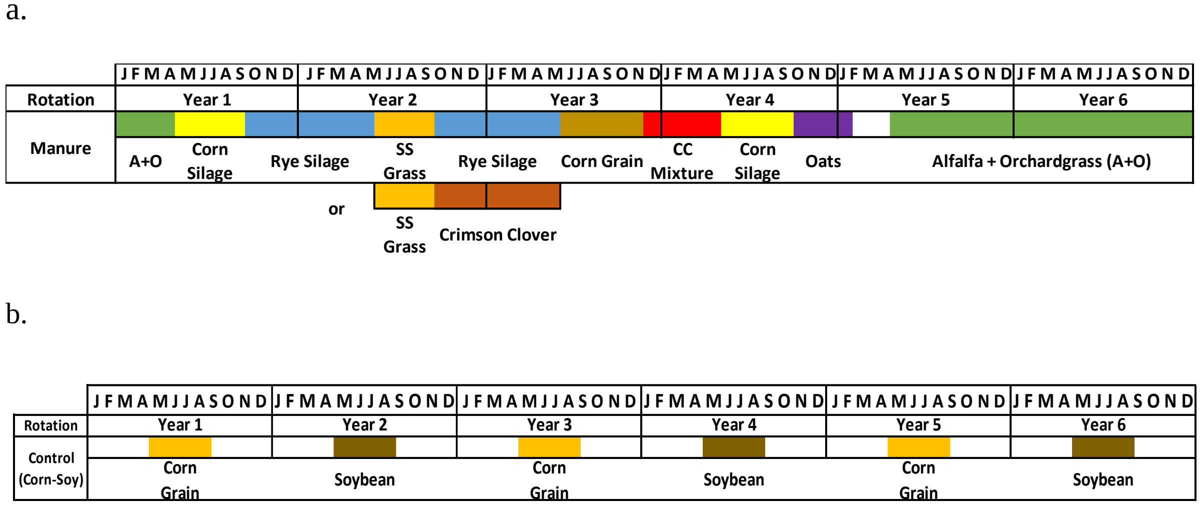

Figure **2-1**. Schematic of the manure rotation (a) and the control rotation (b) in the SDCS.

2017 Treatments

In 2017, we evaluated N_2O emissions and soil properties in five corn entries from 5 May to 4 August. Three corn entries were in the manure rotation, all receiving broadcast manure (BM) but each with a different winter cover prior to Corn: i. Alfalfa+Orchardgrass (AO-AO-BM); ii. Crimson Clover (*Trifolium incarnatum*) (SS-CRIM-BM); and iii. Rye (*Secale cereale*) silage (SS-RS-BM). Two corn entries were in the Control Corn-Soy rotation, with one receiving synthetic fertilizer, iv. (SOY-NONE-UAN) and the other receiving broadcast manure, v. (SOY-NONE-BM). Neither of the two corn entries in the control rotation had winter cover, although Soybean was the prior summer crop for both of these entries (Table 2-1).

Table **2-1**. 2017 treatment details.

Summer Crop prior to Corn	**Winter Cover**	**Fertilizer Type**	**Notation**
Alfalfa+Orchardgrass	Alfalfa+Orchardgrass	Broadcast Manure	AO-AO-BM
Sorghum Sudangrass	Crimson Clover	Broadcast Manure	SS-CRIM-BM
Sorghum Sudangrass	Rye Silage	Broadcast Manure	SS-RS-BM
Soy	None	Synthetic Fertilizer (UAN)	SOY-NONE-UAN
Soy	None	Broadcast Manure	SOY-NONE-BM

All crop rotations were under no-till management. Alfalfa and Orchardgrass was terminated with glyphosate [N-(phosphonomethyl) glycine], 2,4D [(2,4-dichlorophenoxy)acetic acid], and dicamba (3,6-dichloro-2-methoxybenzoic acid) on 26 April (Table 2-2). Crimson Clover and Rye silage were terminated with glyphosate and 2,4D on 04 May. Rye silage was top-dressed with 90 kg N ha^{-1} on 10 April before being harvested on 24 April. Dairy manure was broadcasted in each of the plots receiving manure at different rates based on recommendations from soil tests and manure analysis by The Pennsylvania State University Agricultural Analytical Services Laboratory. AO-AO-BM received 53 Mg ha^{-1} (236 kg total N ha^{-1}) on 15 May. Due to equipment failure, the manure applications in SS-CRIM-BM and SS-RS-BM were split between blocks. Blocks one and two of both treatments received manure on 22 April, while blocks three and four received manure on 19 May. In each plot of these two treatments, manure was applied at a rate of 49 Mg ha^{-1} (219 kg total N ha^{-1}). SOY-NONE-BM received manure at the rate of 29 Mg ha^{-1} (129 kg total N ha^{-1}) on 11 May.

Table **2-2.** Manure application and management events in 2017.

Treatment	Manure Application - Amount	Date of Application	Date of Winter Cover Harvest/ Termination	Additional N Applied	Corn Planted	Sidedress N
SS-RS-BM	Broadcast Manure - 50 Mg ha^{-1}	4/22/17: Block 1& 2 5/19/17: Block 3 & 4	Harvested: 4/24/2017 Terminated: 05/4/2017	Starter: 7-21-7 @ 4 kg N ha^{-1} 56 kg N ha^{-1} on 6 June *Topdressed 90 kg N ha^{-1} on 4/10/2017	5/24/2017	None
SS-CRIM-BM	Broadcast Manure - 50 Mg ha^{-1}	4/22/17: Block 1& 2 5/19/17: Block 3 & 4	Terminated: 05/4/2017	Starter: 7-21-7 @ 4 kg N ha^{-1} 56 kg ha^{-1} N on 6 June	5/24/2017	34 kg N ha^{-1} on 7/3/17
SOY-NONE-BM	Broadcast Manure - 29 Mg ha^{-1}	5/11/2017	N/A	Starter: 12-40-0 @ 22kg N ha^{-1}	5/23/2017	101 kg N ha^{-1} on 7/3/17
SOY-NONE-UAN	None	N/A	N/A	Starter: 12-40-0 @ 22 kg N ha^{-1} 56 kg ha^{-1} N on 6 June	5/23/2017	135 kg N ha^{-1} on 7/3/17
AO-AO-BM	Broadcast Manure - 53 Mg ha^{-1}	5/15/2017	Terminated: 4/26/2017	None	5/18/2017	None

Corn for silage was planted on 18 May in the AO-AO-BM treatment. Corn for grain was planted in the SOY-NONE-BM and SOY-NONE-UAN treatments on 23 May and in the SS-RS-BM and SS-CRIM-BM treatments on 24 May. Additional nitrogen was applied as a starter fertilizer in all of the treatments except AO-AO-BM. The treatments without winter cover (SOY-NONE-UAN and SOY-NONE-BM) received 22 kg N ha^{-1} as 12-40-0 and SS-RS-BM and SS-CRIM-BM received 4 kg N ha^{-1} as 7-21-7. All treatments except AO-AO-BM and SOY-NONE-BM also were fertilized with 56 kg N ha^{-1} on 6 June.

Pre-side dress Nitrate Test (PSNT) was used to quantify soil nitrate available to corn prior to the period of major N uptake (Magdoff, 1991). This test was conducted at the V6 growth stage of corn and 21 ppm was the threshold to determine if supplemental N was required (Beegle et al., 1999). In 2017, the SS-CRIM-BM and the corn without winter cover treatments required additional N. Side-dress application of N occurred on 3 July, and SS-CRIM-BM, SOY-NONE-BM, and SOY-NONE-UAN received 34 kg N ha^{-1}, 101 kg N ha^{-1}, and 135 kg N ha^{-1}, respectively.

2018 Treatments

In 2018, N_2O measurements were made only in the SOY-NONE-BM, SOY-NONE-UAN, and AO-AO-BM treatments from 28 April to 11 July. Alfalfa+Orchardgrass in the AO-AO-BM treatment was terminated on 8 May 2018 with glyphosate [N-(phosphonomethyl) glycine], 2,4D [(2,4-dichlorophenoxy) acetic acid] and dicamba (3,6-dichloro-2-methoxybenzoic acid) (Table 2-3). Dairy manure was broadcasted in SOY-NONE-BM on 25 May 2018 at a rate of 29 Mg ha^{-1} (101 kg total N ha^{-1}) and in AO-AO-BM on 31 May at 41.5 Mg ha^{-1} (143 kg total N ha^{-1}). Corn was planted in all three treatments on 1 June 2018. Both treatments with no winter cover received 7 kg ha^{-1} N as 10-34-0 on 30 May 2018 and AO-AO-BM received the same fertilization on 1 June. Based on the PSNT method previously described, SOY-NONE-BM received 90 kg ha^{-1} N and SOY-NONE-UAN received 67 kg ha^{-1} N, both on 05 July.

Table **2-3**. Manure application and management events in 2018.

Treatment	Manure Application - Amount	Date of Application	Date of Winter Cover Harvest/ Termination	Additional N Applied	Corn Planted	Sidedress N
SOY-NONE-BM	Broadcast Manure - 29 Mg ha^{-1}	5/25/2018	None	Starter: 10-34-00 @ 7kg N ha^{-1} on 5/30/2018	6/1/2018	67 kg N ha^{-1} on 7/5/2018
SOY-NONE-UAN	None	N/A	None	Starter: 10-34-00 @ 7kg N ha^{-1} on 5/30/2018	6/1/2018	90 kg N ha^{-1} on 7/5/2018
AO-AO-BM	Broadcast Manure - 41.5 Mg ha^{-1}	5/31/2018	Terminated: 5/8/2018	Starter: 10-34-00 @ 7 kg N ha^{-1} on 6/1/2018	6/1/2018	None

Environmental Data

Soil volumetric moisture (FieldScout TDR 100 Moisture Meter, Spectrum Technologies) and soil temperature (Analog thermometer, VWR International) were measured on the same six days as N_2O emission measurements from six random sites throughout each plot (Table 2-4). Daily rainfall and air temperature data were gathered from the NRCS-ARS-SCAN site at Rock Springs, Pennsylvania. The weather station was located less than 0.5 km from the SDCS. Table 2-4 lists sampling dates and tests conducted during sampling periods in 2017 and 2018. The data from these sampling events are included in the Appendix.

Table **2-4**. Sampling dates and tests in 2017 and 2018.

Sampling Date	Tests
10 May 2017	N_2O, POXC, TOC, Soil Nitrate, Ammonium
15 May 2017	N_2O
19 May 2017	N_2O
23 May 2017	N_2O
29 May 2017	N_2O
2 June 2017	N_2O, POXC,TOC
7 June 2017	N_2O
13 June 2017	Soil Moisture
20 June 2017	Soil Temp, Soil Moisture
22 June 2017	Soil Nitrate, Ammonium
28 June 2017	N_2O, Soil Temp, Soil Moisture, POXC,TOC, Soil Nitrate, Ammonium
5 July 2017	N_2O, Soil Temp, Soil Moisture
11 July 2017	N_2O, Soil Temp, Soil Moisture
13 July 2017	Soil Nitrate
18 July 2017	N_2O, Soil Temp, Soil Moisture
26 July 2017	N_2O
31 July 2017	N_2O, Soil Temp, Soil Moisture, Soil Nitrate, Ammonium
4 August 2017	N_2O

Sampling Date	Tests
28 April 2018	N_2O, POXC, TOC, Soil Nitrate, Ammonium
2 May 2018	N_2O
11 May 2018	N_2O
14 May 2018	N_2O
23 May 2018	N_2O
31 May 2018	N_2O, Soil Temp, Soil Moisture
2 June 2018	N_2O, Soil Temp, Soil Moisture, POXC, TOC, Soil Nitrate, Ammonium
4 June 2018	N_2O, Soil Temp, Soil Moisture, POXC, TOC, Soil Nitrate, Ammonium
11 June 2018	N_2O, Soil Temp, Soil Moisture
14 June 2018	N_2O, Soil Temp, Soil Moisture
19 June 2018	N_2O, Soil Temp, Soil Moisture, POXC, TOC, Soil Nitrate, Ammonium
29 June 2018	N_2O, Soil Temp, Soil Moisture, POXC, TOC, Soil Nitrate, Ammonium
13 July 2018	N_2O, POXC, TOC, Soil Nitrate, Ammonium

Soil biochemical analyses

Soil samples were collected to a depth of 0-15 cm one time before the application of manure or fertilizer, followed by seven other time points throughout the growing season. In the selected cropping system treatment, six soil core samples were collected and composited from each of four replicated plots and partitioned by depth in the field for carbon analysis (0-5cm and 5-15cm). The remaining soil properties were measured after air-drying and sieving soils (2 mm) from the entire 0-15 cm depth. Soil tests included: ammonium, nitrate, total organic carbon (TOC), and Permanganate Oxidizable Carbon (POXC). Nitrate and ammonium were quantified using a spectrophotometric method described by Doane and Horwath (2003) after the soils were extracted with 2M KCl. POXC was determined using the protocol described by Weil et al. (2003). TOC was measured after ball milling the soil and placing it in tin capsules (12-13.5 mg) for analysis with a CHNS-O elemental analyzer (CE Instruments, Wigan, UK) (Akinsete and Nkongolo, 2016).

N_2O Measurements

Gas samples were collected on 14 sampling dates from 5 May to 4 August in 2017 and 13 sampling dates from 28 April to 11 July in 2018. Measurements were taken from each treatment plot once per sampling date (one chamber per plot) using a vented chamber (32cm x 53cm) placed roughly 45cm from the edge of each plot (four replicate blocks per treatment). Emissions were analyzed using an FTIR Analyzer (Gasmet, Helsinki, Finland). Nitrous oxide fluxes from each plot were measured at approximately the same time of day (9:00am to 12:00pm) for the same amount of time (approximately 5 minutes) to limit temperature and moisture variability. Flux rates were calculated based on a linear regression of chamber deployment time and change in N_2O concentration. Fluxes on days without measurements were estimated using the following equation; $F_n= F_1 + (F_2\text{-}F_1/DOY_2\text{-}DOY_1)$ where F_n is the calculated for a given day, F_1 is the closest measured day prior, and F_2 is the closest measured date prior to F_1. DOY_1 is the Julian day F_1 was measured and DOY_2 is the Julian day F_2 was measured (Adviento-Borbe et al., 2010). Although assuming a linear trend for emissions between sampling dates provides only estimates of actual emissions, this extrapolation provides an estimate of cumulative emissions, which are helpful in making comparisons across treatments (Ponce de Leon, 2017). Nitrous oxide fluxes are reported in units of g N-N_2O/ha/day.

Estimated Available N and Yield Data

Total applied N was calculated based on N contributions from manure, synthetic fertilizers, and prior legume crops and availabilities described in the Penn State Agronomy Guide (2017). Inorganic fertilizer was calculated as kg N/ha. The N contribution from the prior soybean crop in the treatments (no winter cover) was estimated to be 1 lb N/bu of yield. Crimson clover and Alfalfa were estimated at 45 kg N/ha and 90 kg N/ha, respectively. Estimated available N includes the previously described sources of N and considers the availability of N in manure. Manure N availability was calculated by multiplying the application rate by total N (based on manure analysis report) and multiplying by 20%. The Penn State Agronomy Guide estimate for available N is 20% of total N based on application method (broadcasted without incorporation). Corn yield data from the subsequent growing season was also obtained for comparisons.

Statistical Analysis

Statistical analyses of measured N_2O fluxes and soil properties were performed using analysis of variance (ANOVA) with repeated measures (sampling date) in PROC MIXED in SAS (v.9.4), where treatment was considered a fixed effect, block as a random effect. Based on the corrected Akaike information criterion (AICC) and unequally spaced sampling events, spatial power covariance structure was used (SP(POW)). Degrees of freedom were approximated using the Kenward-Roger method and means were compared using LS MEANS. The SLICE option of LSMEANS was used to evaluate differences between treatments by sampling date and Tukey adjustments were made for p-values when testing differences between means. Comparisons were considered significantly different at $p \leq 0.05$. An F-test was performed to compare N_2O variances across blocks where manure application was split (SS-RYE-BM and SS-CC-BM). The variances were not statistically different from each other in either treatment, so it was deemed appropriate to obtain one treatment mean from four blocks. . An F-test was also performed to compare N_2O variances across the no winter cover treatments and AO-AO-BM in 2017 and 2018. The variances were not statistically different so years were combined.

Regression Analysis

To better understand the impact of multiple variables on N_2O emissions, regression analysis was performed in SAS. Potential predictors (Table 2-5) were first plotted against N_2O emissions in Excel to determine if there was a potential linear or quadratic relationship. Selected predictors were then included in the multiple regression using the all possible regressions approach in SAS. Multicollinearity was detected using the Variance Inflation Factor (VIF). For VIF > 15, the relationships between variables were considered to be collinear and removed from the regression.

Table **2-5**. Variables graphed to determine potential predictors.

Variables
Sampling Date (Julian Day)
Soil Nitrate (0-15cm)
Soil Ammonium (0-15cm)
TOC (0-5cm)
TOC (5-15cm)
POXC (0-5cm)
POXC (5-15cm)
Min. Daily Temp
Max. Daily Temp
Soil Temp.
Soil Moisture
Growing Degree Days
Days since Termination
Days since manure
Precipitation 1 day prior
Precipitation 2 days prior
Precipitation 3 days prior

Results and Discussion

Environmental data during two growing seasons

In 2017 soil moisture data, significant differences were observed with sampling date, and there was a significant interaction between sampling date and treatment (p=0.0466). Soil moisture differed significantly among treatments on 20 June, 29 June, and 5 July 2017. On these dates, soil moisture was lower in the SOY-NONE-BM treatments than in all other treatments. Soil temperature data showed no significant differences due to main effects of the five treatments or six sampling dates, but there was a significant interaction between treatment and date (Figure 2-2). On 11 July, temperature was significantly lower in SOY-NONE-UAN than in all other treatments (p=0.0151), and this may have reflected lower overall microbial activity in the treatment with the lowest recent carbon input.

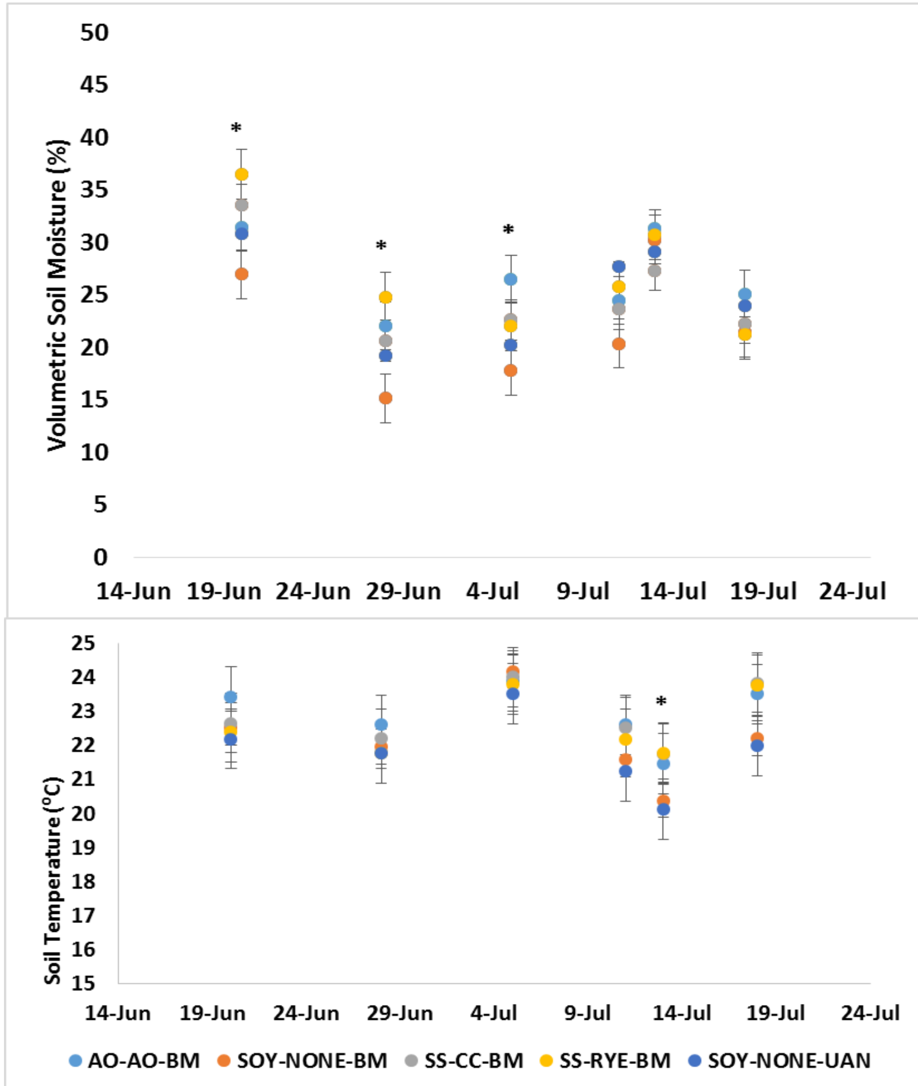

Figure **2-2**. 2017 Soil Moisture and temperature data ± one standard error. *denotes statistical significance between treatments on a sampling date at p<0.05.

In 2018, soil moisture differed across treatments on six sampling dates ($p<0.0001$), 31 May, 2 June, 4 June, 15 June, 29 June, and 11 July (Figure 2-3). On 31 May, soil moisture was not measured in the A+O treatment, but it was significantly higher in the BM treatment than in the UAN treatment. This is likely due to manure application as a liquid slurry on the same date. This was also likely the case on 2 June, where BM was significantly higher than UAN. On the remainning sampling dates, soil moisture in the A+O treatment was significantly higher than in the no winter cover treatments. This is likely due to the crop residues left on the surface after termination of A+O. These increased moisture conditions are conducive for higher denitrification. Soil temperature was significantly higher on the last sampling date than other dates throughout the season ($p<0.0001$) but did not differ across treatments.

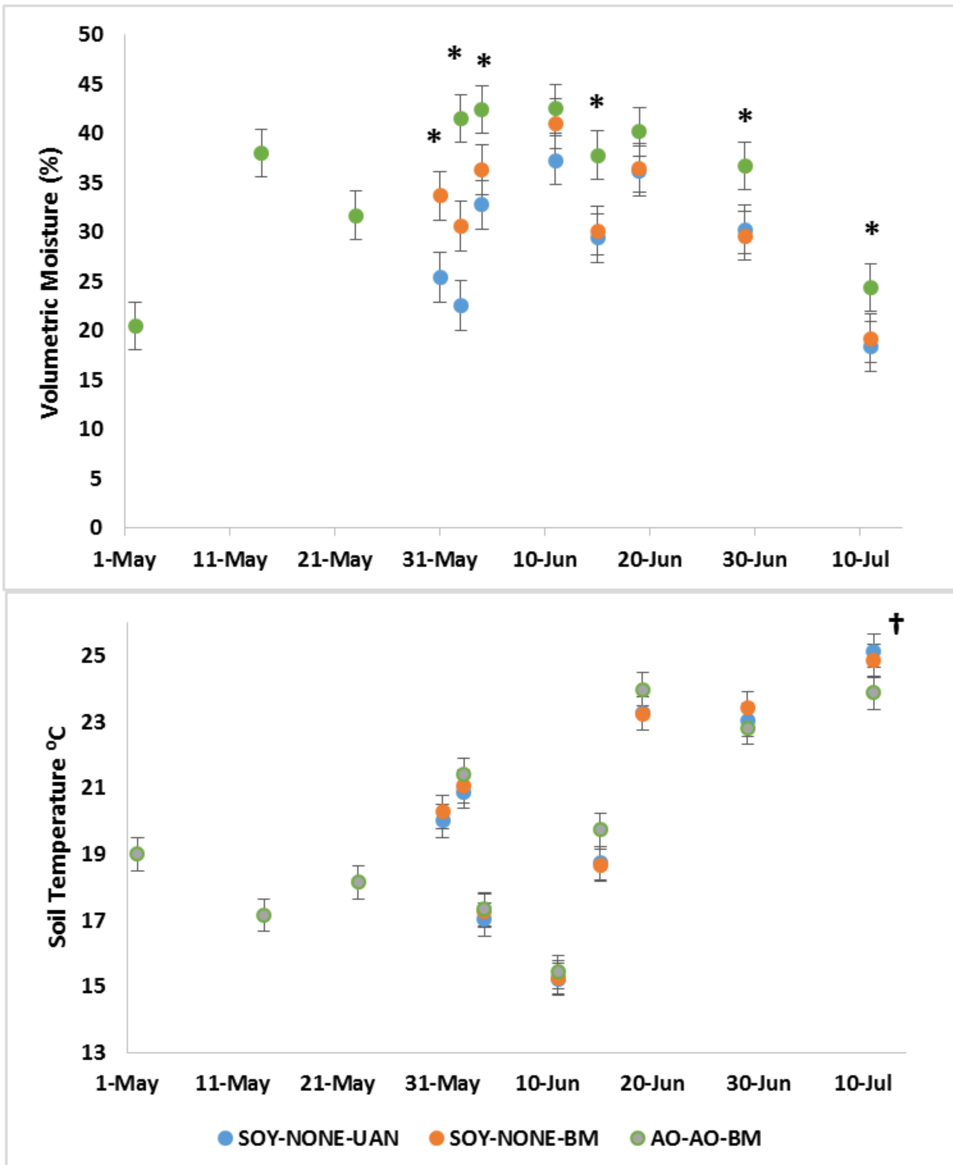

Figure **2-3**. 2018 Soil Moisture and temperature data ± one standard error. *denotes statistical significance between treatments on a sampling date at $p<0.05$. † denotes statistical significance between sampling date at $p<0.05$.

Inorganic N

2017 Ammonium and Nitrate Contents

Treatment means for soil ammonium contents were low, ranging from 0.27-0.84 mg NH_4-N/kg soil, throughout the sampling period. No significant differences in ammonium contents were observed across treatment or sampling date. Treatment means for soil nitrate contents ranged from 1.84-3.09 mg NO_3-N/kg soil. A significant sampling date effect, as well as an interaction between sampling date and treatment, were observed for nitrate measurements ($p<0.0001$) (Figure 2-4). Soil nitrate values were lowest on 10 May, before fertilization took place either by broadcast manure or synthetic fertilizer earlier in the season. The highest soil nitrate contents were observed on 13 July, which was 10 days after a side-dress N application in 3 of the 5 treatments and 51-82 days after initial fertilization events (depending upon treatment and block, Fig. 2-7). Soil nitrate contents were significantly different on 13 July, when nitrate in the AO-AO-BM and SOY-NONE-UAN treatments were higher than in other treatments. This could be in part due to the remaining legume residues in the AO-AO-BM treatment and the side-dress N application received by the synthetic fertilizer treatment 10 days prior to this sampling date. Soil nitrate on the final sampling date, 31 July, was significantly lower than other measured values, except for the sampling date prior to initial fertilization. This is likely due to the growing Corn crop utilizing available soil nitrate.

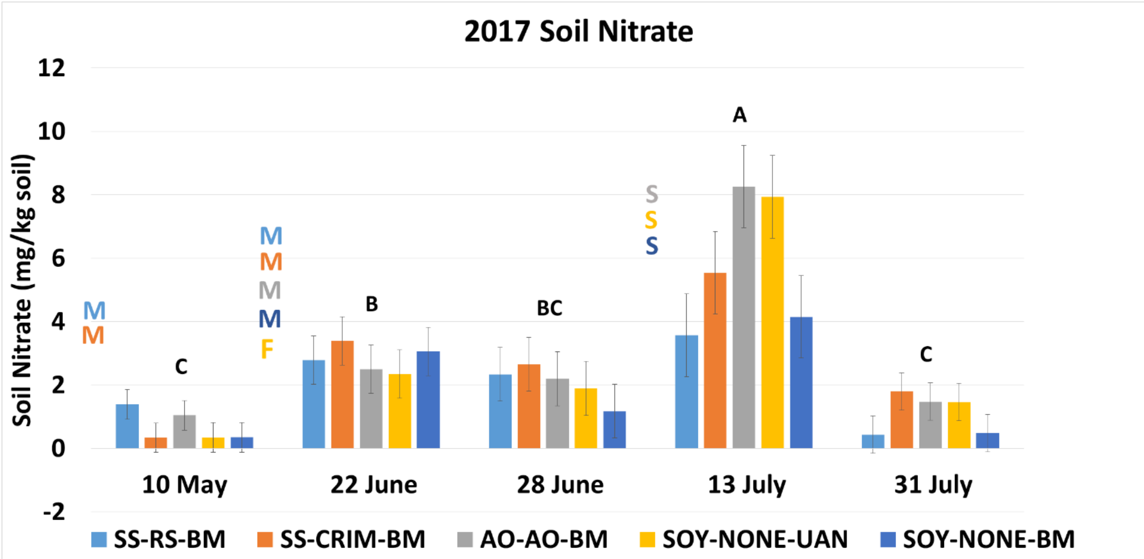

Figure **2-4**. 2017 soil nitrate values for each sampling date (averages from 4 replicated plots in each of five treatments ± one standard error). Manure application occurred on 15 April in AO-AO-BM, on 22 April and 19 May in Blocks 1-2 and 3-4, respectively, of SS-CRIM-BM and SS-RYE-BM. UAN was applied to SOY-NONE-UAN prior to the second measurement date on 22 June. Different letters represent statistical differences in Tukey mean comparisons.

2018 Ammonium and Nitrate Contents

Soil ammonium contents exhibited low treatment means, ranging from 0.58-0.76 mg NH_4-N/kg soil, throughout the sampling period. No significant differences in ammonium contents were observed across treatment and there was no significant treatment*sampling date interaction. However, soil ammonium was determined to be significantly higher (p=0.0192) on the final sampling date, 11 July (Figure 2-5). This appears to indicate that greater rates of N mineralization occurred in between the final two sampling dates.

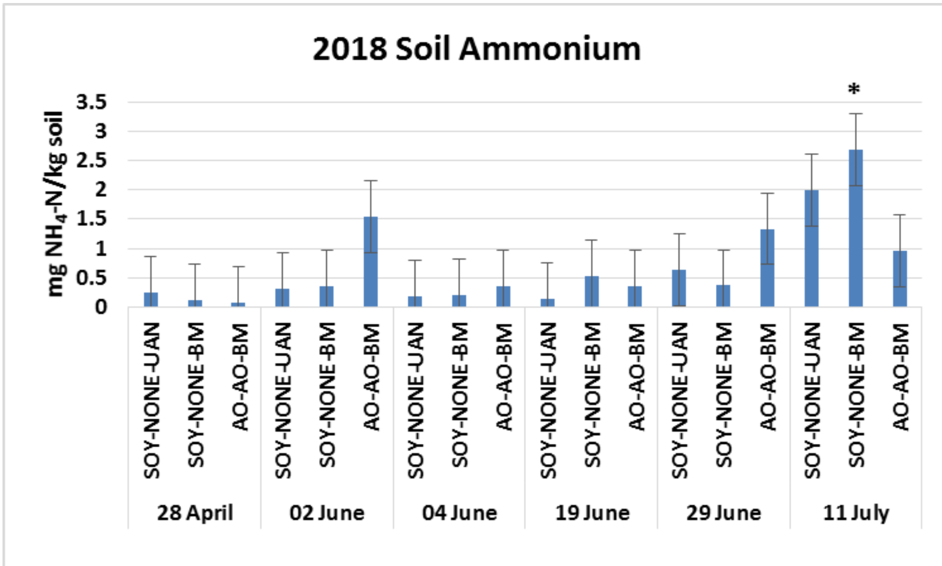

Figure **2-5**. 2018 Soil ammonium averaged by block (n=4) ± one standard error. *denotes statistical difference between sampling date at p<0.05.

Soil nitrate was significantly different among treatments (p=0.0030) and sampling date (p<0.0001). However, the interaction of these two factors was not significant. Soil nitrate values were highest in the SOY-NONE-BM treatment overall (Figure 2-6). The lowest nitrate values were measured on the first sampling date of the season, 28 April. On the last sampling date of the season, 13 July, nitrate values were more than twices as high as the prior sampling date. Nitrate values were higher later in the season compared to nitrate measured in 2017 treatments. This also corresponds with higher N_2O flux measurements later in the 2018 season and trends in soil ammonium.

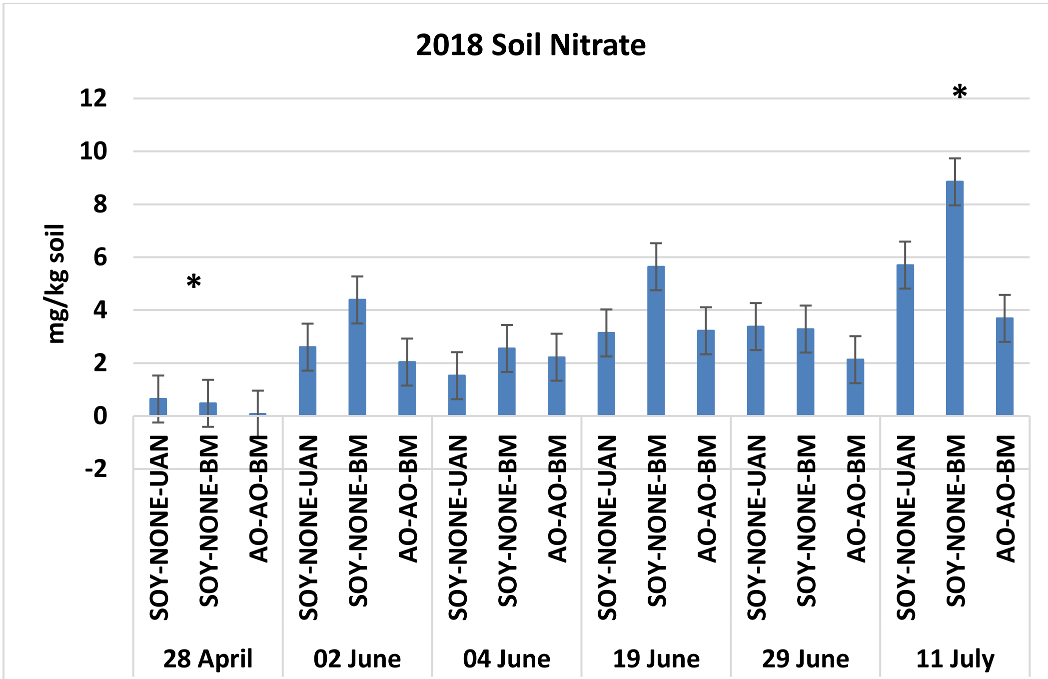

Figure **2-6**. 2018 Soil nitrate averaged by block (n=4) ± one standard error. *denotes statistical difference between sampling date at $p<0.05$.

Soil carbon analyses

2017 TOC and POXC

The treatment*sampling depth interaction for total organic carbon (TOC) was significant ($p<0.001$); TOC was greater at 0-5 cm depth than at the 5-15 cm depth in all treatments, except for SOY-NONE-BM (Figure 2-7). The TOC differences between depths indicated carbon stratification that is typically observed with no-till soils. No differences in TOC were observed among the five treatments on any sampling date.

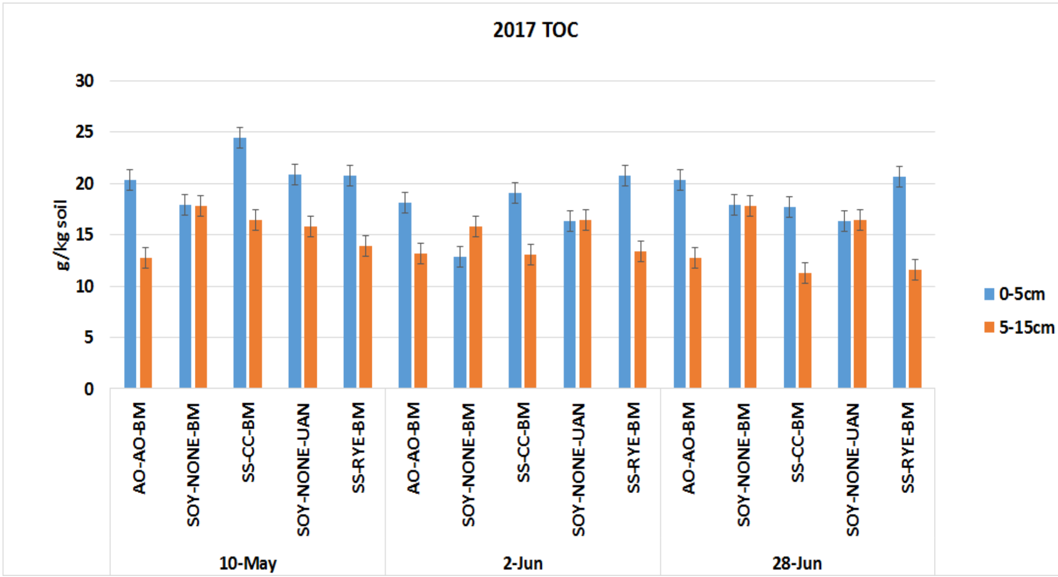

Figure **2-7**. 2017 TOC for each treatment (averaged across four blocks ± one standard error) at the 0-5cm and 5-15cm depths. * denotes statistical differences in treatment within a sampling date at $p<0.05$.

Because TOC has been reported to change little over periods of several years regardless of management (Hurisso et al., 2016), permanganate oxidizable carbon (POXC) has been suggested as a good indicator of the labile soil carbon fraction that may be more responsive to management within shorter time frames. As was observed for TOC contents, the mean values of POXC at 0-5 cm depths were significantly higher than POXC at 5-15 cm depths across all treatments ($p<0.0001$) (Figure 2-8). Significant interactions were also observed between treatment*depth ($p<0.0001$) and treatment*sampling date ($p=0.0002$). These findings are consistent with stratification commonly found in no-till soils, where labile carbon from decomposing residues is concentrated at the surface. As was observed for TOC, treatment differences were found to be significant only on the first sampling date, 10 May. In contrast to 2017, when SS-CC-BM had significantly higher TOC than other treatments, the two treatments with higher POXC were SOY-NONE-BM and SOY-NONE-UAN. The finding that the two treatments without winter cover had higher POXC values than the treatments with winter cover might be attributed to the long-term legacy effect of crop residues left on the soil from Corn grain harvest in the control rotation. The harvest of corn as silage in the manure rotation results in much lower amounts of crop residues left on the soil to decompose.

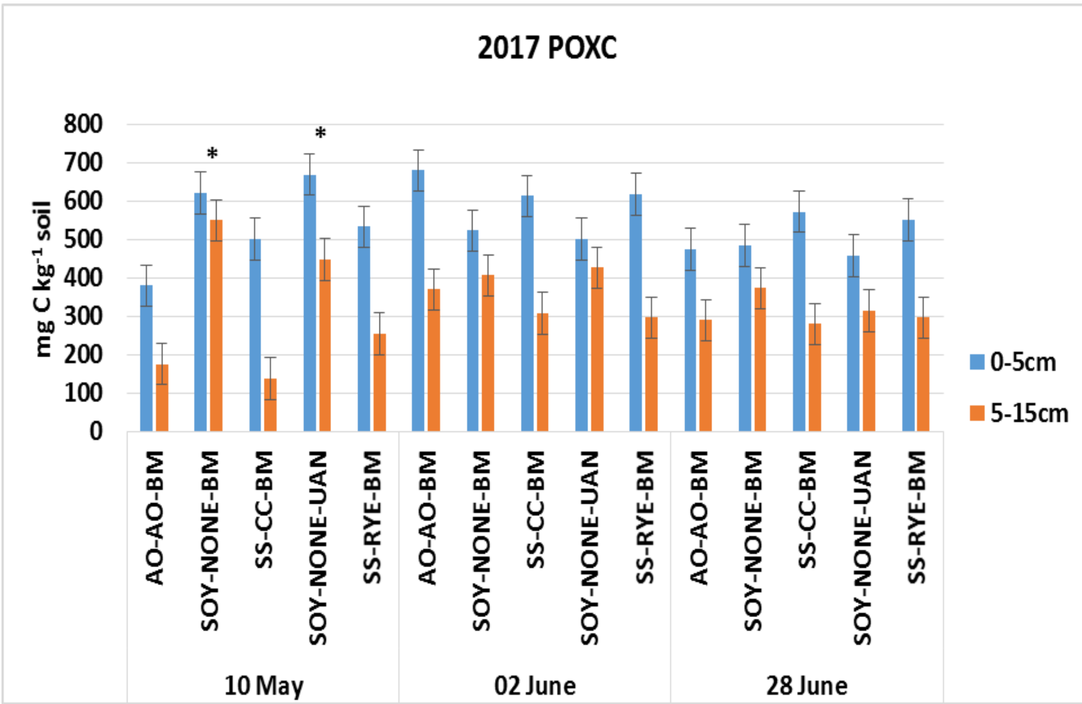

Figure **2-8**. 2017 POXC values for each treatment (averaged across four blocks ± one standard error) at the 0-5cm and 5-15cm depths. Manure application occurred in all treatments (except SOY-NONE-UAN) prior to the second measurement date on 2 June. * denotes statistical significance between treatments within a sampling date at $p<0.05$.

2018 TOC and POXC

In 2018, the effect of sampling depth on TOC contents was significant ($p<0.0001$), indicating higher TOC at the 0-5 cm depth in all treatments across all sampling dates (Figure 2-9). This result was also similar to 2017 TOC data, where there was stratification of TOC in all treatments except for SOY-NONE-BM. There was a treatment*time interaction (p=0.0027) and treatments differed significantly from each other on 28 April, 2 June, 4 June, and 19 June. On 28 April, TOC was significantly higher in both the treatments receiving synthetic fertilizer and A+O prior crop than in the SOY-NONE-BM. TOC was higher in the A+O treatment because of the prior crop and differences in TOC between the no winter cover treatments could have been attributed to residue differences left on the surface from the prior crop (Soybean). On the remaining significant sampling dates, TOC was higher in the AO-AO-BM treatments, once again likely due to the two years of A+O prior to corn planting.

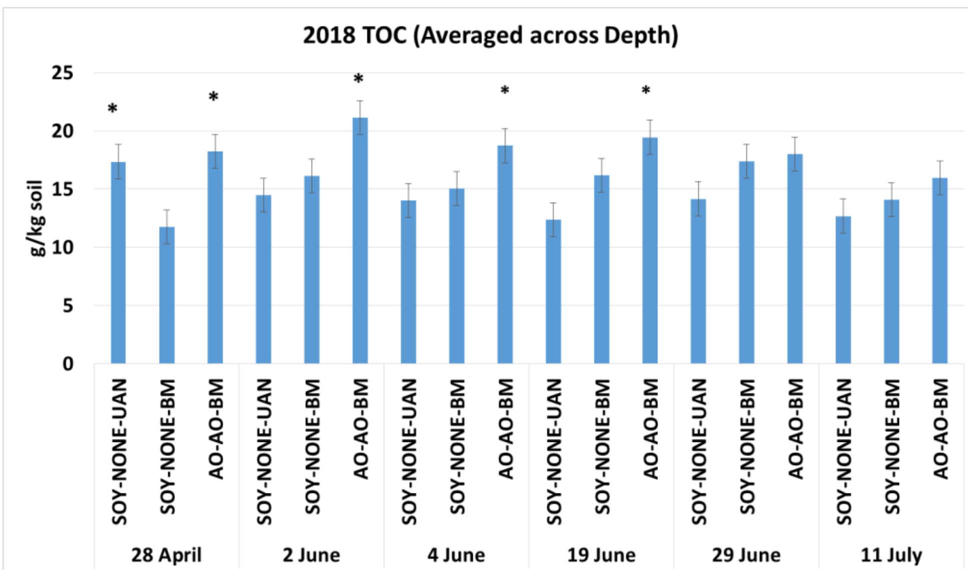

Figure **2-9**. 2018 TOC averaged by block (n=4) and depth ± one standard error. Manure and starter fertilizer application occurred before 2 June sampling date.*denotes statistical difference between treatments within a sampling date at p<0.05.

As was observed in 2017, POXC values at the 0-5 cm depth in 2018 were higher than at the 5-15 cm depth (p<0.0001), indicating labile carbon stratification (Figure 2-10). Sampling date*treatment was significant (p=0.0006), indicating that POXC values differed between treatments on 28 April, 2 June, and 11 July. The significant difference in POXC on 2 June appeared to be due to fertilizer management, where labile carbon would have been added with manure but not with UAN fertilizer. Although both treatments would have received comparable amounts of crop residue, because neither treatment had a winter cover, labile carbon from manure would have been available in the SOY-NON-BM treatment. On the first and final sampling dates, POXC was higher in the AO-AO-BM treatment. This can be attributed to the labile carbon present in the A+O residues left on the soil surface prior to manure application. Because some of the labile carbon detected in the POXC test can be derived from microbial biomass, these results may indicate that some of the prior crop residues, not present in the no winter cover treatments, could have been converted to microbial biomass at this time.

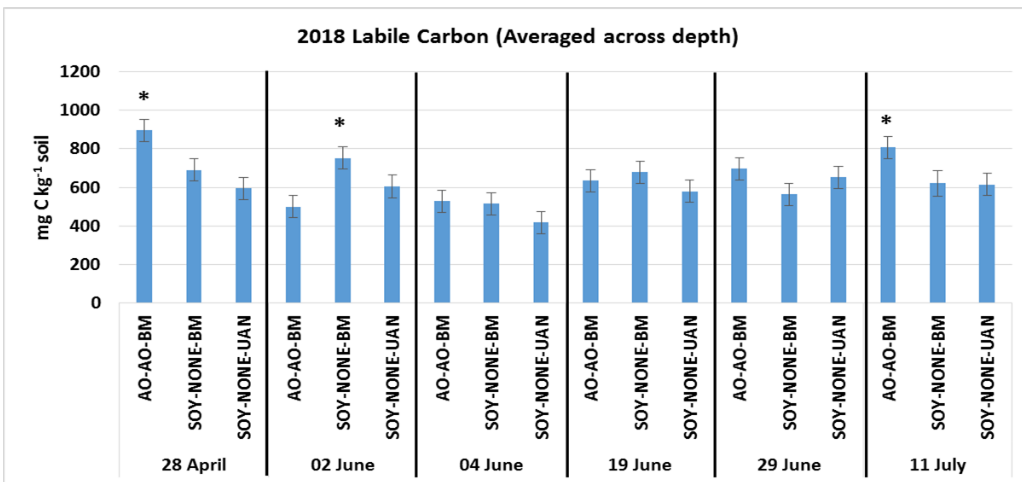

Figure **2-10**. 2018 POXC averaged by block (n=4) and by depth ± one standard error. * represents significant difference between treatments within a sampling date at $p<0.05$. Manure and starter fertilizer application occurred before 2 June sampling date.

N_2O emissions

2017 N_2O emissions

In 2017, the main effects of treatment and sampling date were significant, as well as the interaction ($p<0.0001$). Significant differences across treatment were observed on two sampling dates, 19 May and 9 June 2017. On 19 May, the flux measured in AO-AO-BM was 268 g N_2O-N/ha/day, more than 5 times greater than the next highest flux measured in SS-RYE-BM. On 9 June, flux from the SS-RYE-BM treatment was more than three times higher than the next highest measured flux on the same date. N_2O emissions were in general much higher in the three corn entries of the manure rotation than from the two control treatments which had no prior winter cover crops (Figure 2-11). The AO-AO-BM treatment measured 268 g N_2O-N ha/day on 19 May, five times higher than flux from the next highest treatment. This early spike appeared to result from the combined effects of high rainfall events on 5 May, the manure applications on 15 May, and the presence of decaying, N-rich residues following AO termination on 26 April. This is consistent with findings from two previous studies at this site (Ponce de Leon, 2017 and Adviento-Borbe et al., 2010) where higher flux was measured in Corn after Alfalfa as compared to other treatments. It is likely the large flux seen in AO-AO-BM can be attributed to the addition of labile carbon with manure application, as well as mineralized N from the terminated Alfalfa residue, which was terminated 23 days before (26 April). However, there were no clear trends in C and N pool measurements to explain these patterns in N_2O emissions. Despite being subjected to similar rainfall and manure application timing, no fluxes of the same magnitude were observed in the SS-RYE-BM and SS-CC-BM treatments. The high flux measured in the AO-AO-BM treatment was 56% and 110% higher than the highest fluxes measured in SS-RYE-BM and SS-CC-BM, respectively.

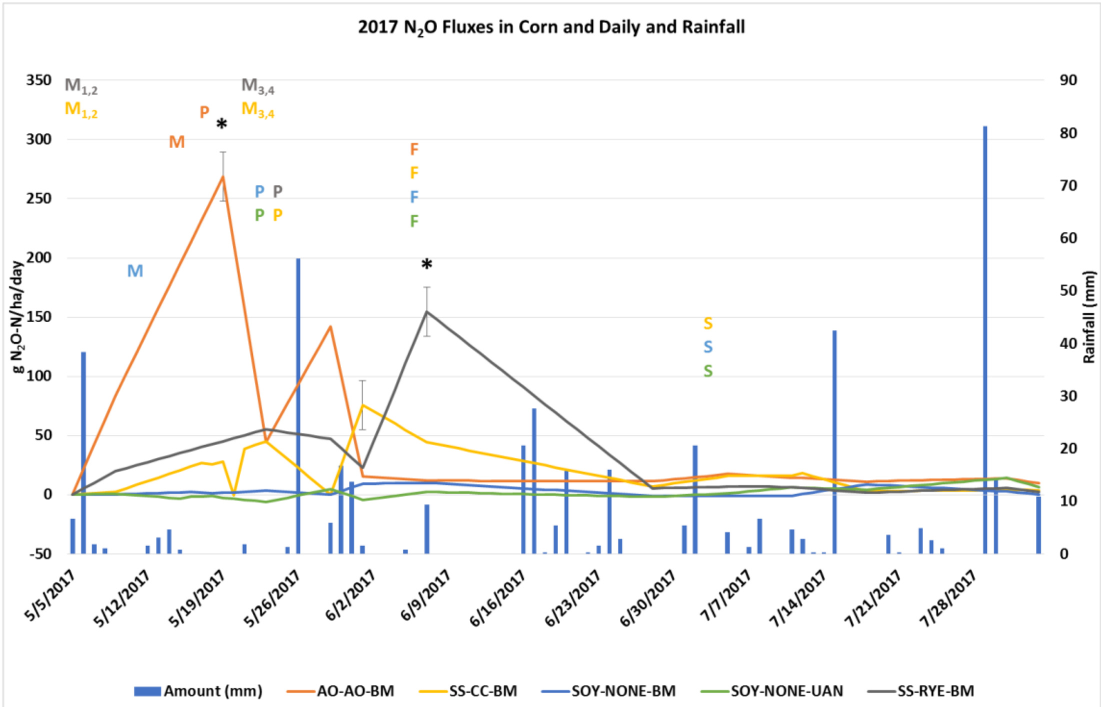

Figure **2-11**. 2017 nitrous oxide emissions in Corn following Alfalfa+Orchardgrass (AO-AO-BM), Crimson Clover (SS-CC-BM), or Rye (SS-RYE-BM), and no winter cover receiving broadcast manure (SOY-NONE-BM), and no winter cover receiving synthetic fertilizer (SOY-NONE-UAN). The left axis represents g N_2O-N/ha/day. Daily precipitation is indicated by the vertical bars and the right axis measures mm of rainfall. Management events for each treatment are indicated by letters of the corresponding color. "M" represents manure application which occurred in blocks 1 and 2 of the SS-CC-BM and SS-RYE-BM treatments on 22 April and in blocks 3 and 4 of the same treatments on 19 May 2017. Manure was applied in the SOY-NONE-BM treatment and the AO-AO-BM treatment on 11 May and 15 May, respectively. "P" represents Corn planting in AO-AO-BM on 18 May, SOY-NONE-BM and SOY-NONE-UAN on 23 May and SS-CRIM-BM and SS-RYE-BM on 24 May. Additional N fertilizer ("F") was applied in all treatments but SS-RYE-BM on 6 June. The SS-CC-BM, SOY-NONE-BM, and SOY-NONE-UAN treatments all were sidedressed ("S") with additional N on 3 July. * denotes significance at $p<0.05$ within a cropping system treatment.

N_2O emissions between winter cover treatments may have reflected differences in the amounts of labile carbon from the respective residues to support heterotrophic denitrification. The SS-RYE-BM had less aboveground residue for decomposition than AO, since the Rye had been harvested for silage on 24 April and sprayed with herbicide on 15 May (three weeks of regrowth to allow herbicide to be taken up). On the other hand, AO had a much longer regrowth period because it had been harvested on 13 Sept, 2016, and allowed to over-winter prior to termination on 26 April. Therefore, the AO residues would have supplied comparatively greater amounts of labile C and mineralized N than Rye residues.

Besides different levels of nutrients in decomposing residues, the timing of early season manure applications also affected N_2O emissions. When comparing SS-RYE-BM and SS-CC-BM in relation to manure application, emissions may have been affected by the fact that manure had not been applied to all plots in these treatments on the same day. Due to weather and equipment constraints, the manure application for these treatments was split between Blocks 1 and 2 (22 April) and Blocks 3 and 4 (19 May). N_2O flux from the SS-RYE-BM treatment was more than three times the next highest flux on 09 Jun. There was a large rainfall event (2nd largest of the season) 8 days after manure application in blocks 3 and 4. Because more of the prior Rye residue was harvested before planting corn, it is likely this flux was promoted by the application of manure timed with a large rainfall event, favorable conditions for denitrification. Aside from these dates, there were no significant differences across treatments. This spike represented the highest flux for the SS-RYE-BM treatment, but it was not as high as the spike observed for AO on 19 May, possibly reflecting a lower supply of mineralized N from RYE than from AO residues. A similar pattern was observed for N_2O emissions from the SS-CC-BM treatment, which were all below 50 g N_2O-N/ha/day through 31 May but increased to 80 g N_2O-N ha/day on 1 June. Fluxes throughout the rest of the season were less than 40 g N_2O-N/ha/day. Emissions were likely low throughout the rest of the season because of the growing Corn crop taking up available N.

Unlike the three treatments with prior winter cover, the two treatments without one (SOY-NONE-BM and SOY-NONE-UAN) showed very low N_2O emissions, not only early in the season but throughout the observation period (Figure 2-11). Typically, a large flux of N_2O is seen 7 to 10 days after manure or fertilizer application (Duncan et al., 2017) but this was not observed. The highest flux from the SOY-NONE-UAN treatment occurred late in the season on 31 July, emitting an estimated 14 g N_2O-N/ha/day, taking place 28 days after sidedress N application, 81 days after initial manure application, but 3 days after the largest rainfall event of the season (81mm on 28 July 2017). On the other hand, a similar increase in N_2O emissions from the SOY-NON-BM was not observed at this time, so that this rainfall event appeared to have more effect on the UAN treatment than the BM treatment. No significant differences were observed across sampling date in the SOY-NONE-BM treatment.

2017 Cumulative N_2O Emissions and Corn Grain Yields

In 2017, means for cumulative N_2O emissions differed significantly across the five treatments (Table 2-6). Mean N_2O emissions from three treatments with a winter cover were at least five times greater than emissions from the treatments with no winter cover, which would have had little biomass on soil surfaces that spring. Cumulative emissions from AO-AO-BM were highest of all five treatments and significantly different from the other two treatments with winter cover. However, the latter two treatments were not significantly different from each other, despite contrasting management of aboveground biomass. For SS-RYE-BM, where most aboveground biomass had been harvested on 24 April after being top-dressed with 90 kg N/ha, mean cumulative emissions were higher than for SS-CC-BM, but not significantly so.

Table **2-6**. Cumulative N_2O emissions in Corn, total available N, estimated available N, N_2O-N emissions per unit of available N, and grain yield, from the selected treatments in 2017.

	Cumulative N_2O emissions in Corn (kg N_2O-N/ha/day)	**Total N Applied N (kg N ha^{-1})**	**Estimated Available N (kg N ha^{-1})**	**N_2O Emissions/Unit of Available N (%)**	**Corn Grain Yield (Mg ha^{-1})**
SS-RYE-BM	3362 ± 850 ba	279	104	3.2	10.1 ± 0.5 ba
SS-CC-BM	1751 ± 850 ba	358	183	1.0	10.7 ± 0.5 a
SOY-NONE-BM	295 ± 850 b	329	226	0.1	8.1 ± 0.5 b
SOY-NONE-UAN	172 ± 850 b	290	290	0.1	8.8 ± 0.5 ba
AO-AO-BM*	4173 ± 850 a	326	137	3	N/A

To estimate N_2O emissions based on units of available N, sources of N were summed for each treatment. Sources included manure N, synthetic fertilizer N, and the N estimated from the prior season's crop and/or winter cover. One N source that was not included in these calculations was the 90 kg N/ha in top-dressing applied to Rye silage in SS-RYE-BM on 10 April 2017, because most of this N was could have been removed with crop harvest, although some of the N might have remained in the rye roots. This assumption may not be valid, since SS-RYE-BM exhibited significantly higher N_2O emissions in June (Figure 2-11). The high percentage of N_2O-N emissions per unit of available N (3.2%) for SS-RYE-BM (Table 2-6) would be considerably lowered if the top-dressed N was included. The calculated percentage of N_2O-N emissions per unit of available N for AO-AO-BM explains that the high emissions can not only be explained by N application, but is dependent on environmental factors as well.

In 2017, mean Corn grain yields were significantly different across treatment based on prior crop (p=0.0246). Corn yield was the highest in the Crimson Clover treatment (10.7 Mg ha-

^{1}), followed by Rye (10.1 kg ha^{-1} N), and no winter cover and synthetic fertilizer (8.8 Mg ha^{-1}), and no winter cover receiving manure (8.1 Mg ha^{-1}). AO-AO-BM was not included in this comparison because it was planted to Corn Silage rather than Corn Grain. The Corn Silage yield for this treatment was 40 Mg ha^{-1} (65% Moisture).

2017 Regression Analysis of N_2O Emissions

All potential predictor variables (Table 2-5) were plotted against N_2O emissions from 2017. However, no variables showed potential as linear predictors as all r^2<0.05. This showed that the variables measured in this study did not capture the factors driving N_2O emissions in 2017. This was likely attributed to sampling frequency and high variability of N_2O emissions.

2018 N_2O Emissions

In 2018, N_2O emissions were compared across three treatments AO-AO-BM, SOY-NON-BM, and SOY-NONE-UAN (Figure 2-12). Treatment (p=0.0270) , sampling date (p=0.0196), and the interaction of these factors (p=0.0247) were significant. As was observed in 2017, N_2O emissions from the treatment with prior winter cover were more than six times greater than emissions from treatments without it. Significantly higher N_2O flux was measured in the AO-AO-BM treatment on 11 June.

As in 2017, fertilizer amendment (synthetic fertilizer or broadcast manure) did not result in differences in N_2O emissions between treatments, which suggests that residue decomposition can be a more influential factor than fertilizer type in determining N_2O flux. Emissions from both treatments, SOY-NONE-BM and SOY-NONE-UAN, were less than 15 g N_2O-N/ha/day on each sampling date until 4 June, 10 days after manure application. SOY-NONE-BM measured 81 g

N_2O-N/ha/day on this date, and emissions increased on the subsequent sampling date, 11 June, to 158 g N_2O-N/ha/day. Emissions from the synthetic fertilizer treatment measured 128 g N_2O-N/ha/day on 4 June and decreased to 74 g N_2O-N/ha/day on 11 June. Emissions from both treatments decreased on 14 June to 8 g N_2O-N/ha/day for SOY-NONE-BM and 7 g N_2O-N/ha/day in SOY-NONE-UAN.

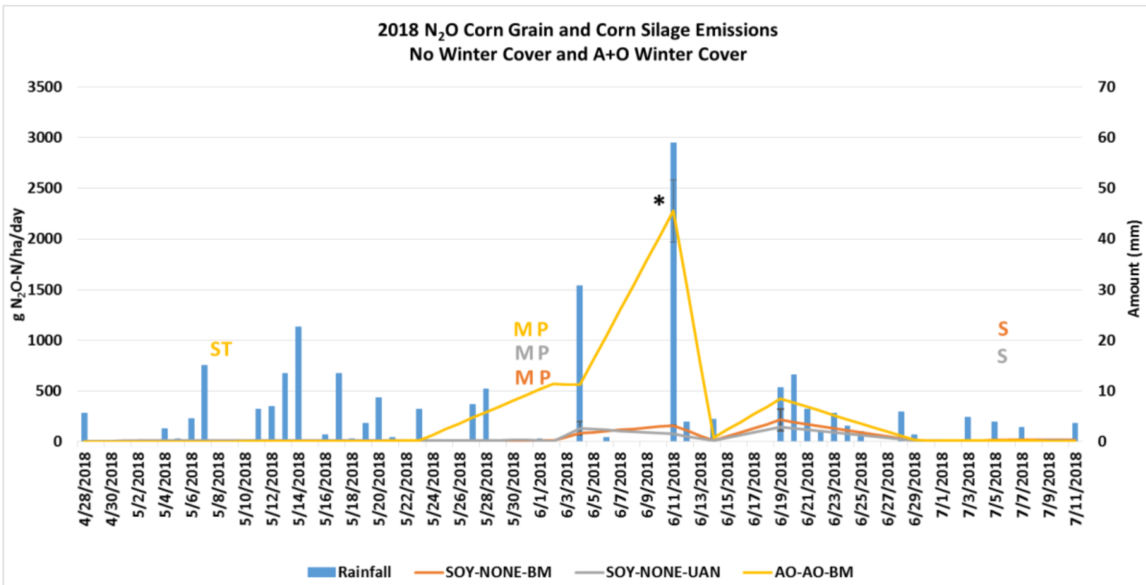

Figure **2-12**. 2018 nitrous oxide emissions from Corn silage after Alfalfa+Orchardgrass and from Corn grain with no prior winter cover and measured rainfall. Values are averaged across four blocks for each treatment and error bars represent ± one standard error. The left axis represents g N_2O-N/ha/day. Daily precipitation is indicated by the vertical bars and the right axis measures mm of rainfall. Management events for each treatment are indicated by letters of the corresponding color. “M” represents manure application which occurred 31 May, and Corn planting (“P”) on 1 June 2018. * denotes significant differences in flux across sampling date at $p<0.05$.

The highest values from the treatments with no prior winter cover in 2018 were measured 19 June, 21 days after starter fertilizer application in both treatments and 26 days after manure in SOY-NONE-BM. These values were not statistically higher because of the large standard errors among dates. This sampling date was also 8 days after a large rainfall event (11 June, 59 mm). Values then decreased to below 11 g N_2O-N/ha/day, in both treatments, on 29 June and remained below 16 g N_2O-N/ha/day for the rest of the sampling period. Like 2017, there were no clear trends in C and N pool measurements to explain measured patterns in N_2O emissions.

2018 Cumulative N_2O Emissions and Corn Grain Yields

Cumulative emissions were more than six times higher in the A+O treatment in 2018 than in the treatments with no winter cover. This is consistent with trends seen in 2017 where cumulative emissions were highest in the A+O treatment. N_2O emissions/unit of available N (%) also reflected the same trends as N_2O emissions. AO-AO-BM N_2O emissions/unit of available N were almost ten times higher than the treatments with no winter cover. This shows that when the amount of N applied is considered, emissions from the A+O treatment were still remarkably high, meaning N application did not drive the high emissions from this treatment.

Table **2-7**. Cumulative N_2O emissions in Corn, total available N, estimated available N, N_2O-N emissions per unit of available N, and grain yield, from the selected treatments in 2018.

		Cumulative N_2O emissions in Corn (kg N_2O-N/ha/day)	**Total N Applied N (kg N ha^{-1})**	**Estimated Available N (kg N ha^{-1})**	**N_2O Emissions/Unit of Available N (%)**	**Corn Grain Yield (Mg ha^{-1})**
2018	**SOY-NONE-UAN**	2.47 ± 2.9 b	144	144	1.7 ± 2.2% b	10.6 ± 0.1 a
	SOY-NONE-BM	3.17 ± 2.9 b	274	193	1.6 ± 2.2% b	8.6 ± 0.1 b
	AO-AO-BM*	20.9 ± 2.9 a	240	126	16.6 ± 2.2% a	N/A

Mean corn grain yields were significantly higher ($p<0.0001$) in the treatment receiving synthetic fertilizer than the treatment receiving broadcast manure. Corn silage yield after A+O was 41 Mg ha^{-1} (65% Moisture).

2018 Regression Analysis of N_2O Emissions

Like the regression analysis for 2017, no variables showed potential as linear predictors (all $r^2<0.08$). This once again demonstrated that the variables measured in this study did not capture the factors driving N_2O emissions in this sampling year.

2017 and 2018 N_2O Emissions

Emissions from the no winter cover and AO-AO-BM treatments were compared across the same time period (5 May to 11 July) in 2017 and 2018. Treatment (p=0.0135), sampling date (p=0.0038), and treatment*sampling date interaction (p=0.0026) were statistically significant. Sampling year and year*treatment were not significant. The flux measured in the AO-AO-BM treatment on 11 June 2018 was significantly higher than all other treatments (Figure 2-13).

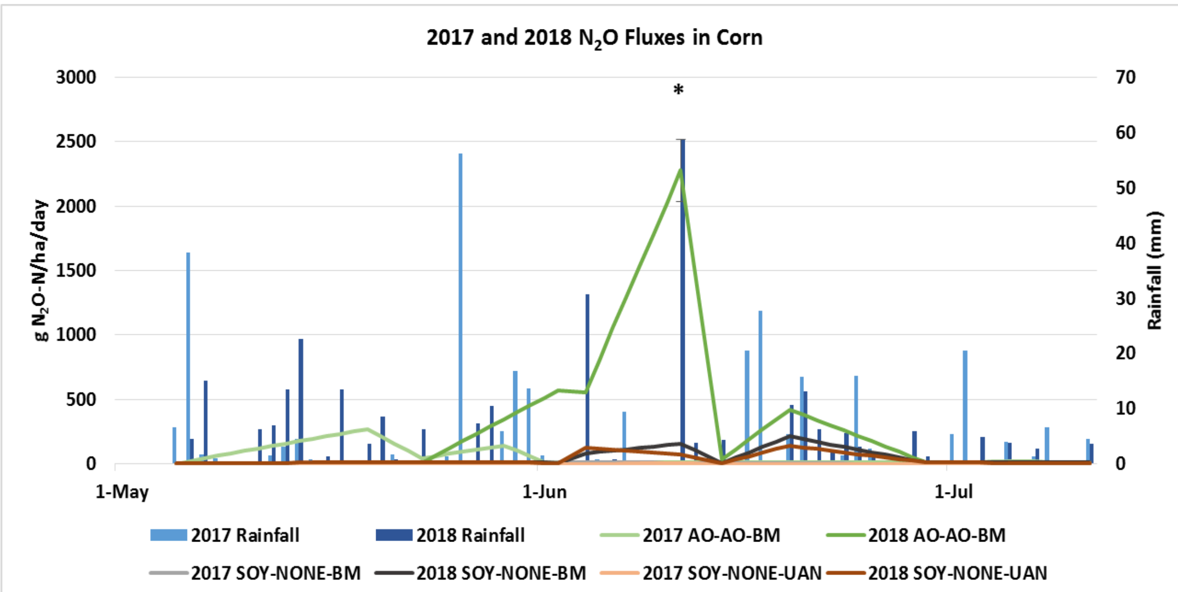

Figure **2-13**. 2017 and 2018 nitrous oxide emissions from Corn silage after Alfalfa+Orchardgrass and from Corn grain with no prior winter cover and measured rainfall. Values are averaged across four blocks for each treatment and error bars represent ± one standard error. The left axis represents g N_2O-N/ha/day. Daily precipitation is indicated by the vertical bars and the right axis measures mm of rainfall. * denotes significant differences in flux across treatment on a sampling date at p<0.05.

Comparing emissions from both years, the two treatments without prior winter cover in 2018 exhibited comparatively high emissions (130-200 g N_2O-N ha/day) between 01 June and 15 June compared to 2017, where fluxes across all treatments in 2017 remained low after 9 June.

These higher emissions coincided with substantial rainfall events and even higher N_2O emissions from AO-AO-BM. The N_2O spike observed in AO-AO-BM prior to 01 June in 2018 was not observed, which may have been due to less rainfall in 2017. This could be attributed to no major rainfall events (greater than 20mm) early in the growing season, followed by multiple large rainfall events in early June, which would have stimulated denitrifying conditions. From 28 April to 01 June, 2017 total rainfall was 195.5 mm, compared to 130.9 mm during the same period in 2018.

2017 and 2018 Cumulative N_2O emissions and Corn Grain Yields

Cumulative emissions were compared across the same time period in 2017 and 2018, 5 May to 11 July. In both 2017 and 2018, mean cumulative N_2O emissions did not differ between the treatments without winter cover, even though they received contrasting forms of fertilizer (manure or UAN) (Table 2-8). However, cumulative emissions in 2018 were significantly higher in these treatments than in 2017, which appeared to be due to annual variations in rainfall frequency, timing and intensity (Figure 2-13). Cumulative N_2O emissions from AO-AO-BM in 2018 were more than five times higher than they were in 2017. Overall, the A+O treatment in 2018 had the highest cumulative emissions across all treatments in 2017 and 2018.

Overall, the emissions per unit of available N values were comparable to those described in previous studies. In the study by Ponce de Leon (2017), values from these same treatments ranged from 0.3 to 1.8% in 2015 and 2016. These percentages were comparable to the range of losses (-0.01 to 2.8%) observed in those treatments in 2017 and 2018, with the exception of AO-AO-BM in 2018 (16.6%). This explains that these emissions cannot be explained by N application alone, and are instead attributed to environmental conditions.

Table **2-8**. Cumulative N_2O emissions in Corn, total available N, estimated available N, N_2O-N emissions per unit of available N, and grain yield, from the selected treatments in 2017 and 2018.

		Cumulative N_2O emissions in Corn (kg N_2O-N ha^{-1})	Total Available N (kg N ha^{-1})	Estimated Available N (kg N ha^{-1})	N_2O Emissions/Unit of Available N (%)	Corn Grain Yield (Mg ha^{-1})
2017	**SOY-NONE-UAN**	-.02 ± 2.9 b	290	290	-0.01 ± 2.2% b	8.8 ± 0.5 ba
	SOY-NONE-BM	0.19 ± 2.9 b	329	226	0.1 ± 2.2% b	8.1 ± 0.5 b
	AO-AO-BM*	3.89 ± 2.9 b	326	137	2.8 ± 2.2% b	N/A
2018	**SOY-NONE-UAN**	2.47 ± 2.9 b	144	144	1.7 ± 2.2% b	10.6 ± 0.5 a
	SOY-NONE-BM	3.17 ± 2.9 b	274	193	1.6 ± 2.2% b	8.6 ± 0.5 ba
	AO-AO-BM*	20.9 ± 2.9 a	240	126	16.6 ± 2.2% a	N/A

Corn grain yields significantly differed across year ($p=0.0323$) and treatment ($p=0.0468$), but the interaction of these two factors was not significant. Overall, yields were 13% higher in 2018 than in 2017. Yields were also 15% higher in the synthetic fertilizer treatment than in the broadcast manure treatment.

2017 and 2018 Regression Analysis of N_2O Emissions

Combining emissions from the three selected treatments in 2017 and 2018 also resulted in no potential predictors for N_2O emissions. The potential predictor with the highest r^2 when plotted against N_2O emissions was days since termination. This variable only explained roughly 14% of the variation in N_2O so it was not included in the regression analysis.

Conclusions

Conservation-based management practices, such as using manure in place of synthetic fertilizer and including legumes and perennials in rotations, have many benefits for soil health. However, these practices also can create soil conditions more conducive for increased nitrous oxide emissions. The planting of a perennial/legume mix prior to Corn receiving manure may be a management combination that is particularly likely to increase N_2O emisisons compared to emissions from Corn after no winter cover that receives synthetic fertilizer instead of manure. Such tradeoffs are important to consider when implementing conservation practices. However, trends in C and N measurements did not appear correlated with N_2O emisisons, implying that other environmental factors influenced N_2O flux to a greater degree. The measured soil and environmental properties in this study did not explain the observed patterns in N_2O emisisons through a regression analysis. This could have been due to high variability in measurements as well as insufficient sampling frequency. However, elevated soil moisture in the AO-AO-BM treatment in 2018 and increasing temperatures later in the season would have created conducive conditions for denitrification and could help explain the high N_2O fluxes measured in this treatment. The findings of this study emphasize the impact of a perennial legume on N_2O

emissions. Chapter three further investigates the high N_2O fluxes measred in AO-AO-BM and a potential strategy to decrease spring N_2O losses.

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Chapter 3

Impacts of Perennial Termination Timing on Nitrous Oxide Fluxes, Soil Carbon, and Inorganic Nitrogen

Introduction

In no-till systems, herbicide application, rather than tillage, is often used to terminate cover crops as well as perennial crops in crop rotations. However, herbicide termination of perennial legumes, like Alfalfa (*Medicago sativa*), may result in greater denitrification losses compared to tillage, because nitrogen-rich residues decay on soil surfaces instead of being incorporated into the soil (Mohr, 1999). Pennsylvania farmers have been increasingly adopting no-till management in an effort to conserve soil, improve water infiltration, and adapt to the changing climate. When no-till farmers employ cover crops, they prefer to terminate them with herbicides rather than disturb the soil. One decision these farmers must make is whether to terminate perennial crops, such as Alfalfa, in the fall rather than in the spring. Fall termination is increasingly being considered because of the higher frequency of wet spring conditions, which delays field operations. When cover crops are terminated in the spring, sufficient time is needed for the systemic herbicide to kill the crop, which can delay planting of the subsequent crop even longer (Bullied et al., 1998). Fall termination permits earlier planting the following spring. However, the presence of a readily available C source in decaying residues during the winter and early spring can promote more N mineralization and nitrification under moist conditions, thus favoring the process of denitrification.

Soluble nitrate moves readily in water by mass flow, making it easily transported and taken up by plants, but also more suceptible to loss. One major loss pathway is denitrification, a microbial respiration process which reduces nitrate to N_2 or incompletely to N_2O, a known greenhouse gas. Denitrification is more likely to occur under saturated, or anoxic conditions when O_2 becomes depleted in soil (Tiedje, 1988). Although agricultural soils are typically unsaturated, denitrification can occur within saturated microsites present in oxic soils, such as in patches of decomposing litter, soil aggregates, and rhizospheres. In addition to nitrate availability, organic carbon availability drives denitrification in unsaturated soils. Carbon is a source of donor electrons for anaerobic respiration by denitrifying organisms, promoting development of anoxic conditions by stimulating oxygen consumption by heterotrophs (Paul, 2014). Thus avoiding the application of N and carbon amendments immediately before the onset of wet conditions can reduce the risk of N losses by denitrification.

One study in in Manitoba, Canada, studied the effect of Alfalfa termination method and timing on N release, uptake and yield of the subsequent crop (Mohr et al., 1999). This study included termination by tillage, herbicide, and tillage+herbicide in the early fall and late fall. There was also an herbicide only treatment in the spring. The authors concluded that method and timing of termination greatly impacted the N release from Alfalfa. Both early and late season herbicide application without tillage delayed N release and reduced the short-term N supply available to plants. Spring herbicide termination of Alfalfa, rather than early or late fall, led to significantly reduced grain yields and delayed N release in 3 of 5 site-years. Compared to tillage and tillage+herbicide, herbicide application alone in the fall allowed for sufficient N mineralization to occur so that crop needs were met, while limiting excess soil inorganic N that would be susceptible to loss. However, this study focused on N uptake from spring wheat in Canada, where weather conditions and soils contrast with those in central Pennsylvania and the timing of high N uptake differs between spring wheat (*Triticum aestevium*) and corn (*Zea mays*). Region-specific studies are needed for more fully evaluating Alfalfa termination method.

With respect to impacts on soil properties and N_2O emissions, relatively few studies have addressed termination timing of perennial crops. The study by Mohr et al. (1999) suggests the need for further research on the broader impacts of this management decision and its implications for more complex cropping systems that also use manure and where soils receive both manure and cover crop residues almost simultaneously. The objective of the present study was to evaluate the influence of perennial crop residues (Alfalfa+Orchardgrass) and termination timing on rates of N_2O emission, soil carbon content, and inorganic N pools in a dairy cropping system. Based on prior studies and findings from the 2017 growing season, we hypothesized that soils under fall terminated Alfalfa+Orchardgrass and spring terminated Alfalfa+Orchardgrass would differ in N_2O emissions, soil carbon fractions, and inorganic nitrogen.

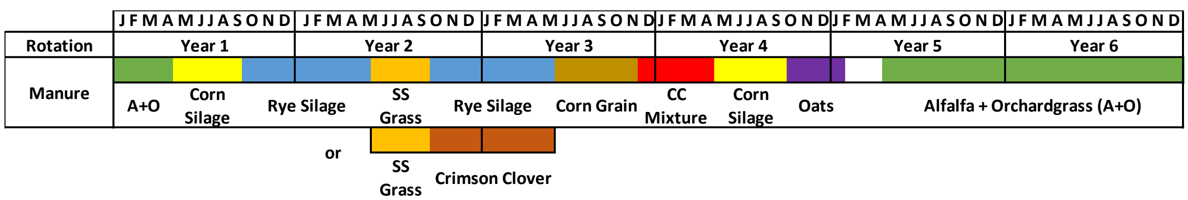
Materials and Methods

Site Description

This study was conducted within the Sustainable Dairy Cropping Systems Experiment (SDCS) at the Pennsylvania State University Agronomy Farm at Rock Springs (Centre County), PA. The soils at this site included well-drained Hagerstown series (fine, mixed, semiactive, mesic Typic Hapludalf) and Opequon series (clayey, mixed, active, mesic Lithic Hapludalf). The SDCS experiment was established in 2010 to represent a dairy farm at 1/20th of the scale necessary to sustain a herd of 65 milking cows (Malcolm et al., 2015). In the SDCS experiment, three different crop rotation series have been maintained (manure management, pest management, and conventional management). For the present study, experimental plots in the manure management rotation were chosen for N_2O emissions and soil tests.

The SDCS manure management rotation is a six-year cropping rotation, with each crop entry represented in each of four blocks, comparing broadcasted manure (BM) and injected manure (IM). For the present experiment, plots in Corn silage receiving broadcast manure in the manure management rotation were sampled after growth of Alfalfa+Orchardgrass (A+O). Since the experiment began in 2010, A+O had been terminated in the spring, prior to Corn planting. This was the first year that fall termination was established as a split-split plot treatment in the manure management series. Soil and gas samples were taken before Corn planting in spring-terminated A+O (AO-SPRTERM) after serving as winter cover, and in fall-terminated A+O which had no live cover in the preceding winter (AO-FALLTERM) (Figure 3-1).

a).



b).

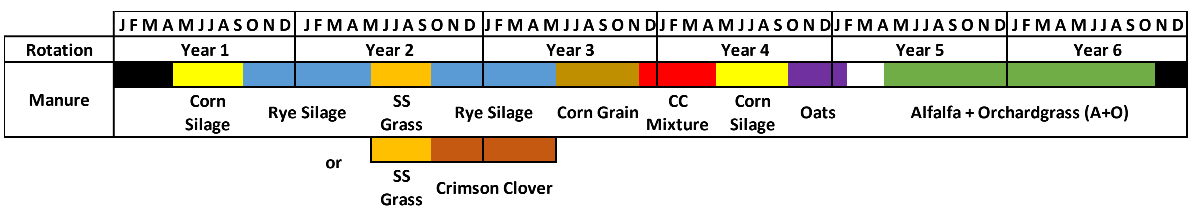


Figure **3-1**. Schematic of the manure rotation in the SDCS. (a) with spring termination (AO-SPRTERM) and (b) with fall termination (AO-FALLTERM).

Spring-terminated Alfalfa and Orchardgrass (AO-SPRTERM) was treated with herbicide on 8 May 2018 and fall terminated Alfalfa and Orchardgrass (AO-FALLTERM) was treated on 15 September 2017 (Table 3-1). Both stands received glyphosate [N-(phosphonomethyl) glycine], 2,4D [(2,4-dichlorophenoxy) acetic acid] and dicamba (3,6-dichloro-2-methoxybenzoic acid). Dairy manure was applied based on recommendations from soil tests and manure analysis by The Pennsylvania State University Agricultural Analytical Services Laboratory. Manure was broadcasted in both treatments on 31 May 2018 at a rate of 41.5 Mg ha^{-1} (143 kg total N ha^{-1}) and Corn silage was planted in both plots on 1 June 2018. A starter fertilizer (10-34-00) was applied at a rate of 7 kg N ha^{-1} at planting to both treatments. Pre-sidedress Nitrate Test (PSNT) was used to quantify soil nitrate available to Corn prior to the period of major N uptake (Magdoff, 1991). This test was conducted at the V6 growth stage of Corn and 21 ppm was the threshold to determine if supplemental N was required. Neither treatment required an additional application of N.

Table **3-1**. Manure application and management events in 2018.

Treatment	**Manure Application - Amount**	**Date of Application**	**Date of Winter Cover Harvest/Termination**	**Additional N Applied at Planting**	**Corn Planted**	**Sidedress N**
AO-FALLTERM	Broadcast Manure - 41.5 Mg ha^{-1}	5/31/2018	9/15/2017	Starter: 7 kg N ha^{-1} as 10-34-00 on 6/1/2018	6/1/2018	None
AO-SPRTERM	Broadcast Manure - 41.5 Mg ha^{-1}	5/31/2018	5/8/2018	Starter: 7 kg N ha^{-1} as 10-34-00 on 6/1/2018	6/1/2018	None

Environmental Data

Each time N_2O flux was measured, soil volumetric moisture (FieldScout TDR 100 Moisture Meter, Spectrum Technologies) and soil temperature (Analog thermometer, VWR International) were determined (apart from 28 April, where no soil temperature measurements were taken). Daily rainfall and air temperature data were gathered from the NRCS-ARS-SCAN site at Rock Springs, Pennsylvania. The weather station was located less than 0.5 km from the SDCS site.

Soil Measurements and Yield Data

Soil samples were collected to a depth of 0-15 cm once, before application of manure or fertilizer application, and seven other times throughout the growing season. Six soil samples were collected and composited from each of four replicated plots of the selected treatments and partitioned by depth in the field for carbon analysis at two depths (0-5 cm and 5-15 cm) for total organic carbon (TOC) and permanganate oxidizable carbon (POXC) tests. TOC was measured using a CHNS-O elemental analyzer (CE Instruments, Wigan, UK) (Akinsete and Nkongolo, 2016), and POXC was determined using the protocol described by Weil et al. (2003). The remaining soil properties were measured using the entire 0-15 cm depth for ammonium, nitrate, and ACE protein. Nitrate and ammonium were quantified using a spectrophotometric method described by Doane and Horwath (2003) after the soils were extracted with 2 M KCl. ACE protein was quantified using the protocol described by Hurisso (2018). Table 3-2 lists dates for all sampling and management events and tests conducted. Corn yield data from the subsequent growing season also were analyzed to provide context for soil measurements.

Table **3-2**. Sampling dates and tests conducted during this study.

Sampling Date	Tests
28 April	N_2O, Soil Moisture, Soil Temp, Nitrate, Ammonium, TOC, POXC, ACE Protein
2 May	N_2O, Soil Moisture, Soil Temp
14 May	N_2O, Soil Moisture, Soil Temp
23 May	N_2O, Soil Moisture, Soil Temp
2 June	N_2O, Soil Moisture, Soil Temp, Nitrate, Ammonium, TOC, POXC, ACE Protein
4 June	N_2O, Soil Moisture, Soil Temp, Nitrate, Ammonium, TOC, POXC, ACE Protein
11 June	N_2O, Soil Moisture, Soil Temp, Nitrate, Ammonium, TOC, POXC, ACE Protein
14 June	N_2O, Soil Moisture, Soil Temp, Nitrate, Ammonium, TOC, POXC, ACE Protein
19 June	N_2O, Soil Moisture, Soil Temp, Nitrate, Ammonium, TOC, POXC
29 June	N_2O, Soil Moisture, Soil Temp, Nitrate, Ammonium, TOC, POXC, ACE Protein
11 July	N_2O, Soil Moisture, Soil Temp, Nitrate, Ammonium, TOC, POXC, ACE Protein

N_2O Measurements

Gas samples were collected on 11 sampling dates between 28 April 2018 and 11 July 2018. Measurements were taken from each treatment plot once per sampling date (one chamber per plot) using a vented chamber (32cm x 53cm) placed roughly 45cm from the edge of each plot (four replicate blocks per treatment). Emissions were analyzed using an FTIR Analyzer (Gasmet, Helsinki, Finland). Nitrous oxide fluxes from each plot were measured at approximately the same time of day (9:00am to 12:00pm) for the same amount of time (approximately 5 minutes) to limit temperature and moisture variability. Flux rates were calculated based on a linear regression of chamber deployment time and change in N_2O concentration. Fluxes on days without measurements were estimated using the following equation; $F_n= F_1 + (F_2-F_1/DOY_2-DOY_1)$. F_n is the calculated flux for a given day; F_1 is the flux on the closest prior measurement day; and F_2 is the flux on the closest measurement date prior to F_1. DOY_1 is the Julian day of F_1 measurement and DOY_2 is the Julian day of F_2 measurement (Adviento-Borbe et al., 2010). Although assuming a linear trend for emissions between sampling dates only provides an estimate of actual emissions, this interpolation gives an estimate of cumulative emissions, which is helpful in across-treatment comparisons (Ponce de Leon, 2017). Nitrous oxide fluxes are reported in units of g N_2O-N/ha/day.

Statistical Analysis

Statistical analyses of N_2O flux measurements and soil properties were performed using analysis of variance (ANOVA) with repeated measures in PROC MIXED in SAS (v.9.4). Two-way ANOVA of treatment and block as independent factors was conducted initially to assess significance of block effects. For all measured properties, block was determined to be non-significant and included in a RANDOM statement. Based on the corrected Akaike information criterion (AICC) and unequally spaced sampling events, spatial power covariance structure was used SP(POW). Degrees of freedom were approximated using the Kenward-Roger method, and means were compared using LS MEANS. The SLICE option of LSMEANS was used to evaluate differences between treatments by sampling date and Tukey adjustments were made for p-values when testing differences between means. Because we hypothesized that treatments would differ, the interaction of sampling date and treatment was analyzed, even if the interaction was determined to not be significant. Comparisons were considered significantly different at $p \leq 0.05$.

Regression Analysis

To better understand the impact of multiple variables on N_2O emissions, regression analysis was performed in SAS. Potential predictors (Table 3-3) were first plotted against N_2O emissions in Excel to determine if there was a potential linear or quadratic relationship. Selected predictors were then included in the multiple regression using the all possible regressions approach in SAS. Multicollinearity was detected using the Variance Inflation Factor (VIF). For VIF > 15, the relationships between variables were considered to be collinear and removed from the regression.

Table **3-3**. Variables graphed to determine potential predictors.

Variables
Sampling Date (Julian Day)
Soil Nitrate (0-15cm)
Soil Ammonium (0-15cm)
TOC (0-5cm)
TOC (5-15cm)
POXC (0-5cm)
POXC (5-15cm)
ACE Protein
Min. Daily Temp
Max. Daily Temp
Soil Temp.
Soil Moisture
Growing Degree Days
Days since manure
Precipitation 1 day prior
Precipitation 2 days prior
Precipitation 3 days prior
Days since Termination

Results and Discussion

Soil Moisture

The total rainfall for the duration of this experiment (28 April 2018 to 11 July 2018) was 294 mm. This is 32 mm less than the measured rainfall for this period in 2017 and greater than the average rainfall for State College during this time period, 229mm. (NRCS-ARS-SCAN)

Soil moisture data was collected on each day N_2O was measured. Measurements were taken from five random locations within a plot and the mean value was calculated. There was a significant interaction between termination timing*sampling date ($p<0.0039$) (Figure 3-2). Termination timing significantly impacted soil moisture on sampling dates: 02 June, 04 June, and 29 June 2018, where soil moisture was higher in the spring terminated treatment. This may have encouraged denitrifying conditions as emissions were significantly higher in the spring terminated treatment on 11 June.

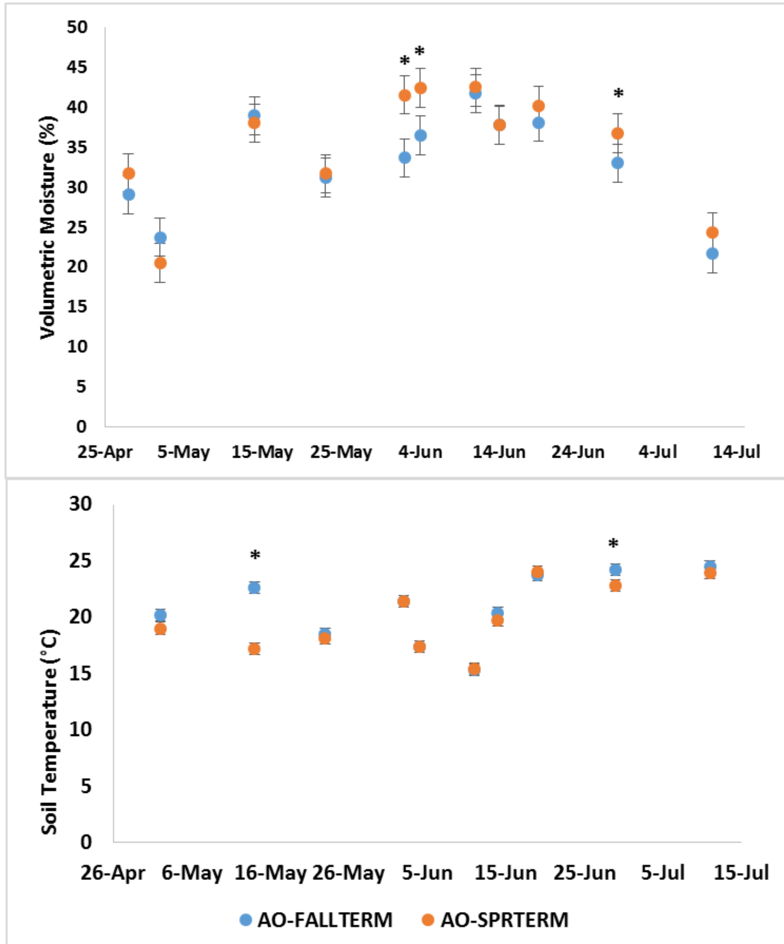


Figure **3-2**. 2018 Volumetric soil moisture and soil temperature averaged across block (n=4) ± one standard error * denotes statistical difference in soil moisture between treatments at p<0.05.

Soil Temperature

Soil temperature data were collected on the same days as soil moisture data except for 28 April 2018 due to equipment failure (Figure 3-2). Measurements were calculated from the mean of three recordings taken within a plot. Termination timing, sampling date, and an interaction of these factors (termination timing*sampling date) were all determined to be significant with $p<0.0001$. Termination timing significantly impacted soil temperature on sampling date 14 May

2018 ($p<0.0001$) and 29 June 2018 ($p=0.0135$), where soil temperature in the fall termination treatment (AO-FALLTERM) was a few degrees higher than in the spring termination treatment (AO-SPRTERM). This temperature difference did not appear to influence N_2O emissions, which were low in all plots those days (Figure 3-8).

Nitrate

Soil nitrate, measured in the upper 15cm of topsoil, differed significantly across sampling dates ($p<0.0001$). Effects of termination timing, treatment and termination timing*treatment were not significant. When averaged across treatment and block, soil nitrate was the lowest on the first sampling date, 28 April, and highest on the final sampling date, 11 July (Figure 3-3). Following manure application on 31 May, nitrate concentration rose on 01 June through 14 June. Soil nitrate dropped slightly on 19 June, and this observation corresponded to the day when the highest measured N_2O flux in the AO-FALLTERM treatment occurred. Soil nitrate values were also highest on the last sampling date of the season while N_2O emissions from both treatments were very low. This could mean that N_2O emissions in this experiment were not driven primarily by soil nitrate availability or that nitrate may have been non-limiting for denitrification throughout the measurement period.

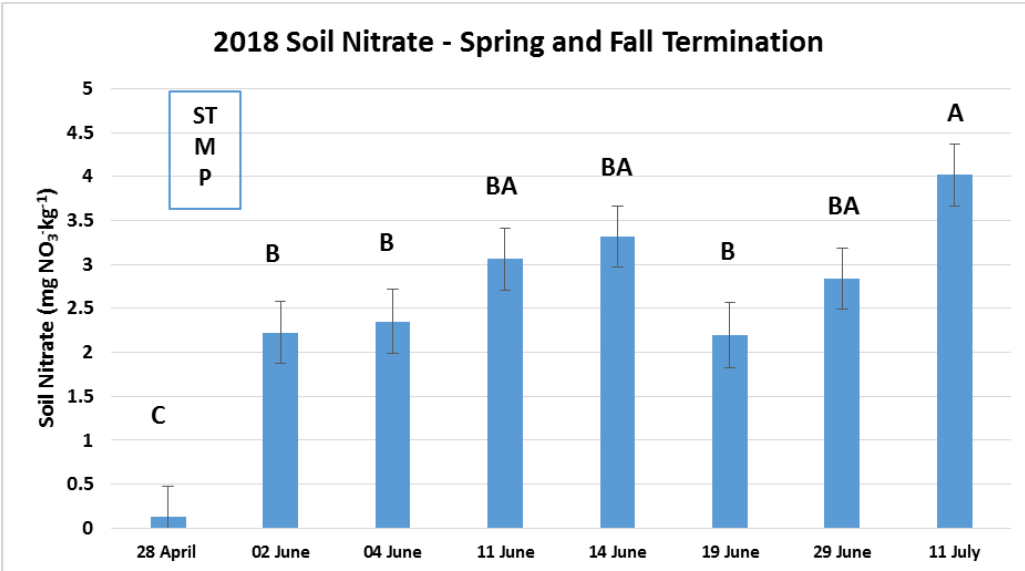


Figure **3-3**. 2018 soil nitrate averaged across treatments (n=2) and blocks (n=4) ± one standard error. ST represents the spring termination of the Alfalfa+Orchardgrass in the AO-SPRTERM treatment on 8 May 2018. M and P mark manure application and Corn planting in both treatments, 31 May and 1 June 2018, respectively. Different letters indicate significant differences at p<0.05.

Ammonium

Soil ammonium, also measured in the upper 15cm, was determined to be significantly different across sampling date ($p<0.0001$) and termination timing ($p=0.0420$). Soil ammonium measured in the AO-SPRTERM treatment was higher than in the AO-FALLTERM treatment (Figure 3-4). Soil ammonium was also highest on 02 June, the first sampling date after manure application (31 May). This is likely due to the fact that the largest fraction of N in dairy manure is ammonium-N and it is possible that most of that ammonium (NH_4^+) volatilized as ammonia gas (NH_3), and did not remain in the soil on 11 June, when soil ammonium was much lower. Studies have estimated that roughly 30 to 70%, even up to 90%, of the ammonium-N in manure can be volatilized from unincorporated, broadcasted dairy manure within 7 days of application (Dell et al., 2012). It is very likely that this was the case between the 02 June and 04 June sampling dates, two and four days after manure application, respectively.

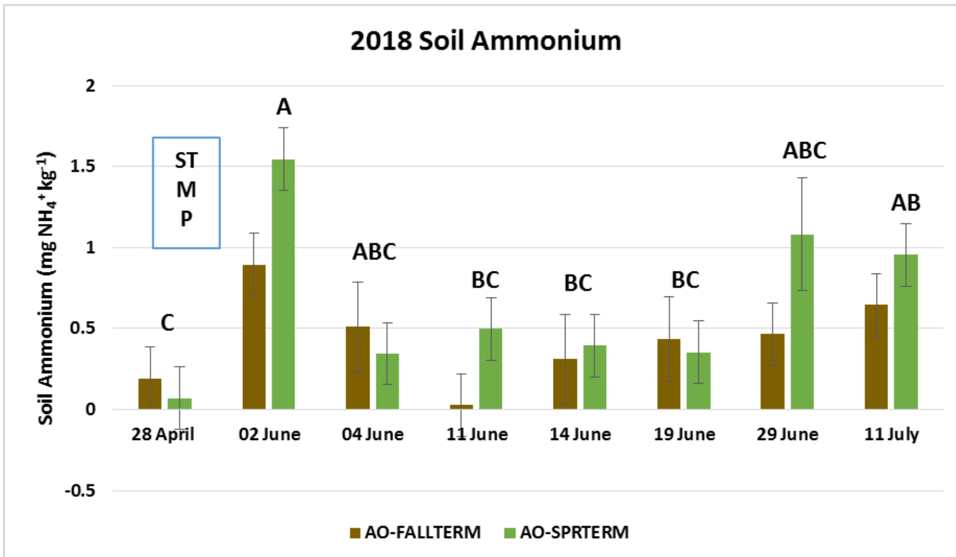


Figure **3-4**. 2018 soil ammonium averaged across blocks (n=4) ± one standard error. ST represents the spring termination of the Alfalfa+Orchardgrass in the AO-SPRTERM treatment on 8 May 2018. M and P mark manure application and Corn planting in both treatments, 31 May and 1 June 2018, respectively. Different letters indicate significant differences at $p<0.05$.

Post-hoc pairwise tests of ammonium concentrations in fall- and spring-terminated soils indicated that fall-terminated soils had significantly lower ammonium than spring-terminated treatments on 02 June, 11 June, 29 June, and 11 July. These results appear to indicate that greater N mineralization was occurring in spring-terminated residues during this sampling period. However, neither N_2O nor soil N was measured in the fall following rye termination, so overall N mineralization cannot be compared in the two treatments.

TOC

TOC values did not differ significantly by treatment, although there was a significant interaction between sampling time*depth (p=0.0396), meaning that TOC at 0-5cm was significantly higher than TOC at 5-15cm on all sampling dates except for 02 June (Figure 3-5). Since these differences were also observed with POXC results, this supports previous findings on the stratification of both carbon fractions in these no-till soils and the impact of surface biomass. The sampling date when depth was not significant, 02 June 2018, occurred two days after manure application and one day after Corn planting. Alfalfa+Orchardgrass was also terminated in the AO-SPRTERM treatment on 8 May 2018. These disturbances may have played a part in homogenizing the soil, disrupting carbon stratification. This effect was short-lived, since clear stratification was observed again only 2 days later.

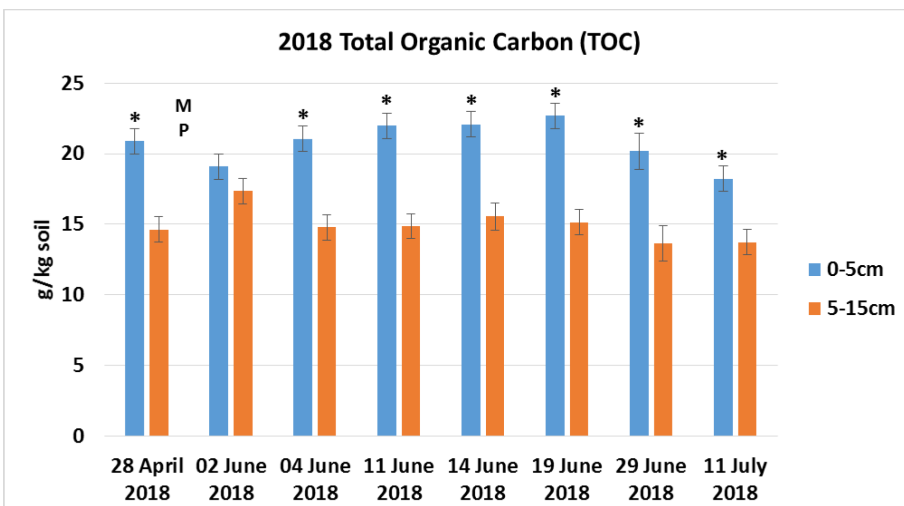


Figure **3-5**. 2018 TOC values averaged across treatments (n=2) and block (n=4) ± one standard error. “M” and “P” mark manure application and Corn planting in both treatments, 31 May and 1 June 2018, respectively.* denotes the sampling dates where TOC was significantly higher at the 0-5cm depth than the 5-15cm depth.

POXC

POXC differed significantly across sampling date ($p<0.001$) and sampling depth ($p<0.001$). There were significant interactions between sampling date*termination timing ($p=0.0002$) and sampling date*sampling depth ($p=0.0218$). The SLICE option revealed that termination timing was significant on 02 June 2018 ($p=0.0007$) and 19 June 2018 ($p=0.0459$). The plots where A+O was terminated in the fall (AO-FALLTERM) had higher values of POXC on these dates (Figure 3-6). Sampling depth was also found to be significant at all time points, POXC values at the 0-5cm depth being higher than those at the 5-15cm depth. This is consistent with carbon stratification typically observed in no-till soils. POXC in the fall terminated A+O plots was significantly higher than POXC measured in spring terminated plots on two sampling dates, 02 June 2018 and 19 June 2018. Because some of the labile carbon detected in the POXC test can be derived from microbial biomass, these results may indicate that more fall-terminated residues were converted into microbial biomass by that time compared to spring-terminated residues. Difference in POXC results were especially notable on 02 June, where much more labile carbon was present at the surface (0-5cm) than at the lower depth (5-15cm).

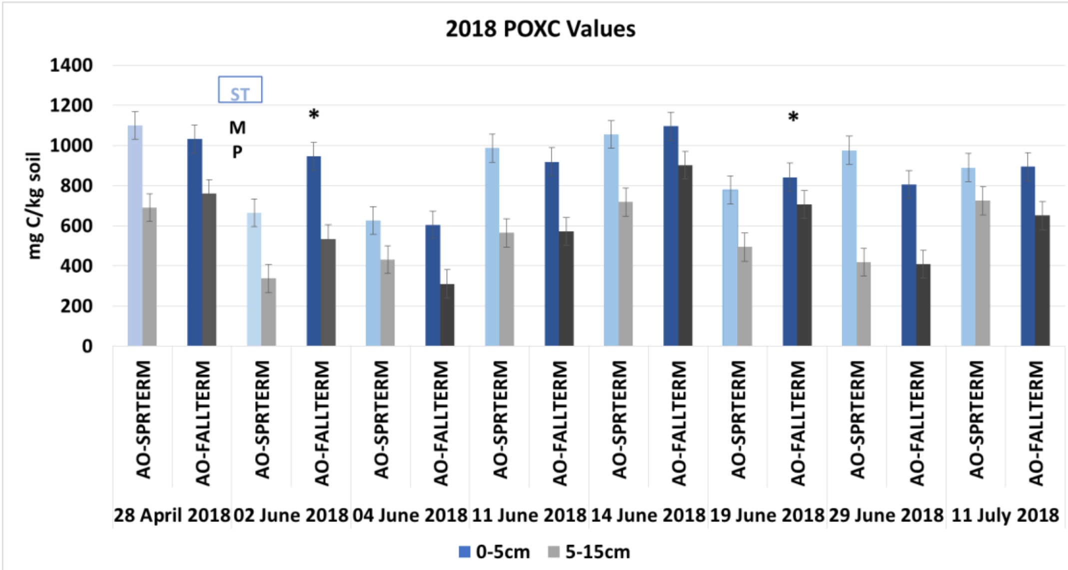


Figure **3-6**. 2018 POXC values averaged across block (n=4) ± one standard error. The lighter colors represent the plots where A+O was terminated in the spring (8 May 2018-denoted by "ST") (AO-SPRTERM) and the darker colors represent fall terminated A+O (AO-FALLTERM) (15 September 2017). "M" and "P" mark manure application and Corn planting in both treatments, 31 May and 1 June 2018, respectively.* denotes the sampling dates where POXC was significantly higher in the fall terminated A+O plots.

ACE Protein

ACE protein did not differ significantly with termination timing, but it did differ between sampling dates ($p<0.0001$). The interaction of termination timing and sampling date was also not significant. On the first three sampling dates, ACE protein values were roughly 23% lower than those measured after the 11 June sampling date, with the exception of 11 July (Figure 3-7). Because this indicator represents an organically-bound form of N, it was not expected to change as much throughout the season as this pool is more recalcitrant than inorganic forms of N, such as nitrate. ACE protein is a relatively new soil health metric based on the rationale that most organic

N is stored in the microbial biomass, a portion of which is protein. A substantial protein fraction in soil (9-20 mg/g dry weight in silt loam textures) would indicate that the processes that mediate the storage and release of N in the organic form are occurring. This is important to consider because inorganic nitrate is highly susceptible to loss while organically-bound N can be a source of slow-release nutrients throughout the growing season. Greater ACE protein measured after 11 June compared to earlier in the season may indicate N retention or immobilization rather than loss as N_2O like earlier in the season.

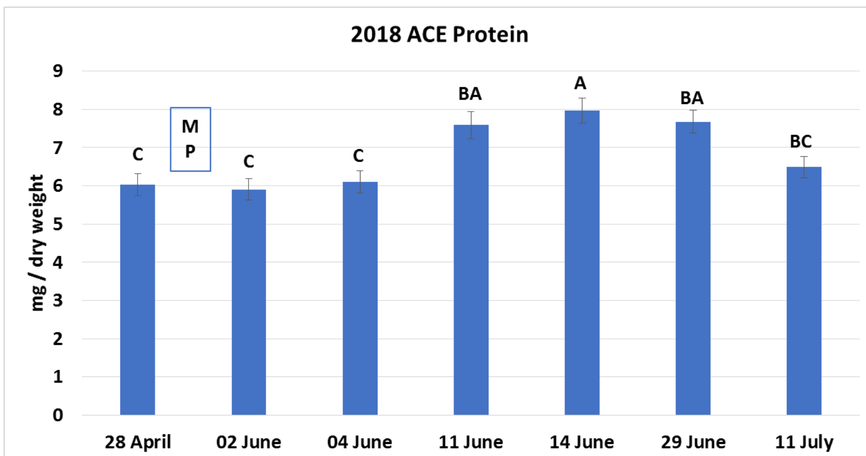


Figure **3-7**. 2018 ACE Protein values averaged across treatment (n=2) and block (n=4). "M" and "P" mark manure application and Corn planting in both treatments, 31 May and 1 June 2018, respectively. Different letters indicate significant differences at p<0.05.

The Cornell Soil Health Testing program uses soil texture in its evaluation of soil health indicators. Based on the silt loam texture of SDCS soils, measured ACE protein values of 5.5-7.5 mg/g dry weight scored at 40-60 out of 100, 7.5-9 mg/g dry weight scored at 60 to 80 out of 100 and 9-20 mg/g dry weight scored at 80 to 100 out of 100. The values measured in this experiment can be scored around 45 out of 100 in the early season, 60 to 75 out of 100 for the June sampling dates, and roughly 50 out of 100 on July 11 (Table 3-4). Overall, these values indicate a moderately sized pool of organically-bound soil N (Mobeius-Clune et al., 2016).

Table **3-4**. 2018 ACE Protein and estimated Cornell Soil Health Score (out of 100) based on scoring function (Cornell University, 2017).

Sampling Date (2018)	**ACE Protein (mg/g dry weight)**	**Cornell Soil Health Score (out of 100)**
28 April	6.0283	45
02 June	5.9082	41
04 June	6.1081	46
11 June	7.5913	64
14 June	7.9752	69
29 June	7.6784	62
11 July	6.4895	49

Nitrous Oxide Emissions

In N_2O emissions, there was a significant interaction between termination timing (fall or spring) and sampling date ($p=0.0221$). On the first three sampling dates, 28 April, 02 May, and 14 May, mean emissions from both treatments were negligible (less than 5 g N_2O-N/ha/day) (Figure 3-8). On 23 May, however, mean N_2O emissions from the spring-terminated AO treatment measured 8 g N_2O-N/ha/day. This is consistent with prior findings in SDCS plots, where in both 2015 and 2016, emissions increased approximately 15 days after the legume crop was terminated (Ponce de Leon, 2017). Three days after manure application (4 June), AO-FALLTERM emissions were 427 g N_2O-N/ha/day, while emissions from AO-SPRTERM were 559 g N_2O-N/ha/day. The highest emissions were observed in the AO-SPRTERM treatment on 11 June, 12 days after manure application, measuring 2.3 kg N_2O-N/ha/day, which was significantly higher than flux measured in the AO-FALL treatment on this date, 230 g N_2O-N/ha/day ($p<0.0001$). This coincided with the highest rainfall during the observation period (59 mm). The following sampling date (14 June), emissions in AO-SPRTERM dropped to 33 g N_2O-N/ha/day. On 19 June, 18 days following manure application, there was a slight peak for both treatments. This date gave the highest flux measurement for AO-FALLTERM (344 g N_2O-N/ha/day). After this date, emissions decreased in both treatments, returning to values similar to those measured in the early season (less than 10 g N_2O-N/ha/day).

Despite the large flux measured in AO-SPRTERM, cumulative emissions were not significantly different between treatments. Cumulative N_2O emissions from AO-FALLTERM throughout the growing season were 7 kg N_2O-N/ha (SE:5) while cumulative emissions from AO-SPRTERM were more than three times emissions from the fall terminated treatment, 21 kg N_2O-N/ha (SE:5).

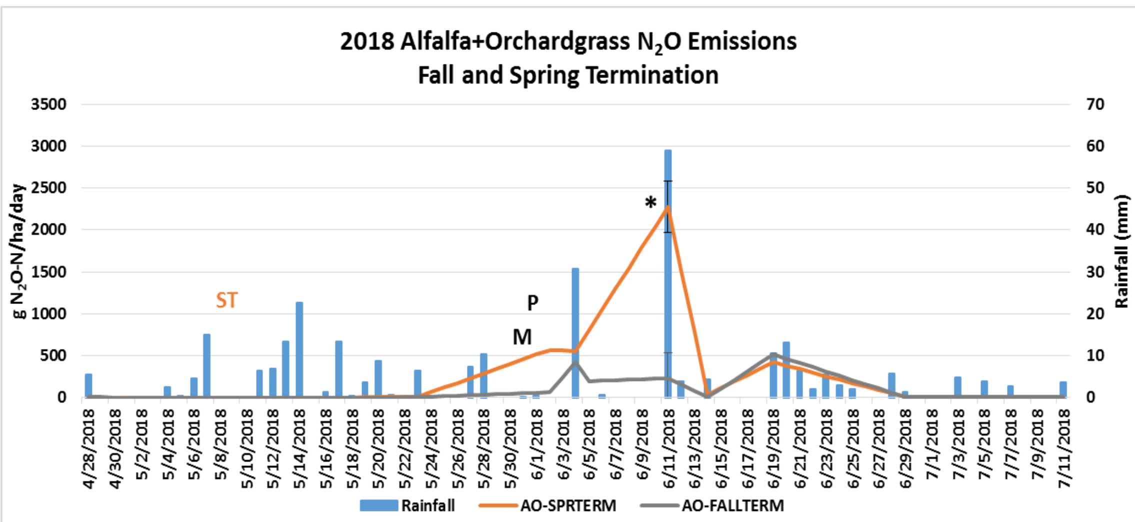


Figure **3-8**. 2018 nitrous oxide emissions from Corn after Alfalfa+Orchardgrass, comparing A+O termination dates. Fall termination in AO-FALLTERM occurred 15 September 2017. ST represents the spring termination of the Alfalfa+Orchardgrass in the AO-SPRTERM treatment on 8 May 2018. M and P mark manure application and Corn planting in both treatments, 31 May and 1 June 2018, respectively. Measured values are means across blocks (n=4) and ± one standard error. *Significant differences between treatments at $p \leq 0.05$.

These measurements can be compared to N_2O emissions made in the 2017 from plots in the manure management rotation. During that season, the highest N_2O flux across five treatments (550 g N_2O-N/ha/day) was observed in spring-terminated Alfalfa+Orchardgrass prior to Corn three days after manure application and one day after Corn planting. Moreover, the next highest measured flux was in the spring-terminated Rye before Corn treatment (250 g N_2O-N/ha/day). This suggests that decaying residues promotes N_2O emissions during the two-week period following termination and that the presence of decaying residues can exacerbate N_2O emissions when manure is added, with legumes associated with higher emissions than non-legumes. This supports the need to better understand soil and environmental conditions that may encourage high N_2O emissions as well as better understand N dynamics in agricultural soils.

Yield Data

Corn silage yield for the 2018 growing season (adjusted for 65% moisture), averaged across all four blocks, did not differ based on termination timing. The AO-FALLTERM treatment averaged 39.9 kg ha^{-1} (SE: 2.6) and the AO-SPRTERM treatment averaged 41.1 kg ha^{-1} (SE: 2.6). This is encouraging for farmers interested in adopting fall perennial termination, but additional years of data are needed to support this choice for cover crop management.

Regression Analysis of Fall Termination Emissions

All potential predictor variables (Table 3-3) were plotted against N_2O emissions from the fall-terminated treatment. Sampling date, POXC (0-5cm), Soil Temperature, growing degree days (GDD), days since manure application, precipitation 1 day prior to sampling, and precipitation 2 days prior to sampling all showed potential as linear predictors ($r^2>0.20$). Looking at the VIF, growing degree days and sampling date were collinear (VIF> 15). Sampling date was removed as one of the variables and the regression was recalculated. The model for predicting N_2O emissions in the fall terminated treatment with the highest r^2 value included all six variables explaining 33% of the variation, but the model was not significant (p=0.098). This model therefore did not capture all the factors driving N_2O emissions.

Regression Analysis of Spring Termination Emissions

All potential predictor variables (Table 3-3) were plotted against N_2O emission from the spring-terminated treatment. Sampling date, POXC (0-5cm). The variables; Soil Ammonium, POXC (0-5cm), maximum daily temperature, soil temperature, and growing degree days were included in the regression because of their potential correlation with N_2O emissions (r^2>0.20). The regression model with the highest r^2 value for spring N_2O emissions included all five selected predictors, but it was not significant or a good fit for the data (r^2= 0.1631, p=0.4297). This demonstrates that the selected measured properties in this model were not good predictors of N_2O emissions in this treatment.

Regression Analysis of Emissions from both Fall and Spring Terminated Treatments

All potential predictor variables (Table 3-3) were plotted against N_2O emissions from both fall and spring terminated treatments. Maximum daily temperature, soil temperature, soil moisture, and precipitation 2 days prior to sampling date were included as predictors because of their potential correlation with N_2O emissions (r^2>0.20). There were no sources of collinearity and including all four predictors in the model explained 22% of variability and was significant at p=0.0010 (Table 3-5).

Table **3-5**. Significance of effects on combined N_2O emissions in corn after fall and spring-terminated Alfalfa+Orchardgrass. Each effect was tested individually against N_2O emissions and then in the multiple linear regression.*VIF: Variance Inflation Factor

	Pr > F	**R^2**	**F**	**VIF***
Maximum Daily Temperature	0.0284	0.546	4.97	2.41
Soil Temperature	0.0052	0.0874	8.23	1.31
Soil Moisture	0.0297	0.0592	4.91	2.09
Precipitation 2 days after	0.1449	0.0245	2.16	1.05
Multiple Linear Regression Model	0.0010	0.22	5.16	-

The equation for the multiple linear regression model was: N_2O Emissions = 3073.05 – 45.15 (maximum daily temperature) + 18.41 (soil temperature) + 7.21 (soil moisture) – 78.24 (precipitation 2 days prior).

Although some of the predictors for N_2O emissions by treatment showed potential linear correlation, the combined regression analysis resulted in the only significant model. Interestingly, soil temperature was the only common potential predictor across three models. This emphasizes the importance of soil temperature regarding microbial processes. The four predictors from the combined model were all abiotic factors, specifically temperature and moisture related. This is consistent with other studies that N alone is not a good predictor for N_2O emissions but environmental conditions, like soil moisture and soil temperature, also play an important role (Conen et al., 2000).

Conclusions

Emissions measured in this experiment were much higher than those measured in 2017, where the highest flux occurred in the spring terminated A+O, 0.55 kg N_2O-N/ha/day. The highest flux measured in the spring terminated A+O in the 2018 growing season was 2.3 kg N_2O-N/ha/day. Comparatively, the highest measured flux from the fall terminated A+O in 2018 was only 0.34 kg N_2O-N/ha/day. Although there were no significant differences in cumulative N_2O emissions between fall and spring termination during this sampling period, the very high flux measured on 11 June 2018 (2.3 kg N_2O-N/ha/day) may indicate higher cumulative N_2O emissions in spring terminated A+O than in fall. However, there were no measurements of fall N_2O emissions from either treatment to confirm this. Although N_2O emissions are thought to be negligible in standing Alfalfa in colder weather months (Wagner-Riddle and Thurtell, 1998), it is possible that fall emissions after A+O termination were significantly higher due to the exceptionally wet fall conditions in 2017 and the addition of labile carbon with A+O residues.

Among measured soil properties, nitrate concentration, TOC, and ACE protein did not differ between treatments at any point during the season. Significant interactions between termination timing and sampling depth or sampling date were observed in N_2O, POXC, and soil moisture and soil temperature. The regression analysis approach revealed that when N_2O emissions were combined across treatments, maximum daily temperature, soil temperature, soil moisture, and precipitation 2 days prior to sampling date were the significant predictors. This showed that environmental conditions, specifically temperature and moisture conditions, played an important role in determining N_2O emissions in this system, which can be altered by management decisions. Other soil properties like N species and carbon fractions did not play a significant role in predicting N_2O emissions in this experiment, but may have played a role in combination with other factors.

The lack of correlation across variables on 11 June, where N_2O emissions in the spring terminated A+O treatment was highest, made it difficult to discern what factors encouraged this large flux of N_2O. The larger pool of ammonium measured on 2 June in the spring terminated treatment may have undergone nitrification prior to 11 June. This would have increased the pool of soil nitrate available to be denitrified. Although this was not necessarily reflected in the soil nitrate data, this could be due to the spatial variability of N.

This study provided some evidence that fall termination of perennial cover crops may be associated with reduced N losses throughout the growing season due to denitrification without a change in yield. However, this study only considered growing season emissions for one site year. Additional years of data should be collected and further research should be done on fall emissions at this site before recommendations are presented to farmers. This management decision also allows farmers to plant earlier in the spring, rather than delaying planting and other field operations until after termination.

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Chapter 4

High Variability and Uncertainty of Soil Redox Potential Measurements

Introduction

Denitrification, which results in gaseous N losses from agricultural soils, is a type of anaerobic microbial respiration that becomes possible when soil pores are filled or nearly filled with water. Because O_2 molecules diffuse through water 10,000 times more slowly than they do through air, dissolved O_2 in soil water is very slowly replenished once it is consumed by aerobic microbes. Once O_2 is depleted, diverse denitrifying soil microbes can use nitrate instead of O_2 as an electron acceptor for respiration. Extensive denitrification, however, is unlikely to occur in the presence of dissolved O_2, because aerobic respiration generates more ATP than denitrification and because of the time lag involved in production of denitrification enzymes. Because oxic soils have redox potential values ranging from +800 mV to +250 mV, the shift in electron acceptor usage from O_2 to nitrate is thought to occur when soil redox potential is sustained below a value of +250 mV (Husson et al., 2014).

Microbes generate ATP by controlling transfers of electrons from donor to acceptor compounds (“redox couples”) via multiple “electron shuttles” in their respiratory membranes. Redox potential, measured as Eh, determines the tendency of dissolved donors and acceptors in soils to give up or acquire electrons, respectively. Bioavailable, labile carbon serves as the main electron donor for soil microbes and represents much of the soil’s capacity to consume O_2. The residence time of carbon in the soil is also a function of Eh, as short as a year in highly oxidized soils, and up to thousands of years under highly reduced conditions (Chesworth 2004).

Electron acceptor usage by microorganisms therefore depends on the Eh of the environment, the availability of electron donors and acceptors, and the thermodynamic favorability of reactions between electron donor-acceptor couples. Table 4-1 shows the order of common soil redox pairs in order of their range of Eh values: Oxygen, Nitrogen, Manganese (IV), Iron (III), Sulfur, and Carbon.

Table **4-1**. Oxidized and reduced forms of important electron-accepting redox pairs during respiration with organic carbon (electron donor) and the approximate Eh values at pH 7.0 (Adapted from Paul 2014).

	Process	**Oxidized Form**	**Reduced Form**	**Approximate Eh (mV)**
Oxygen	Aerobic respiration	O_2	H_2O	+600 to +400
Nitrogen	Denitrification	NO_3-	N_2O, N_2	250
Manganese	Manganic reduction	Mn^{4+}	Mn^{2+}	225
Iron	Ferric iron reduction	Fe^{3+}	Fe^{2+}	+100 to -100
Sulfur	Sulfur reduction	SO_4^{2-}	S^{2-}	-100 to -200
Carbon	Methanogenesis	CO_2	CH_4	Less than -200

Thus, Eh is an important determinant of whether microbes are carrying out aerobic respiration or denitrification in agricultural soils. Eh can be measured as the potential voltage difference between a reference cell and an inert indicator electrode, often Platinum (Pt) inserted into the system being studied. As a system, however, agricultural soils present challenges for measuring Eh, because they are typically unsaturated. This is because pores in unsaturated soils contain both air and aqueous phases, and because air phases physically interfere with electron conductivity. Depending on pore size, aqueous phases in soil pores will contain varied concentrations of dissolved O_2 and enable different degrees of aerobic respiration, thus causing Eh to be highly variable. Eh in unsaturated soils is therefore much more dynamic than in saturated soils having only aqueous phases (Fiedler et al., 2007). Eh is therefore measured more reliably in saturated soils than in unsaturated soils.

Agricultural management intended to build soil carbon, such as cover cropping and reduced tillage, will affect soil conditions and supplies of fresh organic matter that serve as electron donors for microbial activity. An increase in soil organic matter leads to a lowering of soil Eh because it provides an abundant source of electrons (Macías and Camps Arbestain 2010). By increasing soil organic matter, the electrons provided by reduced carbon may also facilitate microbial respiration processes such as nitrate ammonification and complete denitrification to N_2, instead of incompletely to N_2O. Because these microbial processes can help conserve and recycle nitrate, their enhancement could have benefits of reducing costs associated with applied nitrogen.

Despite reported measurement challenges, some studies of Eh in unsaturated soils have been reported in the literature (Husson et al., 2018). Eh measurements have been made in collected soils *ex situ* and in the field *in situ* (Fiedler et al., 2007). In a review of eleven studies on soil redox potentials, two main issues with measuring this property were consistently reported. First, the equipment available to measure Eh varies in electrode type; electrode failures are very common and can provide unreliable measurements. Secondly, unsaturated soil Eh had very high spatial and temporal variability, which limited the repeatability and reliability of these measurements (Husson, 2013). One notable study of Eh in unsaturated soils was conducted by Clay et al. (1990), who demonstrated that Eh in no-till soils was lower at depths of 0-7 cm and 15-22 cm than in plowed soils. These authors showed that surface soil structure and redox potential could be impacted below the depth of tillage.

The objectives of the present study were to assess these challenges and investigate Eh values and carbon fractions in unsaturated agricultural soils at two different experimental sites, the Long Term Tillage (LTT) experiment and the Sustainable Dairy Cropping Systems (SDCS) experiment. Continuously measured Eh values were obtained *in situ* with custom-made redox measurement equipment over several-day periods. Eh measurements were made at two depths to evaluate spatial variability at these sites. The efficacy of conservation practices could be better understood with more knowledge of the relationships between Eh and soil management.

Materials and Methods

Study Site 1: Sustainable Dairy Cropping System

The Sustainable Dairy Cropping Systems (SDCS) experiment is located at the Pennsylvania State University Agronomy Farm at Rock Springs. To study approaches to minimize off-farm purchases by a typical dairy operation in Pennsylvania, the SDCS experiment was established in 2010 to represent a dairy farm at 1/20th of the scale necessary to sustain a herd of 65 milking cows while also producing the required equipment fuel from oil seed crops (Malcolm et al., 2015). The soils at this site included well-drained Hagerstown series (fine, mixed, semiactive, mesic Typic Hapludalf) and Opequon series (clayey, mixed, active, mesic Lithic Hapludalf).This experiment consists of three separate cropping rotations that provide comparisons for pest management strategies, nutrient management strategies, as well as a control rotation. The first two rotations are six-year rotations with each crop entry point being represented in each of four blocks. The control is a two-year Corn Grain-Soybean rotation. The diverse rotations at the SDCS, incorporating various cover crops and manure, enable the study of the impact of sustainable management decisions on important soil properties and changes in soil health on a long-term scale. The treatments of interest were located within the Pest Management rotation. This rotation compares standard herbicide treatment (SH) which entails only herbicide application for weed management, and reduced herbicide management (RH) which uses a combination of tillage, pest resistant variety selection, and minimal herbicide application for weed control.

Two periods of observations of for Eh measurements took place from 10-15 August 2017 in Block 2 and from 22-29 August 2017 in Block 4. Eh was measured in adjacent plots of terminated third year Alfalfa and standing second year Alfalfa in both blocks (all in no-till). Measurements in the Terminated Alfalfa plots (referred to as Canola-NT in plot maps) were made when soils were covered with a thin layer of decaying Alfalfa residues that had been terminated with herbicides after three years of growth. Canola was planted in these plots on 29 August 2017 after the Eh observation period. Measurements in the Standing Alfalfa plots (referred to as A+O-NT in plot maps) were made in standing vegetation during its second year of growth.

Study Site 2: Long Term Tillage (LTT) Experiment

The second study site was the ongoing Long Term Tillage experiment at the Pennsylvania State University Larson E. Russell Agricultural Research Center in Rock Springs, Centre County, PA. This experiment has been in place since 1978 as a randomized complete block design with four replications and six tillage treatments. The plots of interest in this experiment were No-Tillage (NT) and Moldboard plow (Till) and are approximately 230 meters by 14 meters. The “Till” treatments are moldboard plowed to a depth of 30–35 cm in the spring and then disked and harrowed. The soils are primarily Hagerstown and Hublersburg silt loam and were planted in continuous Corn until 2004, when the rotation was switched to three-year Soy-Wheat/legume cover-Corn. All fertilizers and amendments are applied based on Penn State University soil test recommendations. Redox measurements were collected in these plots continuously from 19 September to 3 October 2017. At the time of measurement, Hairy Vetch (*Vicia villosa*) and Oats (*Avina sativa*) were growing after being planted on 29 August 2017.

Redox Measurements

To measure Eh, custom-made equipment from MVH Consult (Netherlands) was used. The fiberglass probes were 8 mm in diameter with platinum sensors at depths of 4-cm and 10-cm from the soil surface. Measurements at these depths accounted for potential influence of surface residue decomposition and encompassed activity within the plow-layer zone of tilled soils. There were 8 probes connected to the data logger labeled 1A, 1B, 2A, 2B, 3A, 3B, 4A, and 4B, each of them having a sensor at the 4- and 10-cm depths. Probes with the same number were not related, they are simply connected next to each other on the datalogger. The custom datalogger (HYPNOS IV, MVH Consult) recorded measurements taken every 15 minutes. To account for variability, probes remained in the soil for a 3-5 day period prior to data collection to allow for stabilization. The data were stored on an SD card and the datalogger itself was entirely battery operated. This equipment provided 48 redox channels (1 per Pt sensor) although only 16 channels were used in this setup, in addition to a channel for one glass AgCl reference probe.

Redox potential was measured in adjacent plots of two separate treatments, 2 probes in each of the SDCS treatments and 4 probes in each treatment of the long term tillage experiment. Probes were randomly placed within a 15 m distance from the datalogger and between crop rows. The newly developed equipment is said to be suited to provide extremely stable measurements and limit any “drift” of Eh values associated with the equipment (Vorenhout et al., 2011).

Carbon Measurements

Six soil samples were collected and composited at depths of 0-5cm and 5-15cm from each plot on the first day of probe placement to quantify soil carbon at the time of sampling. Labile carbon was determined using the Permanganate Oxidizable Carbon (POXC) protocol described by Weil et al. (2003) and Total Organic Carbon (TOC) was measured using a CHNS-O elemental analyzer (CE Instruments, Wigan, UK).

Precipitation

Daily rainfall data were gathered from the NRCS-ARS-SCAN site at Rock Springs, Pennsylvania. The weather station was located less than 0.5 km from the SDCS.

Statistics

Statistical analyses of measured Eh and soil properties were performed using analysis of variance (ANOVA) with repeated measures in PROC MIXED in SAS (v.9.4) to analyze differences between treatments. Degrees of freedom were approximated using the Kenward-Roger method and means were compared using LS MEANS. Tukey adjustments were made for p-values when testing differences between means. Carbon data from the Long Term Tillage experiment were analyzed using T-Tests in R. Comparisons were considered significantly different at $p \leq 0.05$.

Results and Discussion

Measurements of Eh

Sustainable Dairy Cropping System (SDCS)

Block 2: 10 August to 15 August

The focused region in each figure, highlighted by a red rectangle, is from 13 to 14 August 2017, 3 days after probe insertion. Comparisons across treatments were made focusing on measurements during this time period.

In the Terminated Alfalfa treatment at 4 cm (Figure 4-1a), there seems to be no distinguishable pattern or trends within the data. Probes 1A and 1B measured consistently in the oxic zone at this depth. However, at the 10-cm depth (Figure 4-1b), probe 1B was more variable, periodically measuring values in the range of nitrate respiration within the highlighted 48 hour period. Measurements from probe 1B seem to decrease rapidly on 13 August without explanation. There was a rainfall event of 20.6 mm on this date that this could be attributed to. This could also be attributed to equipment malfunction or poor soil contact.

At the 4cm depth in the Standing Alfalfa treatment (Figure 4-1c), the initial measurement from probe 3A was roughly +1250mV. This was exceptionally high compared to other measurements made during this time period, and was attributed to the instrument stabilizing after the probes are inserted. Measurements from probe 3A ranged from roughly 220mV to 620mV in the highlighted 48 hour period. There was little response from probe 3B at this depth.

The two probes measuring at the 10cm depth of the Standing Alfalfa treatment (Figure 4-1d) demonstrated conflicting trends. Probe 3A measured values from roughly -250mV to +400mV within the 48 hour period while probe 3B measured values within the range of +400mV to +700mV. It also seems that when probe 3A measured the lowest values of the series, probe 3B was measuring the highest. These conflicting trends in the data demonstrate that high variability makes associating redox potentials with a specific crop and soil depth is extremely difficult.

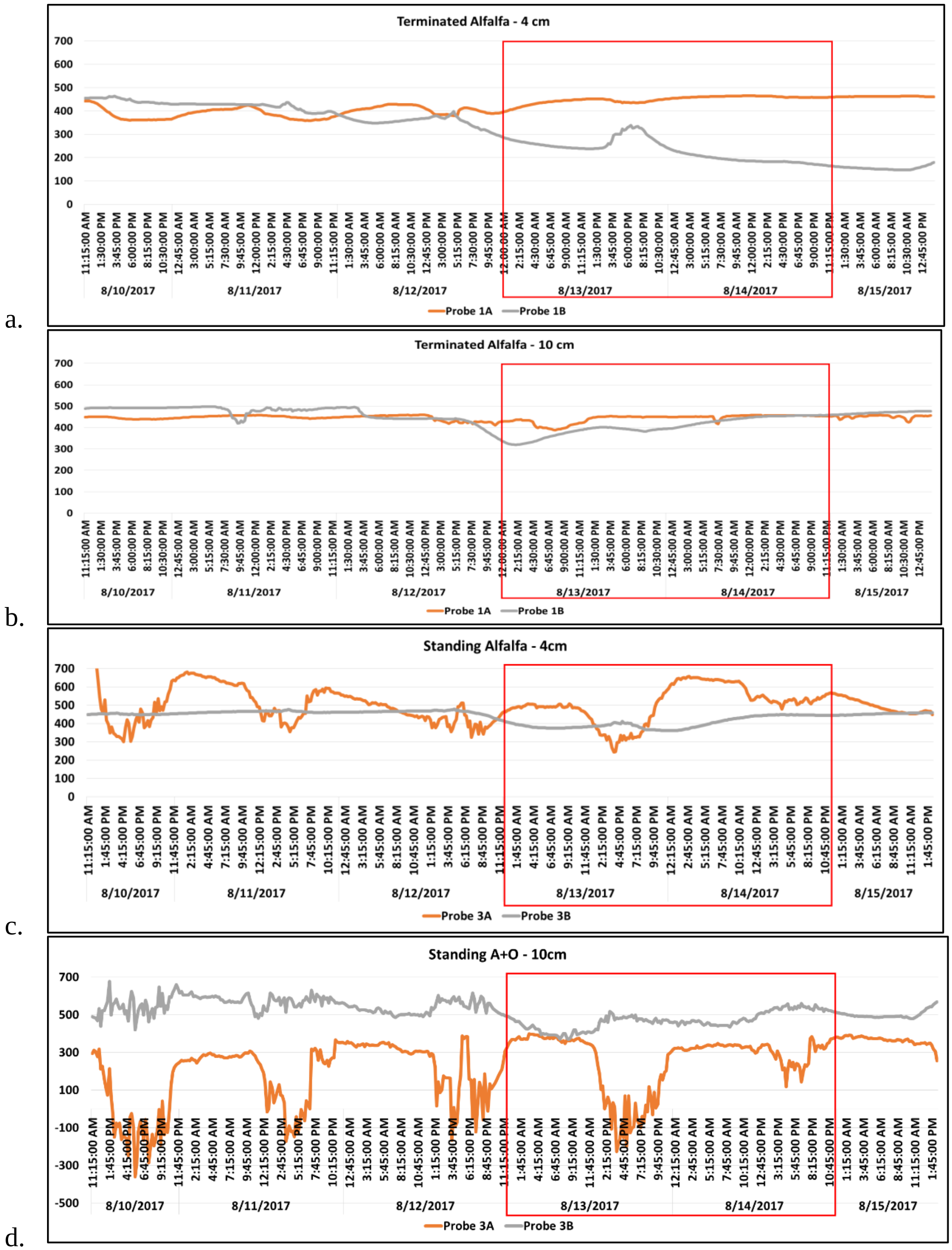


Figure **4-4**. Measurements of Eh in the Terminated Alfalfa treatment-4cm (a) and 10cm depths (b), Standing Alfalfa at 4cm (c) and at 10cm (d) from 10 August to 15 August 2017. Each colored line represents one of four probes at a specific depth within the same plot.

Block 4: 22 August to 29 August

The focused region, highlighted by a red rectangle, is from 25 to 26 August 2017, 3 days after probe insertion. Comparisons across treatments will be made looking at measurements during this time period.

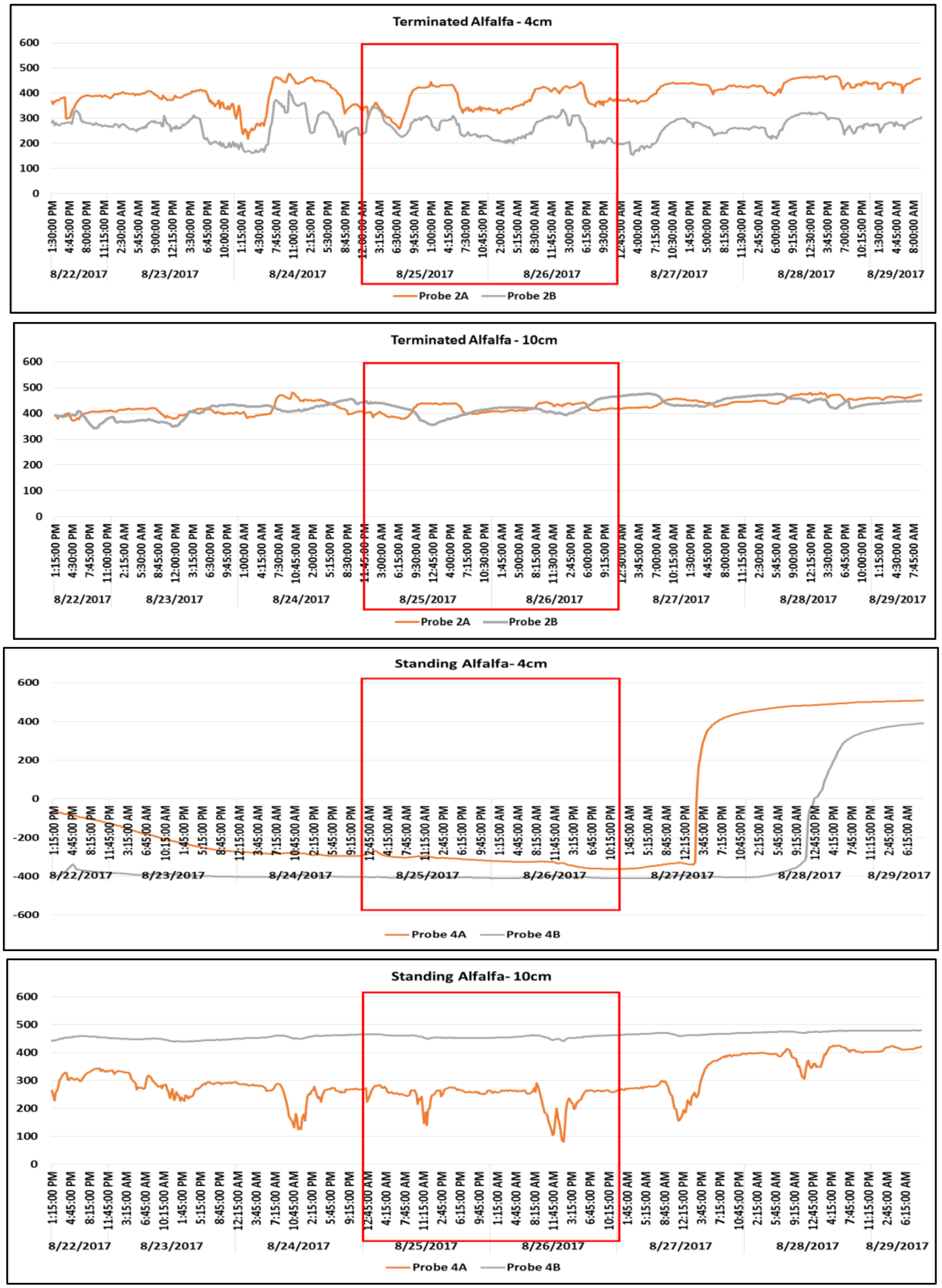
Eh values measured at the 4cm depth in this block of Terminated Alfalfa (Figure 4-2a), showed very similar trends between the two probes. Values ranged from roughly 200 to 430mV for both probes and dropped into the anoxic region within the 48 hour highlighted window.

Values measured at the 10cm depth in Terminated Alfalfa (Figure 4-2b) demonstrated a narrow range of measurements. Eh ranged from 350 to 440mV within the 48 hour period for both probes and the two probes almost seemed to have opposing trends as seen at the 4cm depth. Measurements seemed to overlap for most of the sampling period, remaining in the oxic region.

The 4cm measurements in the Standing Alfalfa (Figure 4-2c) treatment showed very different trends than any of the other measured plots. The measured values from both probes were negative, going as low as -400mV. Without explanation, values measured by probes 4A and 4B greatly increased on 27 August and 28 August, respectively. These unexplained values seem to reflect equipment unreliability rather than actual measurements. There was one small rainfall event on 26 August (6.86 mm) that this sudden increase could be attributed to but this is not clear. Within the 48 hour window, there was little variation in measurement, the values being strictly anoxic.

Probe 4B in the Standing Alfalfa treatment at 10cm (Figure 4-2d) showed almost no variability throughout the period of measurement, remaining at roughly +450 to +475 mV throughout the entire sampling period. It is unclear whether this is equipment error or an accurate measurement. Probe 4A demonstrates some periodic decreases in Eh, dropping as low as +90mV, observed within the 48 hour window.

Throughout these treatments, at the 4 cm depth, whether it's till or no-till, measurements from both probes tend to overlap, roughly falling within the +200 to +500mV range for the highlighted period (excluding Standing Alfalfa, Block 4). For both till and no-till at the 10 cm depth, the lines don't overlap, with each trace having its own separate range, with some traces being in the anoxic range. This demonstrates greater spatial variability in redox potential at 10 cm than at 4 cm, regardless of tillage type.



a.
b.
c.
d.

Figure **4-2**. Measurements of Eh in the Terminated Alfalfa treatment-4cm (a) and 10cm depths (b), and the Standing Alfalfa treatment at 4cm (c) and 10cm (d) from 22 August to 29 August 2017. Each colored line represents one of four probes at a specific depth within the same plot.

Long Term Tillage Experiment

Each of the lines on a graph represents the readings at a given depth from a single probe. The focused region, highlighted by a red rectangle, is from 24 to 26 September 2017, 3 days after probe insertion. Comparisons across treatments will be made looking at measurements during this time period.

At the 4cm depth in the tilled soils (Figure 4-3a), Eh values remained oxic over the highlighted period. There was minimal variation across most of the probes, however, measurements from probe 3A varied by roughly 160 mV within the 72 hour period. Probe 1B shows almost no variation for the rest of the sampling period except for a drop in Eh on 30 September. This was also the day of a small rainfall event (0.25mm) which may explain this change. Also, 21 September, where Eh was the highest, there was another rainfall event of the same magnitude (0.25mm). It is possible these sudden changes in Eh could be due to precipitation. Probes 1A and 3B showed almost no response throughout the 12 days. This could have been due to poor soil-Eh probe contact or an accurate measurement.

In the 10cm depth of this treatment (Figure 4-3b), measurements from probes 1A and 1B remained oxic and steady. Probe 3A exhibited sufficiently low Eh to allow nitrate respiration for the whole two day period, and probe 3B varied enough to support nitrate respiration for a portion of the time. There were conflicting trends between probes 3A and 3B, which made it difficult to discern a clear pattern. There also seemed to be a small shift in the measurement patterns after the rainfall on 30 September.

In the soils receiving no-tillage, at the 4cm depth (Figure 4-3c), both probes demonstrated quite a bit of variation. There seems to be no impact on Eh from the rainfall event on 21 September, but the pattern in both probes changed on 30 September, which is consistent with the tilled treatment at both depths. During the highlighted period, values measured by both probes were low enough to support nitrate respiration for a period of time.

Similar to the tilled soils, at the 10cm depth (Figure 4-3d), there are opposing trends between probes in the same treatment, however, the measured values seem to match more closely after 30 September, which is when one of the rainfall events occurred. The two probes seem to measure minimal changes in Eh and there seems to be no measurable changes after the first rainfall on 21 September.

Within the no-till treatment, Eh values were lower at the 4-cm depth, than at the 10-cm depth. This may be the result of crop residues on the soil surface of these plots. There seemed to be lower variability in the tillage plots at both depths than in the no-till plots. This may be because of the homogeneous nature of tilled soils compared no stratified, no-till soils. However overall, the Eh values from this experiment were variable within a single plot and hard to relate to tillage and measurement depth.

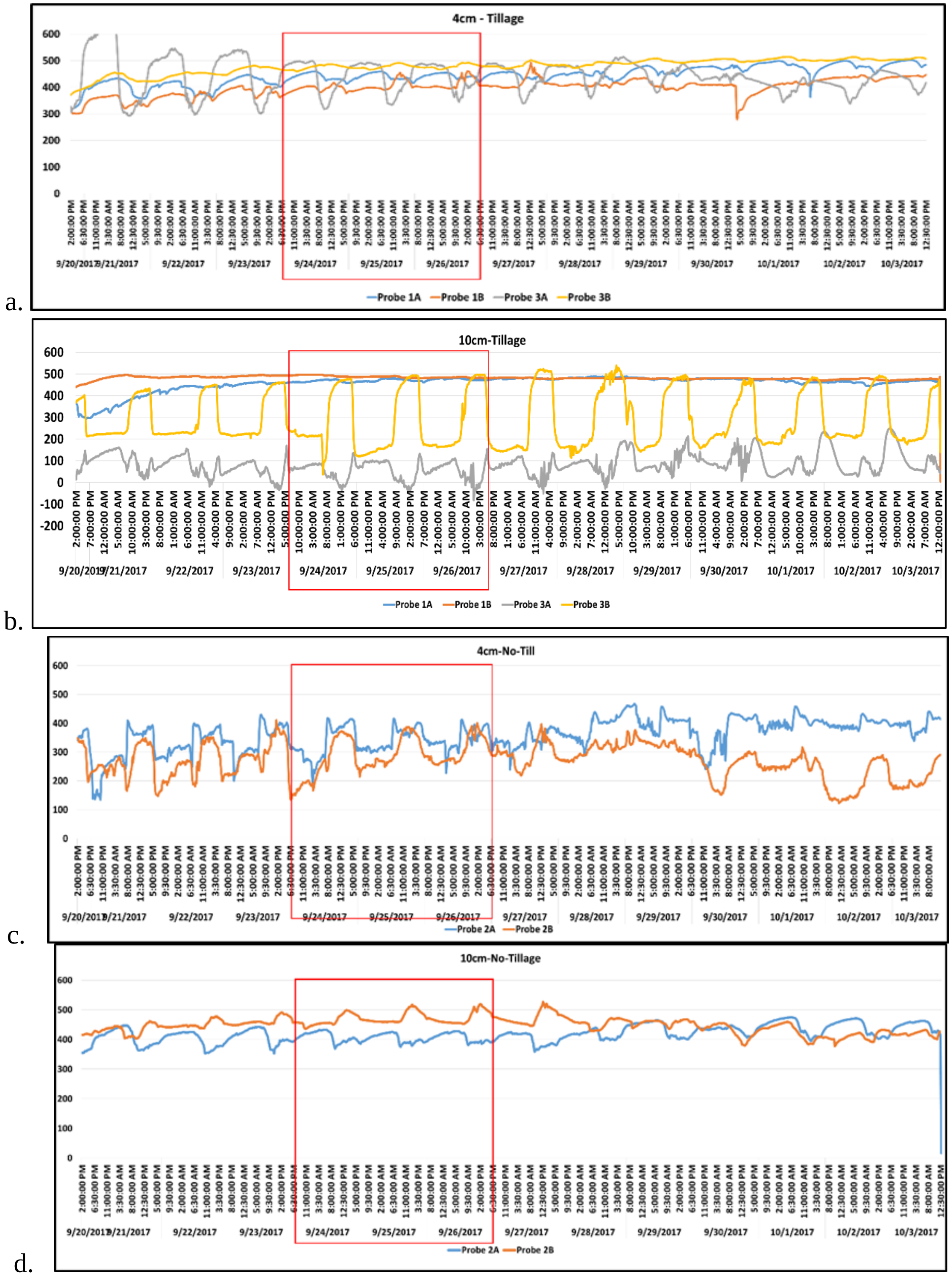


Figure **4-3**. Measurements of Eh at the 4cm depth (a) and 10cm(b) depth in the tilled treatment, and the 4cm depth (c) and 10cm depth (d) in the no-till treatment. Each colored line represents one of two probes within the same plot.

Summarized Eh results

The range, mean, and median for each set of measurements are shown in Table 4-2.

Throughout this project, there were common trends across treatments based on what probe was measuring Eh values. This could have been due to equipment malfunction and is important to note.

Table **4-2**. The measurement range, mean, median, and standard error of each set of measurements collected for this experiment.

Sampling Dates – Location Probe	Measurement Range (mV)	Mean	Median	Standard Error
13 to 14 August 2017 - SDCS				
Terminated Alfalfa NT-4cm				
1A	400-460 (60)	449	452	16
1B	160-340 (180)	231	238	46
Terminated Alfalfa NT-10cm				
1A	310-460 (150)	405	400	41
1B	390-460 (70)	442	451	19
Standing Alfalfa NT-4cm				
3A	220-620 (400)	508	509	100
3B	380-440 (60)	402	364	30
Standing Alfalfa NT-10cm				
3A	(-250)-400 (650)	261	326	149
3B	390-560 (170)	468	467	46
25 to 26 August 2017 - SDCS				
Terminated Alfalfa NT-4cm				
2A	260-430 (170)	371	364	46
2B	190-340 (150)	260	253	41
Terminated Alfalfa NT-10cm				
2A	380-440 (60)	417	415	18
2B	350-460 (110)	414	418	26
Standing Alfalfa NT-4cm				
4A	(-280)-(-380) (100)	-320	-320	23
4B	(-405)-(-395) (10)	-405	-405	4
Standing Alfalfa NT-10cm				
4A	90-300 (210)	245	259	39
4B	440-470 (30)	457	456	5

24 to 26 September 2017 – LTT (Hairy Vetch and Oats)				
Tillage-4cm				
1A	400-430 (30)	440	439	12
1B	390-450 (60)	404	400	19
3A	320-480 (160)	441	472	55
3B	480-500 (20)	477	477	8
Tillage-10cm				
1A	490-500 (10)	471	472	6
1B	490-500(10)	488	487	5
3A	(-50)-120 (170)	54	66	43
3B	180-520 (340)	282	215	140
No-Till-4cm				
2A	200-410 (210)	340	335	46
2B	150-400 (250)	288	277	62
No-Till-10cm				
2A	390-420 (30)	406	404	16
2B	420-500 (80)	471	462	20

Probes 1A and 1B demonstrated very minimal variation regardless of what treatment they were placed in and depth (less than 180 mV). Probes 2A and 2B demonstrated potentially diurnal changes in all of the plots they were placed in for this experiment. The two probes were also very similar and mimicked each other throughout the measurement period, unlike other paired probes which measured very different values. Probes 3A and 3B demonstrated some diurnal variation at the 10-cm and showed very little response at the 4-cm depth. Both probes measured high variability at times, up to 650 mV at 10-cm for Probe 3A and up to 340 mV at 10-cm for Probe 3B in different plots. Probes 4A and 4B within block 4 of the SDCS seemed to be malfunctioning throughout the period of measurement. At the 4-cm depth, both probes measured Eh negative values for most of the sampling period before values randomly increased after 27 August to roughly 400-600 mV. At the 10-cm depth, probe 4B measured almost no variation throughout the sampling period and values remained at roughly 450 mV. Probe 4A seemed to measure some diurnal variation, but measured values remained less than 450 mV. These are the only recorded measurements for these probes within the scope of this project and trends were unlike any measurements from other probes. Observing the patterns measured by each probe, it is possible that these measurements could be compromised because of equipment malfunction.

The high variability in Eh observed in this study is consistent with other studies, where soil Eh varied with soil depth diurnally as well as seasonally (Verhoef et al., 2006). In one report, a very rapid decrease in Eh from + 543 to +70 mV was observed within a few hours after a flooding event, and restoration of the initial values did not occur until days following drainage (Balakhnina et al., 2010). Soil aggregation, which fosters the presence of both oxic and anoxic microsites, further complicates the process of measuring Eh, which can vary up to 200 mV from the inside of a 6-7 mm soil aggregate to the outer periphery (Kaurichev and Tararina 1972, Sexstone et al., 1985).

Nevertheless, there is some evidence that Eh could serve as an indicator of soil health for agricultural soils. Soil health is the “continued capacity of a soil to function” and is especially of interest in agricultural systems (NRCS, 2018). Redox potential has been evaluated as a metric of soil health to track improvement efforts. In one study by Ugwegbu et al. (2001), Eh was measured to evaluate the success of removing excess agricultural chemicals from unsaturated soils. In that study, contaminated soils tended to be electron acceptor or donor limited, and the authors proposed that the addition of a source of electron donors or acceptors would be a strategy for remediation. Eh was used to estimate the type of redox couples (electron donors and acceptors) within the system and effectiveness of the chosen treatment (Ugwuegbu et al. 2001). This study provided support for the idea that Eh could be an indicator of soil health in cultivated soils.

Eh values ranging from 300 mV to 500 mV have been associated with oxic, cultivated soils in studies by Husson et al. (2013) and Macías and Arbestain (2010). In oxic soils with Eh greater than 300 mV, the primary electron acceptor is oxygen, however at lower potentials (300 mV to 0 mV), nitrate may be utilized, possibly altering the microbial community and subsequent

N-cycling processes. In one study, soil water was extracted for Eh measurement to characterize different ecological sites (Snakin et al., 2001). Based on this property, the authors were able to categorize ecological sites by: (i) ecosystem type (agricultural, grassland, and forest); (ii) vegetation type (coniferous, broad-leaved, meadow, meadow-steppe, and steppe vegetation); and (iii) soil type (separately for agricultural and natural communities). This study indicated the relationships between Eh and ecological management.

According to one study, the optimum Eh for plant growth was in the range of +400 to +450 mV (Husson, 2013). This ideal range was proposed to be based on the period of transition between the two major forms of inorganic N, NO_3^- and NH_4^+. Nitrate is more thermodynamically stable under oxidized conditions (Eh>500 mV), while NH_4^+ dominates under Eh conditions less than 400 mV. In many plants, the most growth is obtained with both NO_3^- and NH_4^+ (Marschner 1996). However these linkages are poorly established, and before Eh can be used as an indicator of soil quality, further research is needed on “ideal” Eh ranges for plant growth.

Because unsaturated soils can have both oxic and anoxic microsites, Eh values will be extremely variable spatially and temporally, so that relating Eh to agricultural management is expected to be challenging. According to Husson (2013), four main agricultural practices that can affect soil Eh are: soil amendments, water management, crop rotation, and land preparation. One study evaluated how conservation-based agricultural management altered soil electrical properties, including redox potential (Husson, 2018). In this study, Eh values were measured using an Ag/Cl reference electrode and a voltmeter. These measurements were not continuous, but taken at one time point and values recorded after values stabilized for at least one minute with no change. Values were also adjusted based on the temperature, pH, and the normal hydrogen electrode. In each of the four research sites, all of differing soil types, there were significant differences in Eh between conventional (C) and conservation agriculture (CA) systems. Eh was significantly higher at the 0-5cm depth in C than in CA soils at all four sites. Averaged across four sites, the mean Eh at the 0-5cm depth for the C soils was roughly +550mV, while the mean Eh for CA soils was roughly +497mV. Also, there was an increase in Eh with depth within the CA systems and the opposite trend in C systems. These authors found that redox potential was a “promising indicator” for evaluating soil health and may improve understanding of complex agronomic systems and processes. This study also demonstrated significant differences in electrical behavior between CA systems and conventional systems.

There is evidence that crop selection and rotation can influence soil redox potential. One study in the Czech Republic reported significant differences in Eh with depth and across cropping rotations. Measured Eh values were consistently higher in the upper 30 cm of topsoil than in the subsoil (30 cm-60 cm). The same study also measured significantly higher Eh values in a Sugar Beet/Barley cropping rotation than in an Alfalfa/Wheat rotation (Bohrerova et al., 2004). These authors attributed the difference in Eh values across cropping systems to the influence of the crops on soil moisture conditions and the deeper rooting depth of Alfalfa compared to that of the other studied crops. Researchers were also able to correlate higher Eh values with lower rates of nitrification. This study provided evidence for the influence of crops on soil redox potential and the capacity of redox potential to serve as an indicator of microbial processes, like nitrification.

Carbon Fractions

SDCS

Soil samples were taken on the first measurement date in each respective block (Block 2: 10 August 2017, Block 4: 22 August 2017). There were no significant differences in POXC or TOC across crop or a crop*depth interaction in this experiment (Figure 4-4). However, depth was a significant factor in both carbon fractions. For POXC values, p=0.0108, meaning that the values measured at 0-5cm were significantly higher than values measured at 5-15cm for all treatments. This is to be expected in no-till soils where carbon stratification is common. This was also the case for TOC, where p=0.0072.

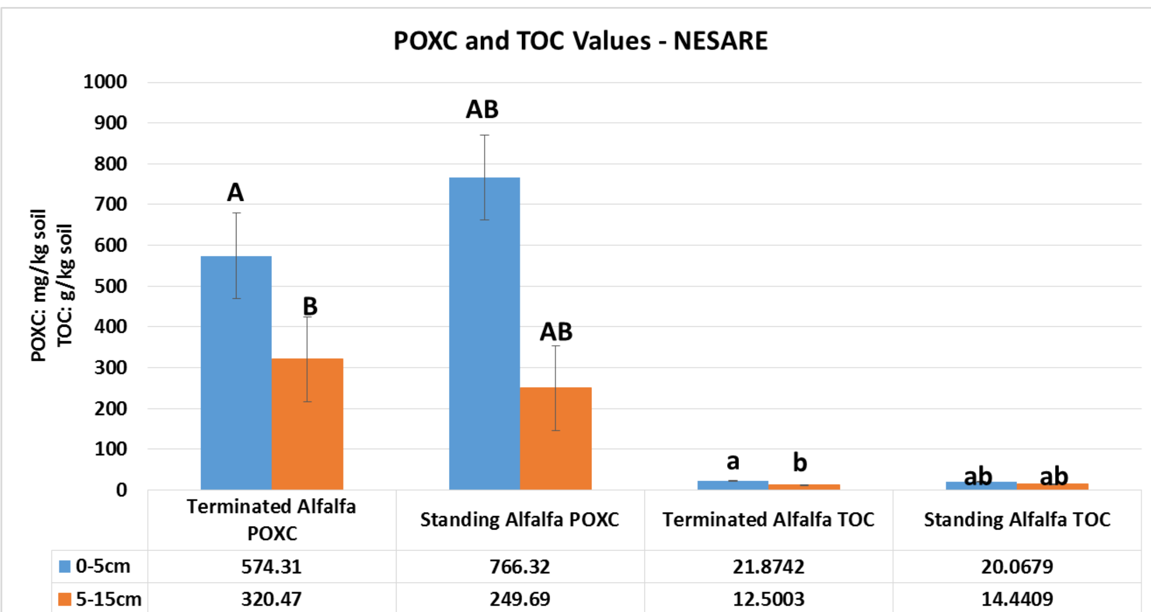


Figure **4-4**. Measured POXC and TOC values averaged across blocks 2 and 4. Different letters indicate a significant difference at p<0.05.

Because of the high variability and inconsistencies in Eh measurements, it's hard to correlate this soil property with either TOC or POXC. POXC is higher at the 0-5cm depth than the 5-10cm depth in Standing Alfalfa, but not in Terminated Alfalfa. This can be attributed to the fact that Standing Alfalfa was an established crop and Canola had not been planted at the time of measurement.

Long Term Tillage Experiment

Soil samples analyzed for POXC and TOC were collected from the long term tillage plots on 20 September 2017. TOC was significantly higher in the no-till treatment (p-0.008) than in the tilled treatment when both depths were combined but there were no significant differences in POXC between the tilled and no-till treatment.

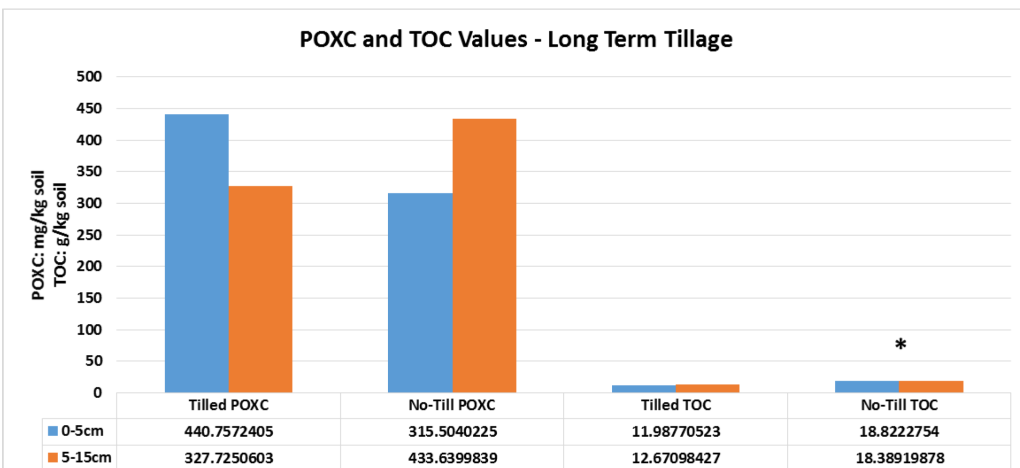


Figure **4-5**. Measured POXC and TOC values from plots in the Long Term tillage experiment. *denotes a significant difference across treatments at $p<0.05$.

It was anticipated that POXC and TOC values at the 0-5cm depth would be higher than values at the 5-15cm depth in the no-till treatment due to stratification. However, there were no significant differences in POXC or TOC across depth. With more replications or additional time points, depth may be significant. Comparing carbon measurements to Eh measurements in the same plots, although these are only single measurements, POXC values at the 0-5cm depth seem higher than values in the 5-15cm depth in the tilled treatment. This mimics Eh values, which were greater at the 4cm depth than at the 10cm depth. This is also the case for the no-till treatment, where Eh and POXC values at the 10cm and 5-15cm depths, respectively, seem higher than values measured at 4cm and 0-5cm.

Conclusions

Eh has been shown to be an indicator of soil health during the remediation processes of contaminated soils (Ugwuegbu et al. 2001). However, current equipment constraints and variability within treatments poses a challenge for using this property to evaluate soil health in agricultural settings. This study sought to measure soil redox potentials in cultivated soils and determine if values were 1) comparable across replications of the same treatment, 2) interpretable, and 3) related to other selected soil properties and agricultural management. Results showed that with the chosen equipment, there was too much variability across the same treatment to make these connections. Also, it is unclear if the minimal response seen in some probes were actual measurements, the result of poor soil contact, or malfunctioning probes.

Prior studies have reported less redox potential variability with depth in Alfalfa plots than in other crops (Bohrerova et al., 2004). This is attributed to the deep-rooting nature of the crop homogenizing the soil and measured redox potentials. The two blocks of Terminated Alfalfa seemed to have more similar measurements across block than those measured in the Standing Alfalfa treatment. Measurements across depth in block 2 of the Standing Alfalfa treatment seem similar, but redox potentials at the 4cm depth in block 4 were much lower than that at the 10cm depth. Further study, under controlled conditions, comparing Alfalfa with other chosen crops would need to take place to study the Eh of soils with Alfalfa compared to other crops.

Within the long-term tillage experiment, it seemed that there were no major differences in Eh values between the tilled and no-till treatments. There did seem to be lower variability in Eh values measured in the tillage plots at both depths than in the no-till plots. This may be because of the homogeneous nature of tilled soils compared no stratified, no-till soils. In the no-till plots, lower Eh values at the 4-cm depth than at the 10-cm depth may be attributed to prior crop residues on the soil surface. Because of the overall high variability, the redox data were difficult to interpret and analyze for differences based on depth or between treatments. Also due to these properties, the relation to the carbon fraction POXC and TOC were not clear. However, this connection could be better understood under controlled conditions and repeated measurements over time.

There is potential for Eh to be used as a soil health indicator with continued research into optimal threshold redox potentials, equipment advances, and understanding of how changes in management impact this property. The production or consumption of the three major greenhouse gases (nitrous oxide (N_2O), methane (CH_4), carbon dioxide (CO_2)) are known to be correlated with redox potentials and other soil properties (Husson et al., 2018). With better understanding of soil Eh, conditions discouraging GHG production could be facilitated.

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Chapter 5

Conclusions

The idea of sustainable intensification is to increase agricultural productivity to meet current and future demands for food and fuel, while encouraging practices that conserve soil quality in the long term. From an environmental standpoint, conservation practices would sustain high yields and economic returns while minimizing environmental impacts normally associated with conventional agriculture. Increasing soil carbon and reducing disturbance are two strategies to mitigate negative effects of conventional agriculture and to improve soil quality. Practices that build soil carbon and water-holding capacity, however, may result in undesirable environmental impacts such as increased N_2O emissions.

Within the SDCS experiment, manure application, cover crops, and perennials are used as strategies to improve soil conditions and mitigate environmental impacts. Despite their soil quality benefits, these strategies did not reduce N_2O emissions in the 2017 or 2018 growing seasons. The greatest N_2O emissions were measured in Alfalfa+Orchardgrass receiving broadcast manure prior to Corn and the lowest in soil with no winter cover receiving synthetic fertilizer. Although incorporating perennials and applying manure are practices known to increase soil health, high N_2O emissions associated with these management decisions represent a tradeoff that needs to be considered with their implementation. The soil properties measured in this experiment were not able to explain patterns in N_2O fluxes. This study did not provide evidence that measured carbon and nitrogen in soil affected N_2O emissions.

In addition to addressing nutrient losses and soil health, farmers in PA are also adapting their management strategies to account for climate change and increasingly wet spring weather conditions. Fall perennial termination is an effective strategy to ensure proper termination before the main crop is planted in the spring and avoid delays in other field operations. No reductions in yield were associated with fall perennial termination and there were no significant differences in cumulative emissions between treatments during the period of measurement. High fluxes from the spring terminated treatment compared to the fall terminated treatment, suggested greater N_2O losses in the spring. However, the lack of N_2O emissions data taken during the previous fall following perennial termination precludes direct comparison of overall N_2O emissions from the two management treatments. Further analysis of these data suggested soil temperature was an effective predictor of N_2O emissions in both spring and fall terminated treatments in this experiment.

Understanding redox potentials in cropping systems would give important insight on nutrient cycling and potential conditions or strategies to mitigate these losses. However, this research has shown the challenges associated with measuring Eh in agricultural soils, including high variability and equipment constraints. With further research on the relationship between carbon fractions and Eh and with equipment advances, soil redox has potential to be used as an indicator of soil health and nutrient cycling. Nutrient retention is one important function of agricultural soils that ultimately depends on the functioning of the microbial community. With more research, redox potential could be a way to evaluate and understand the conditions that facilitate these processes.

Overall, the data from this thesis provide evidence that winter cover prior to Corn does not reduce N_2O emissions compared to soils with no winter cover in addition to evidence that spring termination of the prior crop and application of manure may exacerbate emissions. This represents one of the costs associated with sustainable agricultural practices, although choosing to terminate a perennial in the fall may reduce this effect. The decision to implement more sustainable practices, especially the transition to no-till, can present long-term benefits to farmers under the right climate and soil conditions. Although the initial switch to no-till can present some increased costs (transitioning equipment, increased herbicides, etc.), the soil health benefits, reduced equipment use, and reduced erosion pay off in the long term. Also, in the midst of climate change, the resiliency of soil is of increasing interest. Implementing sustainable practices like no-till and cover cropping are important ways to increase resiliency and protect soil and crops from both droughty and wet conditions. Further research needs to continue to focus on the costs and benefits associated with sustainable agricultural practices and ways to evaluate them.

Appendix A: Chapter 2 Data

Table **A-3**. 2017 Environmental Data.

	Soil Moisture	**Standard Error**	**Soil Temperature**	**Standard Error**
SS-RYE-BM (2017)				
20-Jun	36.5	2.3	22.4	0.9
28-Jun	24.8	2.3	21.8	0.9
5-Jul	22.0	2.3	23.8	0.9
11-Jul	25.8	2.3	22.2	0.9
18-Jul	21.3	2.3	23.8	0.9
13-Jul	30.7	2.3	21.8	0.9
Treatment Mean	**26.8**	**1.6**	**22.6**	**0.7**
SS-CC-BM (2017)				
20-Jun	33.6	2.3	22.7	0.9
28-Jun	20.7	2.3	22.2	0.9
5-Jul	22.6	2.3	24.0	0.9
11-Jul	23.6	2.3	22.5	0.9
18-Jul	22.3	2.3	23.8	0.9
13-Jul	27.3	2.3	21.8	0.9
Treatment Mean	**25.0**	**1.6**	**22.8**	**0.7**
AO-AO-BM (2017)				
20-Jun	31.5	2.3	23.4	0.9
28-Jun	22.1	2.3	22.6	0.9
5-Jul	26.5	2.3	23.9	0.9
11-Jul	24.5	2.3	22.6	0.9
18-Jul	25.1	2.3	23.5	0.9
13-Jul	31.4	2.3	21.5	0.9
Treatment Mean	**26.8**	**1.6**	**22.9**	**0.7**
SOY-NONE-UAN (2017)				
20-Jun	30.8	2.3	22.2	0.9
28-Jun	19.3	2.3	21.8	0.9
5-Jul	20.2	2.3	23.5	0.9
11-Jul	27.7	2.3	21.3	0.9
18-Jul	23.9	2.3	22.0	0.9
13-Jul	29.2	2.3	20.1	0.9
Treatment Mean	**25.2**	**1.6**	**21.8**	**0.7**
SOY-NONE-BM (2017)				
20-Jun	27.0	2.3	22.5	0.9
28-Jun	15.1	2.3	22.0	0.9
5-Jul	17.8	2.3	24.2	0.9
11-Jul	20.4	2.3	21.6	0.9
18-Jul	21.4	2.3	22.2	0.9
13-Jul	30.3	2.3	20.4	0.9
Treatment Mean	**22.0**	**1.6**	**22.1**	**0.7**

Table **A-4**. 2017 Inorganic N data.

	Nitrate	**Standard Error**	**Ammonium**	**Standard Error**
SS-RYE-BM (2017)				
10-May-17	1.3924	0.4675	0.2629	0.5993
22-Jun-17	2.7775	0.7628	0.1992	0.5993
28-Jun-17	2.335	0.8493	0.226	0.5993
13-Jul-17	3.565	1.3039	2.3947	0.5993
31-Jul-17	0.4325	0.5866		
Treatment Mean	**2.10048**	**0.3906**	**0.7707**	**0.3462**
SS-CC-BM (2017)				
10-May-17	0.3375	0.4675	2.1185	0.5993
22-Jun-17	3.385	0.7628	0.3309	0.6672
28-Jun-17	2.6525	0.8493	0.1405	0.5993
13-Jul-17	5.5375	1.3039	0.7721	0.5993
31-Jul-17	1.7975	0.5866		
Treatment Mean	**2.742**	**0.3906**	**0.8405**	**0.3462**
AO-AO-BM (2017)				
10-May-17	1.0403	0.4675	0.6022	0.5993
22-Jun-17	2.5025	0.7628	0.2055	0.5993
28-Jun-17	2.196	0.8493	0.3975	0.5993
13-Jul-17	8.2575	1.3039	1.4124	0.5993
31-Jul-17	1.4725	0.5866		
Treatment Mean	**3.0938**	**0.3906**	**0.6544**	**0.3462**
SOY-NONE-UAN (2017)				
10-May-17	0.3425	0.4675	0.2939	0.7004
22-Jun-17	2.34	0.7628	0.1845	0.5993
28-Jun-17	1.8925	0.8493	1.005	0.5993
13-Jul-17	7.9375	1.3039	0.5972	0.5993
31-Jul-17	1.4575	0.5866		
Treatment Mean	**2.794**	**0.3906**	**0.5202**	**0.3462**
SOY-NONE-BM (2017)				
10-May-17	0.35	0.4675	0.3321	0.5993
22-Jun-17	3.05	0.7628	0.2943	0.5993
28-Jun-17	1.1725	0.8493	0.2307	0.5993
13-Jul-17	4.15	1.3039	0.2183	0.5993
31-Jul-17	0.485	0.5866		
Treatment Mean	**1.8415**	**0.3906**	**0.2689**	**0.3462**

Table **A-5**. 2017 Carbon Data.

	POXC (0-5cm)	Standard Error	POXC (5-15cm)	Standard Error	TOC (0-5cm)	Standard Error	TOC (5-15cm)	Standard Error
SS-RYE-BM (2017)								
10-May-17	534.05	54.352	254.26	54.352	20.704	1.647	13.945	1.647
2-Jun-17	618.17	54.352	297	54.352	20.697	1.647	13.391	1.647
28-Jun-17	552.56	54.352	297	54.352	20.611	1.647	11.568	1.647
Treatment Mean	**568.26**	**13.624**	**282.7533**	**13.624**	**20.6706**	**0.874**	**12.968**	**0.874**
SS-CC-BM (2017)								
10-May-17	501.25	54.352	138	54.352	24.39508	1.647	16.41863	1.647
2-Jun-17	614.12	54.352	309.15	54.352	19.091	1.647	13.008	1.647
28-Jun-17	573.21	54.352	280.8	54.352	17.663	1.647	11.314	1.647
Treatment Mean	**562.86**	**13.624**	**242.65**	**13.624**	**20.3830**	**0.874**	**13.58021**	**0.874**
AO-AO-BM (2017)								
10-May-17	380.65	54.352	176.59	54.352	20.313	1.647	12.722	1.647
2-Jun-17	680.54	54.352	370.31	54.352	18.144	1.647	13.208	1.647
28-Jun-17	474.79	54.352	290.52	54.352	20.313	1.647	12.722	1.647
Treatment Mean	**511.993**	**13.624**	**279.14**	**13.624**	**19.59**	**0.874**	**12.884**	**0.874**
SOY-NONE-UAN (2017)								
10-May-17	670.09	54.352	448.19	54.352	20.827	1.647	15.817	1.647
2-Jun-17	501.93	54.352	427.01	54.352	16.305	1.647	16.388	1.647
28-Jun-17	458.6	54.352	315.63	54.352	16.305	1.647	16.388	1.647
Treatment Mean	**543.54**	**13.624**	**396.9433**	**13.624**	**17.8123**	**0.874**	**16.19767**	**0.874**
SOY-NONE-BM (2017)								
10-May-17	621.37	54.352	550.94	54.352	17.923	1.647	17.828	1.647
2-Jun-17	524.21	54.352	407.57	54.352	15.767	1.647	12.86	1.647
28-Jun-17	484.52	54.352	373.95	54.352	15.56295	1.647	13.64403	1.647
Treatment Mean	**543.366**	**13.624**	**444.1533**	**13.624**	**16.4176**	**0.874**	**14.77734**	**0.874**

Table **A-6**. 2018 Environmental Data.

	Soil Moisture	**Standard Error**	**Soil Temperature**	**Standard Error**
SOY-NONE-UAN				
31-May-18	25.3781	2.8	20.0	0.8
2-Jun-18	22.55	2.6	20.9	0.7
4-Jun-18	32.775	2.6	17.0	0.7
11-Jun-18	37.275	2.6	15.2	0.7
15-Jun-18	29.375	2.6	18.7	0.7
19-Jun-18	36.175	2.6	23.3	0.7
29-Jun-18	30.25	2.6	23.1	0.7
11-Jul-18	18.4	2.6	25.1	0.7
Treatment Mean	**29.0**	**2.1**	**20.4**	**0.3**
SOY-NONE-BM				
31-May-18	33.7	2.6	20.3	0.7
2-Jun-18	30.6	2.6	21.1	0.7
4-Jun-18	36.4	2.6	17.3	0.7
11-Jun-18	41.0	2.6	15.3	0.7
15-Jun-18	30.2	2.6	18.7	0.7
19-Jun-18	36.5	2.6	23.2	0.7
29-Jun-18	29.6	2.6	23.4	0.7
11-Jul-18	19.2	2.8	24.8	0.7
Treatment Mean	**32.1**	**2.1**	**20.5**	**0.3**

Table **A-5**. 2018 Inorganic N Data.

SOY-NONE-UAN (2018)	Nitrate	Standard Error	Ammonium	Standard Error
8-Apr-18	0.6433	1.1184	0.2494	0.71
2-Jun-18	0.4793	1.1184	0.3042	0.71
4-Jun-18	2.6025	1.1184	0.1848	0.71
19-Jun-18	4.3875	1.1184	0.1385	0.71
29-Jun-18	1.5246	1.1184	0.6318	0.71
11-Jul-18	2.494	1.1184	1.9871	0.71
Treatment Mean	**2.0219**	**0.4699**	**0.5826**	**0.3291**
SOY-NONE-BM (2018)				
8-Apr-18	0.4793	1.2914	0.1252	0.8203
2-Jun-18	4.3875	1.1184	0.3474	0.71
4-Jun-18	2.4942	1.2951	0.2061	0.7602
19-Jun-18	5.6416	1.1184	0.5211	0.71
29-Jun-18	3.2862	1.1184	0.3651	0.71
11-Jul-18	8.8526	1.2923	2.6775	0.8284
Treatment Mean	**4.1902**	**0.5058**	**0.7071**	**0.3466**

Table **A-6**. 2018 Carbon Data.

	POXC (0-5cm)	Standard Error	POXC (5-15cm)	Standard Error	TOC (0-5cm)	Standard Error	TOC (5-15cm)	Standard Error
SOY-NONE-UAN (2018)								
28-Apr-18	755.12	72.5455	434.93	72.5455	17.1647	1.6385	12.3705	1.6385
2-Jun-18	720.78	72.5455	489.75	72.5455	15.7018	1.6385	13.2931	1.6385
4-Jun-18	629.71	72.5455	205.23	72.5455	16.5356	1.6385	11.526	1.6385
19-Jun-18	689.01	72.5455	472.48	72.5455	13.5483	1.6385	11.204	1.6385
29-Jun-18	686.29	72.5455	620.13	72.5455	17.0703	1.6385	11.2649	1.6385
11-Jul-18	688.78	72.5455	543.78	72.5455	14.2694	1.6385	11.1154	1.6385
Treatment Mean	**694.9483**	**35.64**	**461.05**	**35.64**	**15.71502**	**0.939**	**11.79565**	**0.939**
SOY-NONE-BM (2018)								
28-Apr-18	821.99	72.5455	560.49	72.5455	17.543	1.6385	11.1257	1.6385
2-Jun-18	964.38	72.5455	539.47	72.5455	19.5943	1.6385	12.6666	1.6385
4-Jun-18	679.65	72.5455	350.15	72.5455	18.012	1.6385	12.1036	1.6385
19-Jun-18	899.98	72.5455	456.98	72.5455	19.838	1.6385	12.5514	1.6385
29-Jun-18	648.32	72.5455	481	72.5455	21.1676	1.6385	13.6268	1.6385
11-Jul-18	690.04	72.5455	555.33	72.5455	17.0559	1.8749	11.4122	1.8749
Treatment Mean	**784.06**	**36.26**	**490.57**	**36.26**	**18.8685**	**0.9524**	**12.2477**	**0.9524**

Table **A-7**. ANOVA table for 2017 N_2O emissions.

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Treatment	4	12.3	5.66	0.0082
Sampling Date	9	25.8	4.55	0.0012
Treatment*Sampling Date	36	89.3	3.63	<.0001

Table **A-8**. ANOVA table for cumulative 2017 N_2O emissions.

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Treatment	4	12	4.69	0.0164

Table **A-9**. ANOVA table for 2018 N_2O emissions.

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Treatment	2	6	7	0.027
Sampling Date	9	27	2.77	0.0196
Treatment*Sampling Date	13	39	2.26	0.0247

Table **A-10**. ANOVA table for cumulative 2018 N_2O emissions.

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Treatment	2	6	8.07	0.0199

Table **A-11**. ANOVA table for 2017 and 2018 N_2O emissions.

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Treatment	2	6	9.59	0.0135
Sampling Date	17	51	2.65	0.0038
Treatment*Sampling Date	30	99	2.16	0.0024
Year	1	99	0	0.9554
Treatment*Year	2	99	0.01	0.9658

Table **A-12**. ANOVA table for 2017 and 2018 cumulative N_2O emissions.

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Treatment	2	6	11.17	0.0095
Year	1	3	7.71	0.0692
Treatment*Year	2	6	5.13	0.0502

Appendix B: Chapter 3 Data

Table **B-1**. ANOVA table for Spring and Fall terminated A+O N_2O emissions.

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Treatment	1	52.4	3.71	0.0594
Sampling Date	10	56.6	3.22	0.0025
Treatment*Sampling Date	10	56.6	2.02	0.0478

Table **B-2**. ANOVA table for Cumulative Spring and Fall terminated A+O N_2O emissions.

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Treatment	1	6	4.03	0.0915

Table **B-3**. 2018 Environmental Data.

	Soil Moisture	**Standard Error**	**Soil Temperature**	**Standard Error**
AO-FALLTERM				
28-Apr	29.1	2.4	.	.
2-May	23.7	2.4	20.2	0.5
14-May	38.9	2.4	22.6	0.5
23-May	31.2	2.4	18.5	0.5
2-Jun	33.7	2.4	21.4	0.5
4-Jun	36.5	2.4	17.4	0.5
11-Jun	41.7	2.4	15.3	0.5
14-Jun	37.8	2.4	20.4	0.5
19-Jun	38.1	2.4	23.7	0.5
29-Jun	33	2.4	24.2	0.5
11-Jul	21.7	2.4	24.5	0.5
Treatment Mean	**33.6**	**1.1**	**20.8**	**0.2**
AO-SPRTERM				
28-Apr	31.8	2.4	.	.
2-May	20.5	2.4	19.0	0.5
14-May	38.0	2.4	17.2	0.5
23-May	31.7	2.4	18.2	0.5
2-Jun	41.5	2.4	21.4	0.5
4-Jun	42.4	2.4	17.3	0.5
11-Jun	42.5	2.4	15.4	0.5
14-Jun	37.8	2.4	19.7	0.5
19-Jun	40.2	2.4	24.0	0.5
29-Jun	36.7	2.4	22.8	0.5
11-Jul	24.4	2.4	23.9	0.5
Treatment Mean	**35.60**	**1.1**	**19.9**	**0.2**

Table **B-4**. 2018 N Species Data.

	Nitrate	**Standard Error**	**Ammonium**	**Standard Error**
AO-FALLTERM				
28-Apr-18	0.19	0.44	0.19	0.19
2-Jun-18	2.42	0.44	0.89	0.19
4-Jun-18	2.48	0.44	0.51	0.28
11-Jun-18	2.90	0.44	0.03	0.19
14-Jun-18	3.17	0.44	0.31	0.28
19-Jun-18	2.25	0.44	0.43	0.26
29-Jun-18	3.23	0.44	0.47	0.19
11-Jul-18	4.32	0.44	0.65	0.19
Treatment Mean	**2.62**	**0.21**	**0.44**	**0.69**
AO-SPRTERM				
28-Apr-18	0.07	0.44	0.07	0.19
2-Jun-18	2.04	0.44	1.55	0.19
4-Jun-18	2.22	0.44	0.35	0.19
11-Jun-18	3.22	0.44	0.50	0.19
14-Jun-18	3.47	0.44	0.39	0.19
19-Jun-18	2.13	0.44	0.35	0.19
29-Jun-18	2.44	0.44	1.08	0.35
11-Jul-18	3.69	0.44	0.95	0.19
Treatment Mean	**2.41**	**0.21**	**0.66**	**0.69**

Table **B-5**. 2018 Carbon Data.

	POXC (0-5cm)	Standard Error	POXC (5-15cm)	Standard Error	TOC (0-5cm)	Standard Error	TOC (5-15cm)	Standard Error
AO-FALLTERM								
28-Apr-18	1032.81	69.6086	759.14	69.6086	20.2744	1.2738	14.3951	1.2738
2-Jun-18	945.96	69.6086	534.24	69.6086	18.1177	1.2738	14.9991	1.2738
4-Jun-18	603.27	69.6086	310.49	69.6086	20.3853	1.2738	13.752	1.2738
11-Jun-18	918.82	69.6086	571.23	69.6086	21.4118	1.2738	15.0736	1.2738
14-Jun-18	1095.18	69.6086	901.71	69.6086	23.0273	1.2738	16.0699	1.2738
19-Jun-18	841.64	69.6086	706.04	69.6086	22.6682	1.2738	14.046	1.2738
29-Jun-18	804.99	69.6086	407.98	69.6086	18.9669	1.2738	13.3032	1.2738
11-Jul-18	893.37	69.6086	650.4	69.6086	18.8982	1.2738	13.2331	1.2738
Treatment Mean	**892.01**	**30.3847**	**605.16**	**30.3847**	**20.8647**	**0.5074**	**14.359**	**0.5074**
AO-SPRTERM								
28-Apr-18	1099.5	69.6086	690.72	69.6086	21.5108	1.2738	14.8627	1.2738
2-Jun-18	664.23	69.6086	336.93	69.6086	20.0488	1.2738	19.6912	1.2738
4-Jun-18	626.77	69.6086	430.44	69.6086	21.647	1.2738	15.8015	1.2738
11-Jun-18	986.57	69.6086	564.58	69.6086	22.5586	1.2738	14.6513	1.2738
14-Jun-18	1054.66	69.6086	717.38	69.6086	21.1294	1.2738	15.0268	1.2738
19-Jun-18	777.57	69.6086	494.18	69.6086	22.6795	1.2738	16.2377	1.2738
29-Jun-18	976.01	69.6086	418.69	69.6086	21.3623	1.2738	13.9986	1.2738
11-Jul-18	889.4	69.6086	724.05	69.6086	17.6654	1.2738	14.2494	1.2738
Treatment Mean	**884.4**	**30.3847**	**547.12**	**30.3847**	**21.0752**	**0.5074**	**15.5649**	**0.5074**