Nitrogen dynamics and biological response to dairy manure applications

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Academic Abstract

Animal manures are land applied in agronomic systems to supply essential crop nutrients and decrease dependency on chemical fertilizers. Liquid manures are traditionally surface broadcast to fields and sometimes incorporated to reduce odor and nutrient losses; however, incorporation is incompatible with no-till agriculture. Subsurface manure injection is a no-till compatible alternative application method which addresses these concerns, but likely changes the dynamics of nutrient cycling. Comparison of the two application methods has yielded mixed results and warrants further research. Therefore, the objectives of this research were to contrast the surface broadcast and subsurface injection of dairy slurry on nitrogen and carbon cycling, crop yield, and biologic responses to proxy soil health. In a forced air-flow laboratory incubation, manure injection reduced ammonia volatilization by 87% and 98% in a sandy loam and clay loam soil, respectively. The increased ammoniacal nitrogen recovery resulted in increases of soil nitrate of 13% for the sandy loam and 26% for the clay loam after 40 days of incubation. Microbial measurements were inconclusive in the laboratory. In 7 site-years of field study, soil nitrate was greater in 7 of 25 measurements under manure injection and 30% higher under injection on average during the corn pre side-dress nitrate test (PSNT) time. Soil nitrate sampling methods were assessed for fields injected with manure; a standard random sampling method had a coefficient of variation (C.V.) of 28% and was as equally repeatable as utilizing an equi-spaced distribution of cores taken across an injection band, C.V. of 30%. Both biological responses, carbon mineralization

(C-min) and substrate induced respiration (SIR), were not different between application methods; both were highly variable and C-min was especially intensive logistically. Corn yield showed no consistent response to application method, but probably was not nitrogen limited. In 2 years of field study conducted on a university research farm injection resulted in greater 0-15cm soil nitrate levels than surface broadcast 1 week after application and persisted for 9 additional weeks. In injected plots, nitrate was concentrated in the injection band; nitrate movement was significant only 10cm lateral to the injection band but overall distribution fit well to a second degree polynomial, especially 2 and 4 weeks after application, $R^2>0.80$. Evidence of leaching was observed in one year after receiving considerable rainfall in weeks 1 and 2 after application. When corn grain yield was averaged year over year, injection was 26% greater than the no-manure control, and 15% greater than surface application. Both biological metrics, C-min and microbial biomass, were stratified by depth; C-min was concentrated within the manure band leading to greater mineralization under injected applications. Microbial biomass was significantly higher under injection at the 15-30cm depth. Overall biological response to manure application method was inconclusive, however manure injection is superior to surface application in terms of nitrogen recovery.

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General Audience Abstract

Animal manures supply nutrients essential to crop growth (notably nitrogen and phosphorous); liquid manures (pigs and dairy cattle) are commonly applied by spraying them on soils before tillage. Where no-tillage is used as a conservation measure subsurface injection can be used as an alternative to leaving manure on the soil surface. The purpose of this research was to assess nutrient cycling, crop yield, and soil health impacts of surface applied and injected dairy manure applications. Manure injection greatly reduces a nitrogen loss pathway, and as a result supplies more plant available nitrogen to the crop. Methods of soil sampling fields using injection were compared and a recommended sampling method was defined. Transport of a form of nitrogen vulnerable to movement in the ground was found to only travel 10cm away from where manure was injected. Transport of this form of nitrogen below the injection area was observed after abundant rainfall. Crop yields were sometimes higher under injection however, yields are also determined by factors other than nitrogen. Soil health was not repeatably improved under one application method, but microbial activity was greater at shallower soil depths.

Dedication

To my grandparents,
Michael Wayne Bierer and Frances Marcella Bierer,
I can only aspire to be as decent and loving as you,
Thank you for everything

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Introduction

In 2000, the U.S. population was estimated at 281 million and by 2010 had grown to an estimated 309 million people (U.S. Census Bureau, 2010). An expanding population presents challenges to agriculture in the production and management of crops and livestock. Progressively more crops, animals, and fibers will be needed to meet a growing population's demands, often having adverse effects on the environment. Therefore, it is of importance to progress technologies associated with animal and crop production to meet this growing demand while remaining indefinitely sustainable.

Food animal production, i.e. swine, cattle, and poultry production, has increased since 2012 (USDA, 2019). One of the challenges faced with increasing animal production is an increase in waste production. In 2012, it was reported that 275,420 farms utilize animal manures on over 22 million acres of land (USDA, 2014). In just 5 years, this number increased 8% to 297,297 farms using manures on nearly 24 million acres (USDA, 2019). While manure is a valuable fertilizer resource, its use can have adverse environmental impacts if precautions aren't taken with its use.

One of the main environmental concerns regarding land application of manure is that the nutrients it contains will not entirely be used by the plant or stored in the soil. Some nutrients in manure will be transported to waterways by surface runoff, subsurface water movement, erosion, and atmospheric deposition. The United States Environmental Protection Agency cites nitrogen and phosphorous as the leading cause of water quality degradation (USEPA, 2015). Nutrient loss from manure can be minimized; under proper management the manure source, rate, timing, and method of application are carefully selected to abate nutrient losses. One way nutrient losses are reduced is by incorporation of manure after application however, this is incompatible with no-till and conservation-till agriculture. Manure injection is a no-till compatible alternative application method that has been shown to reduce nitrogen and phosphorous leaching losses (Maguire et al., 2013; Kulesza et al., 2014) as well as ammonia volatilization (Misselbrook et al., 1996; Rahman et al., 2001; Chen et al., 2014). Although, the degree of reductions has varied and is partly dependent on site and temporal conditions (Delaby et al., 2014). Injection of manure is accomplished using several different implements (Maguire et al., 2011) but all place manure beneath the soil surface to reduce atmospheric contact. However, doing so may fundamentally change the dynamics of decomposition and nutrient release from manure, ultimately effecting crop production.

Studies comparing manure injection to surface application have reported various levels of yield response. Bittman et al., (2005) reported no increase in forage yield with injection over surface applied manure and attributed this to the damage to the sward by the injection implement. Powell et al., (2011) reported no significant differences in corn silage yields between injected and surface applied diary slurry, although nitrogen losses in the injection application, 9.1%, were lower than the surface application, 27.1%. Rahman et al., (2001) reported injection increased yield but only when the rate of manure application was high. Elsewhere, Russelle et al., (2008) reported greater corn yields under injection due to greater capture of ammoniacal nitrogen and Sutton et al., (1982) reported yields increased by 14% under injection relative to surface application of swine slurry. Clearly, the inconsistency of yield response warrants continued study of application methods to draw further conclusions.

Apart from yield responses to nitrogen cycling, the dynamic of carbon decomposition may change under injected manure applications because the manure is placed beneath the soil surface. It is accepted that the practice of manure application can result in substantial carbon sequestration (Risse et al.,n.d.; Follett, 2001), and that carbon sequestration depends on the climatic regime and rate of manure application (Sommerfeldt et al., 1988; Gupta et al., 1992). Authors have also reported that the rate of decomposition tends to slow at depth (Wildung et al., 1971; Gill and Burke, 2002), and the pools of carbon storage change with depth due to differences in microbial physical and chemical interactions (Rumpel and Kögel-knabner, 2011). Thus, it is plausible that application technique has an impact on the carbon cycling of manure. Measuring changes in soil carbon in the short term is notoriously difficult; respiration based metrics are used as short term estimates of carbon fluxes from soils, although this method has been criticized as microbes must partition carbon between growth and respiration (Conant et al., 2011).

The frequency of soil respiration measurements has increased in recent years as it is associated with climate change and soil health. Soil health is defined by the Natural Resources Conservation Service as “the continued capacity of a soil to function as a vital living ecosystem that sustains plants, animals, and humans.” (Natural resources Conservation Service, n.d.). Practically, it is being used as a manageable extension of intrinsic soil properties or soil quality. There is an abundance of research into the measurement and improvement of soil health. The Soil Health Institute was founded in 2013 and identifies 19 tier 1 physical, chemical, and biologic soil properties which they consider effective indicators of soil health. Of significance to manure application method and included in these 19 tier 1 indicators are carbon and nitrogen mineralization. Therefore, because manure application method likely impacts nutrient cycling dynamics, it may also impact soil health.

Objectives

The general focus of this dissertation was to consider dairy slurry application methods of surface broadcast and subsurface injection on the cycling of nitrogen and carbon, crop yield, and soil health. There are three chapters of study that were focused as:

Chapter 1

A. Quantify the ammoniacal nitrogen losses from injected and surface applied dairy slurry applications to soils of coarse and fine textures using a recently developed forced air-flow incubation technique.

B. Measure soil nitrogen transformations after surface broadcast and injected applications of dairy slurry in soils of coarse and fine textures.

C. Gauge soil biological response as a proxy of soil health to surface broadcast and injected applications of dairy slurry on soils of coarse and fine texture.

Chapter 2

A. Evaluate methods of pre-sidedress nitrate test (PSNT) soil sampling fields using manure injection equipment.

B. Observe nitrogen recovery and crop yield between surface broadcast and injected applications of dairy slurry on working farms.

C. Measure soil biological response as a proxy of soil health between surface broadcast and injected applications of dairy slurry in the field.

Chapter 3

A. Measure seasonal dynamics of soil nitrogen and crop yield response to surface broadcast and injected applications of dairy slurry while isolating nitrogen application in the field.
B. Measure soil biological response as a proxy of soil health between first year surface broadcast and injected applications of dairy slurry.
C. Characterize the spatial distribution and seasonal changes in the above parameters around an injected band of dairy slurry.

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Chapter 1: Effects of Dairy Slurry Injection on Carbon and Nitrogen Cycling

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Abbreviations:

ANOVA: analysis of variance, **HSD**: honest significant difference, **PAN**: plant available nitrogen, **TKN**: total Kjeldahl nitrogen, **TKP**: total Kjeldahl phosphorous

Abstract

Surface broadcast of dairy slurry is a common practice, however, concerns over nuisance odors and nutrient losses have prompted research into alternatives. Manure injection is one practice that addresses these concerns but is not widely adopted. Therefore, two studies were conducted to quantify NH_3-N loss by volatilization, impacts on soil N cycling, and microbial response between surface broadcast and subsurface injection of dairy slurry. A constant air flow volatilization chamber system measured NH_3-N losses and soil inorganic N, mineralizable carbon, and active microbial biomass. A 40-day static air incubation was performed to study nitrogen transformations over a longer period of time after application. Statistical significance was evaluated at the $\alpha=0.05$ level. In the volatilization study, subsurface injection reduced NH_3-N losses by 98% and 87% in a clay loam and sandy loam, resulting in greater soil inorganic nitrogen compared to surface application. There were no significant differences in active microbial biomass between treatments. Surface application prompted greater microbial respiration in the sandy loam, but there were no significant differences between treatments in the clay loam. In the static incubation study differences in soil NO_3^--N became significant on day 28 and by day 40 injection showed increases in soil NO_3^--N of 13% and 26% in the sandy loam and clay loam, respectively, relative to surface application. While the effect of subsurface injection on soil microbial response was unclear, it remains a tool that can greatly reduce NH_3-N losses by volatilization and increase soil plant available nitrogen.

Introduction

The application of animal manure in agricultural systems removes manure from animal production sites, and restores valuable nutrients and organic materials that aid crop growth and soil quality. Nutrients provided by the manure reduce soil nutrient requirements that are otherwise fulfilled by expensive chemical fertilizers. While the application of animal manure provides these benefits at a low cost, there are several concerns over the land application of animal manures. Liquid manures, such as swine and dairy, are most often applied to agricultural fields by surface broadcast. However, the practice of leaving manures on the soil surface has prompted reevaluation of current application technologies (Maguire et al., 2011). Manure left sitting on the soil surface causes nutrient losses of nitrogen by NH_3-N volatilization, and increases N and P losses by surface runoff. Losses of N due to volatilization can be large; 45-80% of ammonical-N applied can be lost as NH_3-N (Thompson and Meisinger, 2002; Maguire et al., 2011). Losses of N decrease the N use efficiency of the agricultural system, and increase the need for N inputs via chemical fertilizer. Additional concerns of manure application are the associated odor problems, and farmers are commonly reported by neighbors for their application of animal manures due to nuisance odors (Hardwick, 1985).

In response to these concerns, incorporation by tillage can be used to minimize atmospheric contact with manure, but this is not feasible in no-till systems. In no-till environments and where available, manure injection is suggested as an alternative application technique. Injection of dairy manure has been shown to decrease odor emissions by 33% relative to surface broadcast (Chen et al., 2014), while the injection of poultry litter can decrease NH_3-N losses by 98% relative to surface application (Kulesza et al., 2014). Manure injection can also decrease nutrient losses in surface runoff, with injection of poultry litter reducing TKN and TKP in runoff by more than 50% compared to surface application (Kulesza et al., 2014). Despite the benefits associated with subsurface injection, widespread adoption of this technique has been slow due to concerns related to application speed and equipment cost (Maguire et al., 2013). Indeed, Chen et al. (2014) studied the costs of different application techniques and agrees that injection has a higher "start up" cost, however, the total cost of injection may be lower due to retention of nutrients and reduced need for chemical fertilizers. Similarly, Rotz et al. (2011) modeled different application techniques and claims that the increased costs of shallow disk injection are leveled with economic return, maintaining profit.

Although greater microbial biomass was reported in soils treated with manure than on unfertilized (Tessier et al., 1998) and chemically fertilized plots (Fraser et al., 1988; Edmeades, 2003), less is known about the impact of manure application technique on soil microorganisms. Generally, no-till practices are thought to increase soil enzyme activity over time when compared with tillage. Still, the shortage of literature on manure application techniques and microbial response has been noted (Acosta-Martinez and Waldrip, 2014). One recent study did consider the application technique of swine manure on the soil arthropod community and found significant interactions, $\alpha=0.05$, between application technique and collembolan populations (Schuster, 2015). Land application of manures has been shown to increase soil carbon, with higher rates of application resulting in larger amounts of carbon storage (Ding et al., 2012).

Therefore, this study was performed to expand the knowledge of nitrogen cycling dynamics and microbial responses between application methods of liquid dairy manure.

Specifically, the objectives of this study were to quantify NH_3-N losses, track changes in soil nitrogen, and observe responses of microbial biomass & mineralizable carbon between surface broadcast and subsurface injection of dairy manure.

Methods

Soil and dairy manure collection and analysis

The following studies were performed using two soil types. One was a Braddock clay loam (Clayey, mixed, mesic Typic hapludult), and the other was a Dragston sandy loam (Coarse-loamy, mixed, thermic, Aeric Ochraquults). From here onwards the Braddock and Dragston soils will be referred to as the clay loam and the sandy loam, respectively. Field capacity, the soil water content after soil saturation and 24 hours free drainage, was 41% and 29% for the clay loam and sandy loam, respectively. Dairy manure used in the studies was gathered from a stirred slurry tank on a working dairy farm in Virginia. Manure analysis performed by the agricultural service laboratory of Clemson University indicated a moisture content of 94.13%, TKN of 2.21 kg Mg^{-1} and Organic Nitrogen 1.23 kg Mg^{-1} (Bremner and Breitenbeck, 1983; Peters et al., 2003). The manure was refrigerated prior to use to minimize changes in properties.

Volatilization Chamber Study

Ammonia volatilization was measured in a laboratory setting using enclosed *Kimble Chase* glass jars, hereafter “chambers”, 8.8 cm inside diameter x 15 cm depth, as described by Woodward et al. (2011) and Kulesza et al. (2014). Briefly, humidified air is pumped through chambers in temperature controlled boxes into acid traps. Chambers are designed with threaded tops and are fitted with lids housing gaskets in order to create an airtight seal. Polytetrafluorethylene thread seal was wrapped around the chambers threads to aid sealing. Temperature of the boxes were set and maintained at 26˚ C, air flow was calibrated to 1 L min^{-1} using a digital flow meter. Air traveled from the pump, through distribution lines, and through external and internal humistats filled with DI water to humidify air entering the chambers. Air passing through the chambers is directed through bubbling stones into 4 oz bottles containing 100 mL 0.04 M phosphoric acid. The phosphoric acid removed NH_3-N from the passing air and trapped it in solution as NH_4^+-N. A detailed description of box design and schematics can be found in Woodward et al. (2011).There were three treatments; surface applied dairy manure, injected dairy manure, and a no manure control. Two soils (clay loam, sandy loam) x 3 treatments (surface, injected, and control) x 3 replicates = 18 samples. Field capacity, determined gravimetrically by free draining saturated soil samples for 24 hours, was obtained in order to predict water needs to establish 70% field capacity at the beginning of the experiment. Five hundred grams of soil was added to each chamber; injection slits were simulated by inserting 2 rectangular metal plates vertically into the soil to a depth of 2.5 cm from the bottom of the jar. The plates were then shifted and arranged in a "V" shape and wooden shims placed between plates (removed before manure application) to hold form. Soils were brought to 70% field capacity excluding the water content to be added by dairy slurry in treated soils. Jars were left covered overnight for water infiltration. In treated soils manure was applied based on surface area, at a common Virginia application rate of 56,123 L ha^{-1} , approximately 34 mL jar^{-1} (Virginia Department of Conservation and Recreation, 2005). In surface treatments, manure was poured on top of undisturbed soil. In injected treatments, after pouring manure in the simulated slit the metal plates were gently removed, afterwards the plates were used to carefully close injection slits with soil that had mounded outside the “V” created by the metal plates. This method was used to mirror field conditions created by a shallow disk manure injector (Maguire et al., 2011). Jars were placed in temperature controlled boxes in a randomized complete block design with three blocks and the lids were secured. Acid trap bottles were changed at 1, 3, 6, 9, 12, 18, 24, 36, 48, 60, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312, and 336 hour$_{(s)}$ from the beginning of the study to establish a time series of NH_3-N emissions. Acid collected was weighed and refrigerated until analysis. The samples were analyzed for NH_3-N using a Lachat Instruments flow injected colorimeter (QuickChem 8500 FIA Automated Ion Analyzer, Lachat Instruments, Hatch Company, Loveland Colorado, USA) using QuickChem phenol method 10-107-06-1-G (Prokopy, 1993). Upon study completion, soils within each jar were homogenized, a portion of each soil was retained moist and refrigerated at 1.1° C until microbial analyses were performed. All other soils were laid out thinly on drying racks to air dry. After drying, soil samples were ground to pass a 2 mm screen and 4 g subsamples were placed in 50 mL centrifuge tubes and shaken with 40 mL of 2 M KCl for 30 minutes to extract soil inorganic N. The extract was vacuum filtered through Millipore S-PAK .45 μm membrane filters and analyzed for NH_4^+-N and NO_3^--N on a LACHAT instruments Quickchem 8500 autoanalyzer using QuickChem Salicylate Method 12-107-06-2-A and QuickChem Method 12-107-04-1-B, respectively (Hofer, 2001; Knepel, 2001).

A carbon mineralization study was performed with the moist soils from the volatilization study in order to estimate the amount of mineralizable carbon present in each sample. The analysis was done following the procedures of Strickland et al. (2010)

and was conducted after the volatilization study with soil from each chamber that was homogenized prior to subsampling. Samples were sieved to pass a 4 mm screen and 6 g dry weight sub samples were placed in 50 ml centrifuge tubes and adjusted to 65% field capacity. Samples were placed in an incubator for a total of 30 days with samples collected at 1, 5, 10, 20 and 30 days. Twenty four hours prior to each sampling the headspace was flushed with CO_2 free air for 3 minutes to clear the tubes of CO_2. At each sampling time, 5 mL of air was withdrawn and analyzed on a LI-COR CO_2 H_2O gas analyzer (LI-7000 CO_2/H_2O analyzer, LI-COR, Lincoln Nebraska, USA). Samples were taken after 1, 5, 10, 20, and 30 days; flushing of headspace was repeated 24 hrs before each sampling time. Measurements taken across time generated a curve with which the area underneath was used to estimate total CO_2 emitted over the 30-day period.

Soil Incubation

Active microbial biomass was estimated on the soil samples that had been stored wet following the volatilization study. Microbial activity was approximated via CO_2 respiration measurement using the method of Fierer and Schimel (2002). Four grams dry weight soil was placed into 50 mL centrifuge tubes and placed in a 20˚ C incubator over night to condition. The next day, 8 mL of autolyzed yeast solution (concentration of 12 g yeast : 1 L H_2O) was added to each tube, after which samples were shaken for 1 hr. Tubes were capped, flushed, and incubated at 20° C for 5 hrs after which samples were analyzed for CO_2 following the procedure described above.

Since the volatilization study only allowed analysis of soil nitrogen at the end of a 14-day period, the same three dairy manure treatments (surface, injected and control) on the same two soils were studied in a 40-day incubation. Five sampling times (0, 7, 14, 28, and 40 days), and 3 replicates were prepared to total 90 incubation cups that were placed in a climate controlled laboratory at 22.2° C. Three hundred grams of dry soil was placed in 10.5 cm x 10.5 cm x 8.5 cm (depth) planter cups lined with coffee filters to prevent soil loss from drainage holes. Control soils were then brought to 70% field capacity using DI water. Manure treated samples had the moisture from manure addition taken into account before wetting and received the difference in DI water before manure application. Manure injection was performed using two rectangular metal sheets to form a "V" opening in the soil, in which manure was applied, by surface area, at the common VA application rate (56,123 L ha^{-1}), approximately 62 mL slurry. After pouring manure in the slit, soil from each side of the slit was pushed loosely over the "V", simulating field injection of manure. In surface applied soils, manure was poured on top of undisturbed soil. After manure application was completed, laboratory cellophane was used to cover each planter cup. Three small holes were made in each cellophane lid to allow gaseous exchange but reduce water loss. Samples were weighed to assure adequate water content and moisture was adjusted to 70% field capacity every 2-3 days using DI H_2O. Soils were then incubated at 22.2° C for 40 days and analyzed on days 7, 14, 28, and 40 for NO_3^--N and NH_4^+-N. At each sampling time, samples were spread out thinly on paper in the lab to dry, excluding the coffee filter. All samples were set out to dry in this manner on their respective day. Once dried, soil samples were ground to pass a 2 mm sieve and 4 g subsamples were placed in 50 mL centrifuge tubes. Forty mL of 2 M KCl was added to each tube, after which the tubes were shaken for 30 min. The resulting extract was vacuum filtered through a Millipore S-PAK 0.45 μm membrane filter and processed on a LACHAT instruments Quickchem 8500 autoanalyzer for NH_4^+-N and NO_3^--N using the aforementioned colorimetric methods.

Statistical analysis

Data were analyzed using JMP Pro 12 software (SAS Institute, 2016). One-way ANOVA was performed by soil and treatment means separated using the Tukey-Kramer HSD test. In the volatilization study analysis was performed on the cumulative NH_3-N loss at the end of the 14-day period. One-way ANOVA was performed on microbial measurements by soil type; where applicable, treatment means were separated using Tukey-Kramer HSD. In the 40-day incubation study, sample dates were analyzed separately and means compared within a sampling time. All analyses were considered significant at the α=0.05 level and error bars in figures represent the standard deviation of the mean. The REG procedure in SAS 9.4 was used to evaluate the trends in soil NO_3^--N and NH_3-N dynamics over time and to compare the slopes and intercepts of those regressions (SAS Institute Inc., 2013).

Results and Discussion

Chamber Study

Ammonia Volatilization

Throughout the 14-day volatilization study, manure injection decreased NH_3-N losses relative to surface application (Fig. 1). After 14 days, NH_3-N losses were reduced by 98% and 87% relative to surface application in the clay loam and sandy loam soils, respectively (Table 1). Ammonia losses from both soils and all treatments fit well to a logarithmic model, injection and surface treatments ranged from $R^2=0.85$ to $R^2=0.98$, $\alpha=0.05$, $p<0.001$ (Fig. 1). Control treatments fit a logarithmic model due to rewetting prior to NH_3-N measurement. Ammonia losses from the injection treatment were indistinguishable and marginally elevated from the no-manure control in the clay loam and sandy loam, respectively. These results are similar to those seen in Kulesza et al. (2014) where poultry litter was injected and surface applied. In their study, NH_3-N losses from litter injection were statistically the same as the no-treatment control. Additionally, Powell et al. (2011) reported lower NH_3-N volatilization in injection (40-95%) and aeration-incorporation (26-64%) treatments compared to surface application in three of four years of field studies. Powell et al. (2011) also reported that most of the cumulative NH_3-N volatilization occurred in the first 48 hrs after manure application. This is similar to our study, where 70% of total NH_3-N loss had occurred from surface applications of manure 36 and 48 hours after application of manure for the sandy loam and clay loam, respectively (Fig. 1). In manure injected treatments 70% of total NH_3-N loss had occurred 96 and 144 hours after application for the sandy loam and clay loam (Fig. 1).

Soil Nitrogen

The total organic and inorganic-N applied via surface application or injection was the same. Both methods of manure application increased soil NO_3^--N to concentrations well above the control (Table 1). Nevertheless, injection increased soil NO_3^--N by 23% in the clay loam and 110% in the sandy loam over surface application after subtracting the control, presumably due to NH_4^+-N conservation (Table 1; Fig. 1). This is beneficial for crop production as non-legumes such as corn (*Zea Mays*) rely on NO_3^--N additions for economic yield. The sandy loam showed greater differences in soil NO_3^--N between surface application and injected treatments, due to more NH_3-N losses from surface application in the sandy loam (Fig. 1). Total inorganic N (calculated as NH_3-N + NO_3^--N + NH_4^+-N) was the same for injection and surface application in both soils, indicating there was no impact of management on organic N mineralization over 14-days (Table 1.). These results are similar to those obtained by Kulesza et al. (2014) where poultry litter injection increased soil NO_3^--N by 47% in a loam and 58% in a sandy loam. In that study a large portion of nitrogen captured was in the NH_4^+-N form, possibly due to the shorter study length of 7 days. If nitrification were allowed to continue by increasing study length, it is expected this NH_4^+-N would be converted to NO_3^--N. In the present study the soil total inorganic-N was increased by 31% and 108% in the clay loam and sandy loam relative to the control, respectively. This is again similar to Kulesza et al. (2014) who found inorganic-N to increase by 71% and 105% for a loam and sandy loam, respectively.

Soil Carbon

In the clay loam soil there were no significant differences in mineralizable-C detected between treatments although variability was high (Fig. 2). In the sandy loam the surface application of manure increased mineralizable C by 68% relative to the control while injection resulted in an increase of 20% relative to the control (Fig. 2). Additionally, injection reduced mineralizable carbon by 70% relative to surface application after subtracting the control (Fig. 2). The increases in microbial respiration are presumably due to a larger mineralizable carbon pool originating from manure application, alternatively, addition of manure could have provided the nitrogen necessary to metabolize carbon already present in the soil, or both. Indeed, Liang et al. (1996) reported that adding manure extracted organic carbon to soils resulted in higher mineralizable carbon than soils receiving no additions of carbon, however, this was probably from carbon already present in the soil. Similarly, Tessier et al. (1998) reported higher soluble carbon and total carbon in plots fertilized with incorporated manure than an untreated control. Conversely, Wander et al. (2007) reported that particulate organic matter carbon did not accumulate in a system amended with dairy manure and attributed it to excess labile N stocks provided by manure. In the present study, manure application in the sandy soil resulted in higher mineralizable-C than the no treatment control, presumably due to the organic carbon and nitrogen added from manure. Additionally, surface application lead to greater mineralizable-C than injection application, possibly indicating that mineralization of organic carbon had already occurred or is not occurring as quickly in the injection treatment. However, this higher mineralizable-C did not translate into greater mineralization of N, as total inorganic N was the same for surface and injected manure (Table 1). Anecdotal evidence exists that injection of manure may cause decomposition of added carbon to slow, as it can be identified visually in the injection slit several months after application (Fig. 3). No treatment effect was present in the clay loam soil; other than high variability, this could be attributed to the soil nearing its effective carbon saturation point as described by Stewart et al. (2007). Briefly, the closer a soil is to its effective carbon saturation point, the smaller the observed increases in soil carbon will be.

Active Microbial Biomass

No significant differences were detected in the active microbial biomass estimates among any of the treatments in either soil (Fig. 4). This could be explained, in part, by the nature of the volatilization study. During the NH_3-N volatilization study (14-days) the soils were kept in an environment conducive to microbial growth, this opportunity for growth before the active biomass estimate occurred could explain the lack of differences between treatments. Future endeavors looking at microbial parameters should consider monitoring microbial activity directly after manure application. Nevertheless, microbial biomass has been shown elsewhere to increase after being treated with manure, Tessier et al. (1998) noted that great variability was seen in manure treated plots, probably due to inconsistencies in both the incorporation of manure and the properties of the manure itself. Likewise, Kuzyakov and Blagodatskaya, (2015) agree that animal manures are a source of microbial “hotspots” and these “hotspots” are likely to have greater total and active microbial biomass. In a study by Rochette et al. (2000), long term (18 year) application of swine manure did not have a significant effect, α=0.05, on year end residual microbial biomass carbon, however, microbial biomass carbon peaked directly after manure application and varied by application rate. In theory, microbial biomass should follow carbon mineralization rates; biomass increases during the mineralization of carbon and decreases when little mineralization is occurring.

40-Day Incubation

Over the course of the 40-day incubation, manure injection resulted in higher soil NO_3^--N concentrations relative to both surface application and the control in both soil textures (Fig. 5A and C). In both soils, changes in soil NO_3^--N concentration due to manure application became significant on day 7 compared to the control, however separation between application methods was not significant until day 28 (Fig. 5A and C). On day 28, injection had increased soil NO_3^--N by 30% and 34% relative to surface application in the sandy loam and clay loam, respectively. Conjunctively, mineralization and nitrification followed a quadratic relationship with strong R^2 relationships, all $p<0.05$ (Fig. 5). The intercept of the regression for NO_3^--N was similar for all three treatments but the slope was different between the control treatment and either injection or surface application. Both injection and surface application resulted in continually increasing NO_3^--N through day 40 (Fig. 5A and C). By day 40, the increase in soil NO_3^--N by injection over surface application had changed to 13% and 26% for the sandy loam and clay loam, respectively. Similarly, a study done in Saskatchewan found that the injection of liquid swine manure resulted in consistently higher pre-seeding soil N over broadcast application and subsequent incorporation (Mooleki et al., 2002). Additionally, Schmitt et al. (1995) showed that manure injection increased soil NO_3^--N in the top 30 cm of soil by 17% relative to surface broadcast. As discussed for the volatilization study above, increases in soil NO_3^--N were due to nitrogen retained by reducing NH_3-N volatilization losses. These increases in NO_3^--N are present in both studies performed, however they are probably greater in the volatilization study due to the forced airflow design. Soil NO_3^--N is one parameter of plant available nitrogen, and increasing NO_3^--N at the proper time could result in increased crop yield. Application of manure resulted in higher soil NH_4^+-N concentrations, especially early in the 40-day incubation. Generally, there were no significant differences in soil NH_4^+-N between manure application methods throughout the study, the exception being a relatively small difference on day 40 of the clay loam soil where the injection treatment was more similar to the control than the surface treatment (Fig. 5B and D). Comparison of the slope of the regressions from the NH_4^+-N over time were similar for both injected and surface-applied manure but both different from the control. Both the injected and surface-applied manure exhibited a decline in soil NH_4^+-N over time reaching near zero on day 40 for the clay loam soil (Fig. 5B) and on day 28 for the sandy loam soil (Fig. 5D). In the sandy soil complete nitrification had occurred by day 28, with no significance in NH_4^+-N seen afterwards (Fig. 5D). In the clay loam nitrification had not been completed entirely by day 40. This was probably due to clay soils having a higher cation exchange capacity and ability to hold NH_4^+-N. This phenomena is discussed in a study by Fortuna et al. (2012), suggesting that increasing NH_4^+-N fixation due to mineralogy is negatively correlated to NH_3-N oxidizing bacteria. The temporal dynamics of soil N in this study indicate that manure application method did not largely impact nitrification or mineralization rates (Fig. 5). Thus, there is no need to alter the timeframe of manure application when using manure injection due to concerns over nitrogen availability relative to crop growth.

Conclusions

The results of this study indicate that manure injection leads to reductions of N losses by NH_3-N volatilization relative to surface application, and these are coupled with increases in plant available nitrogen as NO_3^--N. This increase in soil NO_3^--N could be beneficial for crop production, and could help cover the extra costs associated with manure injection. Application technique did not affect microbial biomass estimates. Mineralizable-C increased in the sandy soil where manure was applied, with surface application having the greatest mineralizable-C. If more conclusive effects of application technique on carbon cycling are found in the future, the preference of one manure application technique may increase. Nevertheless, injection remains a viable choice for manure application and appears superior to surface broadcast in terms of N retention.

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Tables

Table 1.1: Soil nitrogen after 14-day volatility study including NH3-N loss, soil NO3--N, soil NH4+-N, and total inorganic-N capture.

Soil Type	Treatment	NH_3-N loss	Soil NO_3^--N	Soil NH_4^+-N	Total inorganic-N
		mg jar^{-1}			
Clay loam	Control	0.0 b	18.9 b	1.4 b	20.3 b
	Injection	0.2 b	36.4 a	7.8 a	44.3 a
	Surface	8.9 a	33.1 a	5.5 a	47.4 a
Sandy loam	Control	0.0 c	11.4 c	0.6 a	12.1 b
	Injection	1.9 b	40.2 a	0.8 a	42.9 a
	Surface	15.3 a	25.2 b	0.8 a	41.3 a

Within each soil type, different letters in each column indicate significant differences, $\alpha = 0.05$.

Figures

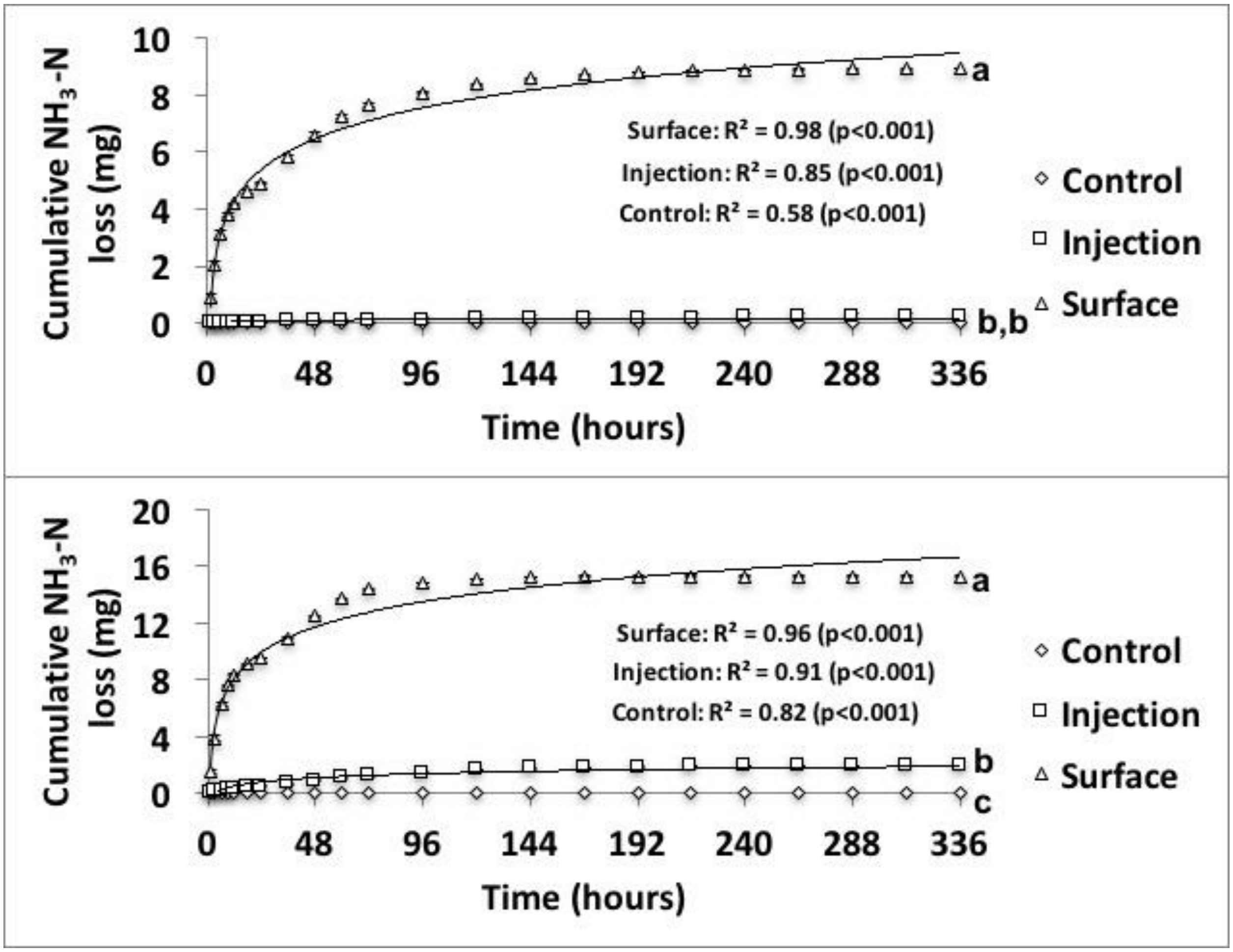


Figure 1.1: Cumulative NH3-N loss during a 14-day forced airflow volatilization study. Panel A is the clay loam and panel B is the sandy loam. Different letters signify significant differences at the end of the two-week period α= 0.05. Error bars indicate the standard deviation. P-values indicate an F-test for overall significance.

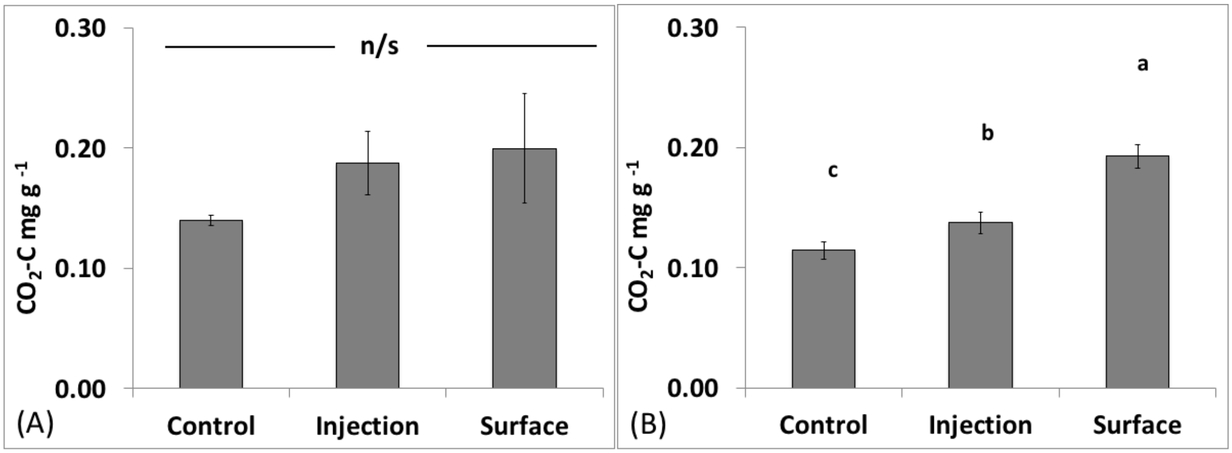


Figure 1.2: Cumulative mineralized carbon during 30-day incubation performed on soils run through volatilization chambers, clay loam (A) & sandy loam (B). Data shown represents the integration of 5 points in time on days 1, 5, 10, 20, & 30 of the incubation. Significant differences are indicated by different letters, n/s indicates no significant differences, α=0.05. Error bars indicate the standard deviation.

Figure 1.3 Photo of a manure injection slit 4 months after application. Manure added carbon is seen in the center of the photo (Maguire et al., 2013).

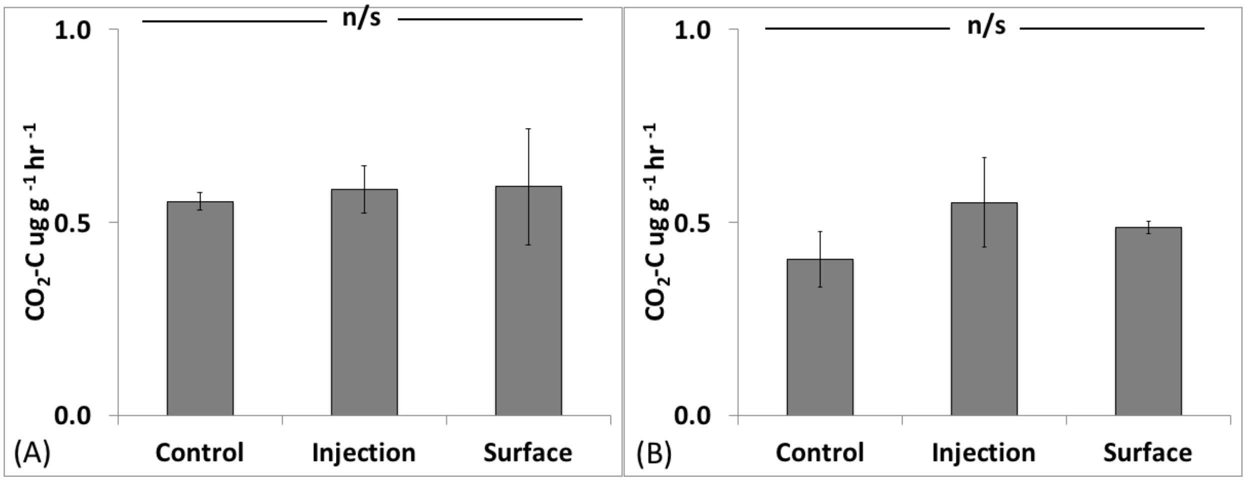
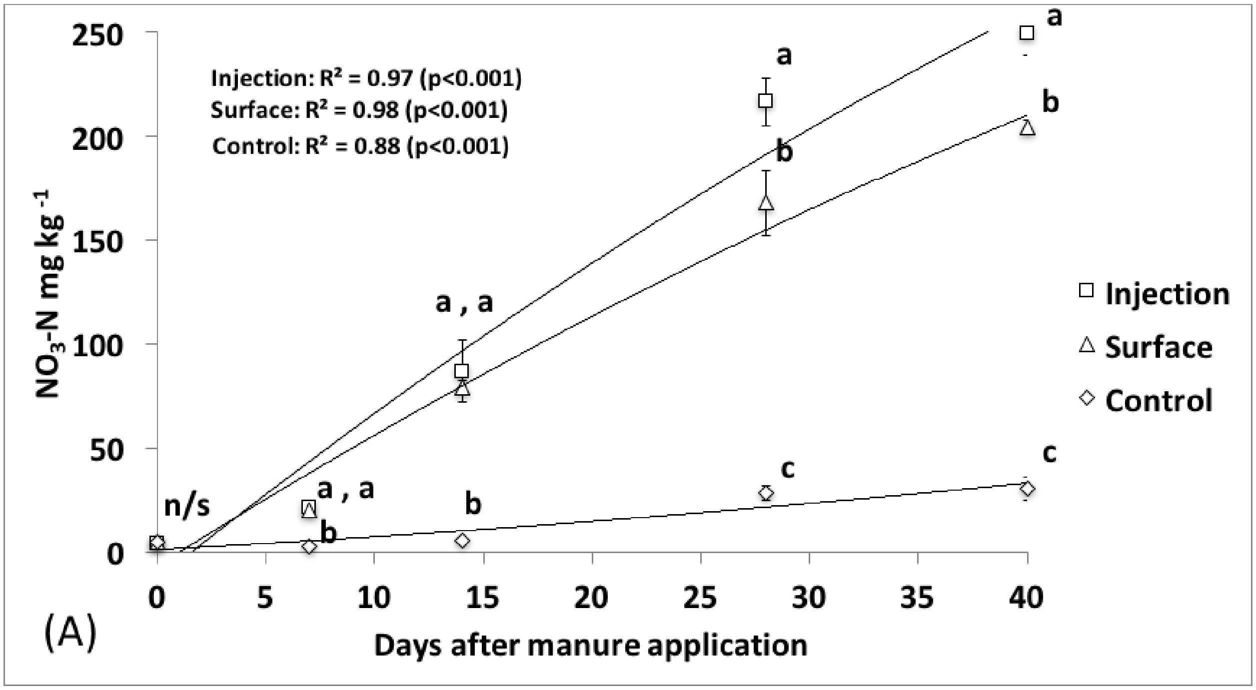
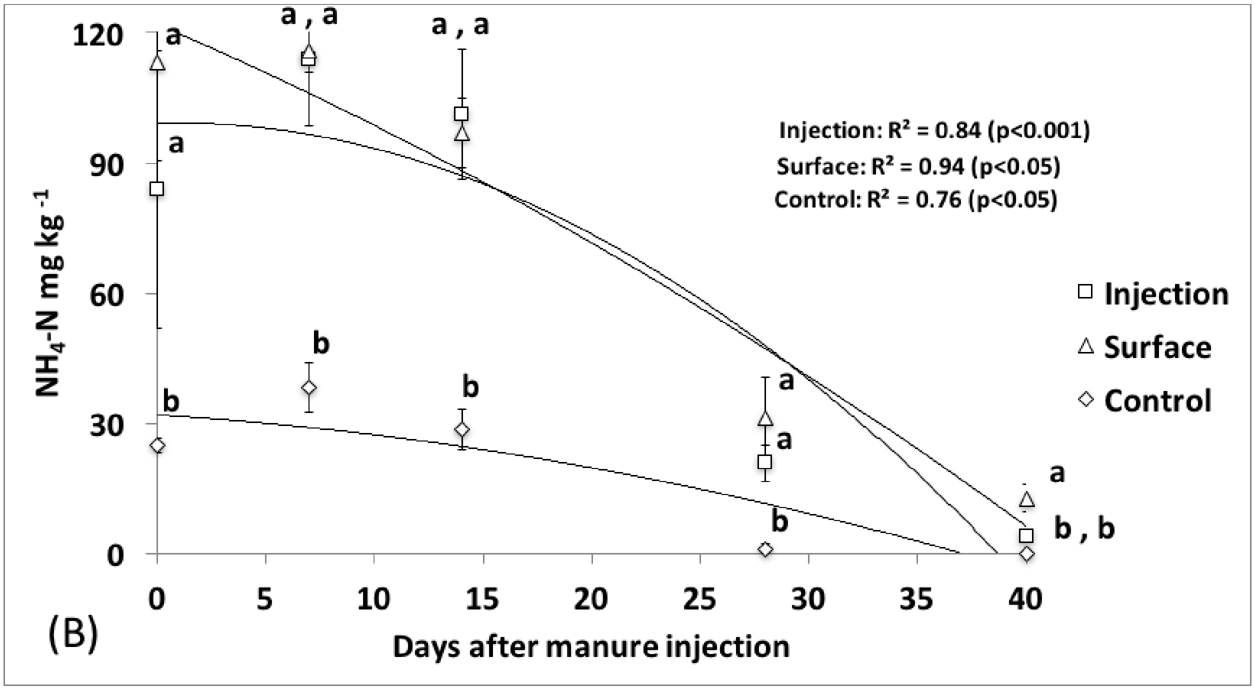


Figure 1.4: Active microbial biomass estimate from an induced respiration incubation using an autolyzed yeast substrate on a clay loam (A) & sandy loam (B). The n/s indicates no significant differences, α=0.05. Error bars indicate the standard deviation.


250
200
150
100
50
0
NO_3-N mg kg $^{-1}$
Injection: R² = 0.97 (p<0.001)
Surface: R² = 0.98 (p<0.001)
Control: R² = 0.88 (p<0.001)
a
a
b
b
a , a
n/s
a , a
b
b
c
c
□ Injection
△ Surface
◇ Control
0
5
10
15
20
25
30
35
40
(A)
Days after manure application


120
90
60
30
0
NH_4-N mg kg $^{-1}$
a
a , a
a , a
a
Injection: R² = 0.84 (p<0.001)
Surface: R² = 0.94 (p<0.05)
Control: R² = 0.76 (p<0.05)
□ Injection
△ Surface
◇ Control
b
b
b
b
a
a
b
a
b , b
0
5
10
15
20
25
30
35
40
(B)
Days after manure injection

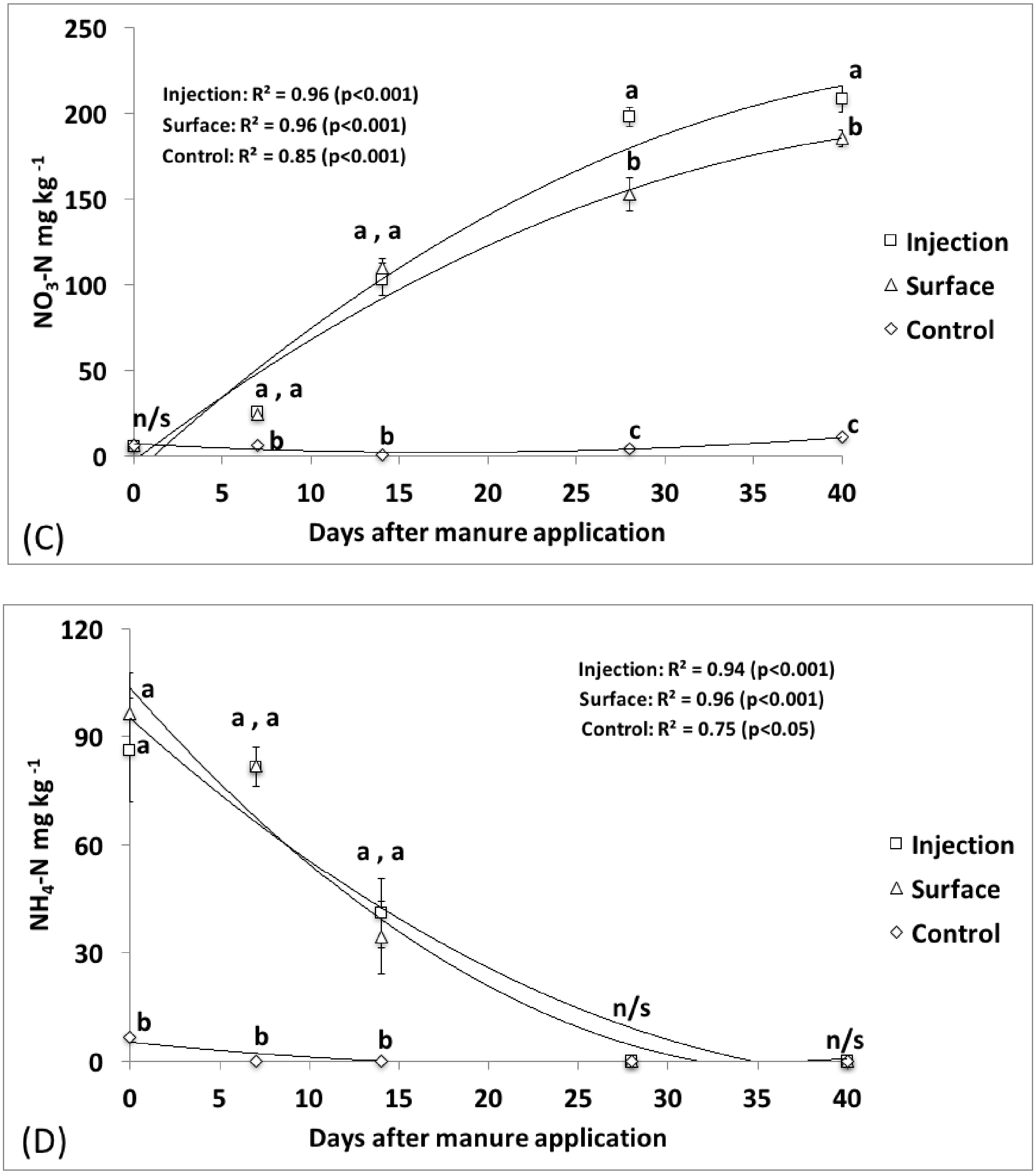


Figure 1.5: Soil nitrogen dynamics as NO3--N and NH4+-N between manure application methods over the course of a 40-day static air incubation. Graphs A & B are the clay loam and C & D are the sandy loam. Significant differences are noted by differing letters at each time point, n/s indicates that no significant differences exist, α=0.05. Error bars indicate the standard deviation. P-values indicate an F-test for overall significance.

Chapter 2: Evaluating dairy manure application method on soil health and nitrate

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Abstract

Liquid manures are typically applied via surface broadcasting; however, subsurface injection is an alternative characterized by greater nutrient retention and a spatially distinct application pattern, potentially altering management strategies and nutrient cycling dynamics. Thus, a field study was conducted from Spring 2016 through Fall 2018 on 7 Sites to assess Pre Side-dress Nitrate Test (PSNT) methodology, seasonal soil NO_3-N trends, corn silage and grain yield, estimated milk production via Milk 2006, and biological soil health among surface broadcast and subsurface injection applications of dairy slurry. A weighted sampling method had a coefficient of variation of 37%, ~8% higher relative to random (28%) and equi-spaced (30%) sampling methods. Soil NO_3-N was sometimes higher with subsurface injection, and at PSNT time an average of 30% higher than surface application, however, soil NO_3-N was not always higher with injection across Sites. There were no significant differences in crop yield or milk production between surface and injected slurry applications, but means were always higher for injection. Biological soil health tests were highly variable and analyzing carbon mineralization took considerably more time than other tests. There were no significant differences in carbon mineralization between manure application methods, although mineralization values increased with soil organic matter. Estimated microbial biomass was on average 46% lower under subsurface injection relative to surface broadcast in 2017, but results were inconsistent in 2016 and 2018. Overall, the biological indicators of soil health were not productive in showing differences between application methods. Nevertheless, it is apparent that injection can decrease chemical side-dress N applications, and either the standard method of PSNT soil sampling or an equi-spaced method can be used in injected fields.

Introduction

Manures used in agronomic systems offset the chemical fertilizer needed for optimal plant growth. Liquid manures that contain upwards of 90% water by weight are commonly surface broadcast by splash plate on agricultural fields for growing crops. However, alternatives to broadcast application such as banded surface application and subsurface injection can alter the spatial distribution of applied nutrients (Maguire et al., 2011). Injection of manure has advantages over surface broadcast from several aspects (Maguire et al., 2011, Brandt et al., 2011, Chen et al., 2014). Nitrogen use efficiency is improved by reducing ammonia (NH_3) volatilization when manure is placed below the surface rather than surface applied (Bierer et al., 2017). Additionally, when manure is below the soil surface, N and P losses to runoff are reduced (Kulesza et al., 2014, Watts et al., 2011). Odor is reduced by preventing atmospheric contact/transport of the gases (NH_3, H_2S, and VOC_s) commonly released from manure (Pfost, 2018). Conversely, injection has the potential to increase N losses through leaching (Pote et al., 2003). Despite the identified benefits of injection yield response has varied in field studies. A study conducted in Sweden found injection halved NH_3-N emissions but failed to increase ley yield compared to surface banding of dairy slurry (Rodhe and Etana, 2005). Similarly, Misselbrook (1996) reported no difference between injection and surface broadcast application on grass/clover yield despite significant reductions in NH_3-N emissions under shallow injection. For soils that have received manure, the corn pre-sidedress nitrate test (PSNT) estimates N availability and suggests any additional sidedress fertilizer in corn crops; the test relies on analyzing soil cores taken when the corn is 15-30cm (Magdoff and Ross, 1984; Maguire et al., 2019). However, values of soil NO_3-N are magnitudes different when taken from manure bands and the inter-band space; this variability may complicate nutrient management tools such as the

PSNT that rely on random soil sampling. For example, grid sampling techniques in proximity to an injected manure band have shown variations >100 mg kg^{-1} in measured soil N, making a reportable value difficult to obtain (Sawyer and Hoeft, 1990).

Soil health is comprised of physical, chemical, and biological parameters essential for sustainable plant production (Natural Resources Conservation Service, 2019). In some cases several metrics are compiled into a composite score that gauges soil health; the two most common are the Haney Test and the Comprehensive Assessment of Soil Health (CASH). The Haney soil health test (Equation 2.1) uses a 1-day microbial respiration response to rewetting of dry soil and water extractable organic carbon and nitrogen to form a composite score from 1-50 with values above 7 being considered healthy (Gunderson,2017). The development of the Haney soil health test originated from work concluding water can be used as an extractant for microbial carbon in lieu of 0.5M K_2SO_2 (Haney et al., 1999). Subsequent study on inorganic N extractants, ultimately resulting in Haney's H^3A extract, reported high correlations (R^2 >0.90) between water, KCl, and H^3A soil extracts (Haney et al., 2006). Researchers in the Midwest reported the Haney test health score was partially correlated to the economic optimum N rate (R^2=0.54) but preferred the one day CO_2 burst test (R^2=0.55) alone as the cost of processing samples was lower (Yost et al., 2018). Others have found the Haney Test unreliable due to random methodological variance and the failure to validate the recommendations it makes (Sullivan and Granatstein,). The "CASH" approach by Cornell University uses multiple chemical, physical, and biological indicators that are scored and composited between soils. A normal distribution curve is drawn for each indicator and a raw score given according to the percentile the sample is located within, raw scores are averaged for an overall quality score (Moebius-Clune et al., 2017). Roper et al. (2017) compared both composite measures of soil health on soils of differing long term management and regional origin, and reported a mixed ability of indicators to respond to long term management and a failure to correlate soil health values to crop yield. Biological parameters of soil health are believed to be the most sensitive to changes or disruptions in management since physical indicators are also tied to intrinsic properties and chemical indicators such as pH and nutrient concentrations change more slowly. Isolating biological indicators among the 19 “tier 1” indicators endorsed by the Soil Health Institute identifies carbon and nitrogen mineralization and soil organic carbon as metrics of soil health (Soil Health Institute, 2019).

Although multiple studies have been conducted on aspects of manure injection, few have analyzed the impact on soil health or sampling protocols for injected fields. Therefore, field trials were established in spring of 2016 and carried through fall 2018 comparing the surface application of manure to manure injection on working dairy farms. The objectives of this study were to determine the optimal PSNT sampling method for injected fields, and evaluate the impact of injection on seasonal soil NO_3-N, crop yield, milk production, and biological soil health.

Methods

Site Setup and Properties

Research plots were established on working dairy operations in spring of 2016-2018; locations were chosen based upon injection equipment availability and producer willingness to participate. All Sites were located within the Ridge and Valley physiographic region of Virginia, USA. In all cases manure was gathered from a stirred slurry storage lagoon during emptying. Manure total Kjeldahl nitrogen and ammonical-N analyses were completed by the agricultural service laboratory at Clemson University (Table 2.1) (Bremner and Breitenbeck, 1983) (Peters et al., 2003). Plots were established prior to planting corn (*zea mays*) or soybean (*glycine max*) with the treatments of surface broadcast manure and manure injection. In 2016, 3 Sites were planted in corn and harvested as silage; in 2017, 2 Sites were established, one in corn for grain and another in soybean. In 2018, 2 Sites were planted in corn, one for grain and one for silage; no location was repeated for a second year. All sites used a 76cm row spacing except for Site 3 which used a 38cm spacing, and Site 2 which was planted in twin rows 86cm for outside and 57cm for inside rows. For surface application treatments the injection equipment was raised from the soil with the pump still running to create a broadcast application with the same manure application rate. Manure application rate was decided by the land owner and is reported in Table 2.1. Manure plant available nitrogen (PAN) was calculated using Virginia availability coefficients, 35% of total organic N, 25% of surface applied ammonical N, and 95% of injected ammonical N. In 2016 a shallow disc injector (Vertical Till Injector, Washington, IA) was used at all Sites with a band spacing of 76cm and injection depth of ~15cm. Sequentially, a fluted opening disc created a slit in the soil, manure was pumped in, followed by slit closure with two angled discs. In 2017 and 2018 a Dietrich® footed shank injector (DSI incorporated, Goodfield, IL) was used at a band spacing of 61cm and depth of ~20cm. Sequentially, a disc cut surface residue, a footed shank resembling an inverted "T" opened the slit and manure was pumped in creating a subsurface band of manure. Treatments were applied in field long strips 1 or 2 passes wide (~9m per pass) in the area selected for study. Plots were sampled a minimum of 1.5m away from plot edges to prevent border effects.

Pre Side Dress Nitrate Test & Soil Nitrate Sampling

The routine PSNT must be conducted when the corn is ~30cm tall, but the same method was used 4 times throughout the growing season to measure soil NO_3-N for comparison between surface and injected applications of manure (Maguire et al., 2019). Time of sampling varied by date of manure application but closely adhered to the following schedule: Time 1= one month after manure application, Time 2= routine PSNT when corn was ~30cm tall, Time 3= four months after application, Time 4= six months after application. In 2016, Sites were only sampled three times, with the third sampling date after crop harvest. Three soil sampling methods were compared, which we called the standard, weighted, and equi-space methods. The standard method used current recommendations for ten, thirty-cm deep soil cores distributed randomly within each plot (Maguire et al., 2019). The weighted method was by far the most intensive and called for ten (2016) or eight (2017) thirty-cm deep soil cores, based on band spacing, centered across the injection band and the inter-band space in one inch increments with four subsamples per plot. The resulting soil NO_3-N concentrations from across band and inter-band samples were combined based on the area they were hypothesized to represent (Equation 2.2). The equi-spaced method (Meinen and Beegle, 2015), used five (2016) or four (2017 & 2018) thirty-cm deep soil cores, based on band spacing, taken fifteen-cm apart and perpendicular to the direction of injector travel, four subsamples per plot. In 2016, the equi-spaced and weighted methods were used, in 2017 and 2018 the standard method was added, and in 2018 the weighted method was removed due to the excessive work it required. Surface applied plots utilized the standard sampling method for all years. For NO_3-N analysis soil samples were spread thinly to air dry and ground to pass a 2-mm sieve. Four grams were weighed into 50mL centrifuge tubes and 40mL 2M KCl added. Tubes were shaken for 30 min and vacuum filtered through Millipore S-PAK 0.45-μm membranes. The samples were processed on a Lachat Instruments QuickChem 8500 autoanalyzer for NH_4^+-N and NO_3^--N using QuickChem Salicylate Method 12-107-06-2-A and QuickChem Method 12-107-04-1-B, respectively (Hofer, 2001; Knepel, 2001).

Crop Harvest

Crop harvest was performed, when applicable, using a combine/weigh wagon or chopper and ground scale. When equipment was limiting, hand harvest was performed by harvesting two rows for a distance of 3m. Dry matter yields are shown as the crop harvested varied; Sites 1,2,3, and 6 were harvested as corn silage, Site 4 and 7 were harvested as corn for grain and Site 5 was soybean. Forage analysis was performed using Near Infrared Reflectance Spectroscopy (NIR) with a FOSS XDS Rapid Content Analyzer (XM-1100 series; FOSS, Eden Prairie, MN). Forage analysis was used in conjunction with yield to estimate milk production when silage was harvested at Sites 1,2,3, and 6 using the MILK 2006 program (Shaver, 2006).

Biological indicators of soil health

Soil samples for biological indicators were taken with the same methods used in the soil NO_3-N sampling noted above, and then were 4-mm sieved and refrigerated moist until analysis. Two respiration-based metrics of soil health were used in the study. Mineralizable carbon (C-min) – an estimate of bioavailable soil C – was determined following the methods of Strickland et al. (2010) and Fierer et al. (2005). Briefly, C-min was determined by measuring total CO_2 emissions over the course of a 30 d incubation. Soils (6 g dry weight) were maintained at 65% water-holding capacity and 20° C with respiration across this time period determined (soils were measure 5 times [i.e. incubation days 1, 5, 10, 20, and 30) on an infrared gas analyzer (IRGA; Model LI-7000, LiCor Biosciences, Lincoln, NE, USA) using a static incubation technique. Total mineralizable-C was estimated by integrating CO_2 production across time. The second metric of soil health, substrate induced respiration (SIR) estimates active microbial biomass. Briefly, we amended 4 g dry weight equivalent soil with 8 mL solutions of an autolyzed yeast solution following the work of Fierer and Schimel (2002). After a 1 h pre-incubation with shaking, the soil slurries (i.e., soil and solution combinations) were incubated for 4 h at 20 °C. After incubation, respiration for each amendment was determined as described for C-min above.

Statistical Analysis

Data were analyzed using JMP Pro 14 software (SAS Institute Inc., 2019). Analysis of variance was performed by Site and time if applicable; treatment means were separated using the Tukey-Kramer honestly significant difference test. The PSNT methods were compared using Time 2 PSNT data and analyzed by Site, with means separated using the Tukey-Kramer honestly significant difference test. All further soil analyses on injected plots were conducted using samples from the equi-space method. Soil NO_3-N and biological soil health were analyzed by manure application method at each Site and time, with means separated using the Tukey-Kramer honestly significant difference test. Crop yield and milk production were analyzed by manure application method at each Site, with means separated using the Tukey-Kramer honestly significant difference test. All analyses were considered significant at the $\alpha=0.05$ level; error bars in figures are the standard deviations of the means.

Results and Discussion

Pre-Sidedress Nitrogen in the Injected Plots

PSNT numbers when corn reached a height of ~30cm in injected plots varied greatly across Sites and years, from a low of 5.25 mg kg^{-1} at Site 5 in 2017, to a high of 47.57 mg kg^{-1} in 2016 (Table 2.2). Comparing PSNT between years, PSNT numbers were always higher in 2016 than 2017 and 2018, and year was a significant effect ($P<0.0001$). Site also had a significant effect ($P<0.0001$); however, within each year, Site was only significant in 2016 ($P=0.0065$) and 2017 ($P=0.0022$). Soil PSNT numbers are made up of captured ammoniacal-N plus mineralized soil and manure organic-N, minus NO_3-N lost to leaching, plant uptake, and denitrification.

These factors are greatly affected by weather, soil properties, and management history which influenced the PSNT values observed in this study. Captured ammonical N was shown to be greater in finer-textured soil when NH_3-N volatilization was quantified for injected applications of dairy slurry versus surface applied slurry (Bierer et al., 2017). Nitrification is conducted by obligate aerobes and thus dependent on water-filled pore space, making precipitation and soil texture regulators of mineralizable N (Paul, 2007). Mineralizable N was elevated up to 355% in soils having a history of manure applications (8 years) when compared to a no application control (Sharifi et al., 2014). All Sites in the current study have an extensive history of manure application except for Site 3; further, soil textures varied from Site to Site (Table 2.1). In 2016, all Sites were located in soils high in organic matter, that, in conjunction with a wet spring, led to overall high PSNT readings (Table 2.1). In 2017, PSNT results reflected average weather conditions, whereas in 2018, yearly precipitation was 68% higher than average and spring temperatures were warmer than average (NOAA, 2019), resulting in elevated PSNT levels. Our PSNT values can be compared with Virginia guidelines for additional side-dress N applications. Virginia PSNT guidelines, revised in 2019, use 3 brackets for additional side-dress N applications; <15mg kg^{-1} apply full rate, 15-26mg kg^{-1} apply 50-75% of full rate, >26mg kg^{-1} N sufficient (Virginia Department of Conservation and Recreation Division of Soil and Water Conservation, 2014; Maguire et al., 2019). There were no significant differences in sampling methods tested except at Site 5 in 2017 where the standard method was higher than both the equi-spaced and weighted methods (Table 2.2). Using the revised Virginia guidelines results in consistent recommendations across sampling methods. Nevertheless, the weighted sampling method resulted in higher standard deviations than the equi-space and standard methods, which elevated the coefficient of variation (C.V.) of the weighted method to 37% (Table 2.2). Both the equi-spaced and standard methods resulted in similar C.V. values to those obtained in the Surface applied plots. All methods had acceptable C.V. values when compared to other studies that examined general grid sampling of soil N in fields. A C.V. of 45% was obtained in the top 30cm of three large fields while another study reported a C.V. of 16% in smaller 90m x 40m plots (Goderya et al., 1996; Długosz and Piotrowska-Długosz, 2016). Zebarth et al. (1999) assessed soil N using systematically spaced cores and random sampling after sidedress applications of N and reported similar C.V. between methods; however, increased N rate raised the C.V. of the random sampling method. Similar to the present study, Assefa and Chen, (2007) reported localized elevated soil NO_3-N within an injection band 3, 6, and 19 weeks after manure application, and suggested the use of "directed paired sampling" in injected fields. However, the recommendation was based on simulation of soil N values between directed paired samples, not observed field testing. They go on to note that an ideal sampling method would account for lateral positioning of the manure band, but could be labor intensive. In the present study the C.V. of the standard and equi-spaced methods were low (Table 2.2) and the labor of sampling was not greatly increased using the equi-spaced method. The present study in addition to prior research would recommend using the equi-spaced method as a more dependable method of sampling injected fields when the direction of injector travel is known.

Soil Nitrate Trends with Time for Injected and Surface Applied Manure

Across sampling times, soil NO_3-N was influenced by N mineralization, N additions, crop uptake, and miscellaneous losses; values ranged from a low of 1.49 mg kg^{-1} at Site 3 post-harvest to a high of 47.57 mg kg^{-1} at PSNT time at Site 1 (Table 2.3). Year had a significant effect on soil NO_3-N at all sample times ($P<0.01$); however, no Sites were repeated year to year. Soil NO_3-N was higher 1 month after manure application in 2016 and 2018 compared to 2017, resulting from higher applications of manure N in addition to higher soil organic matter (Table 2.1). When corn was ~30cm tall, PSNT was >40 mg kg^{-1} at Sites 1 & 2, (Table 2.3), indicating substantial PAN stores. Site 1 soil NO_3-N remained elevated in the post-harvest sampling at 23.72 mg Kg^{-1} for injection and 16.58 mg Kg^{-1} for surface application, which indicated possible excess N application and non-N based yield limitation. In other states, when post-harvest soil NO_3-N tests >20 mg kg^{-1}, fields are under consideration for reductions in manure or side-dress N applications, however, Virginia uses the corn stalk nitrate test to assess N application suitability (Sullivan and Cogger, 2003).

In 2017 and 2018 soil nitrate generally declined from Time 1 to Time 2, likely due to crop uptake; Site 5 was planted in soybean and reported a marginal but insignificant increase in soil nitrate from time 1 to time 2, potentially due to N fixation supplementing crop N uptake (Table 2.3). Soil nitrate increased from Time 3 to Time 4, except at Site 4, as net mineralization of organic-N occurred simultaneously with the decline of crop N uptake. Trends between manure application methods were inconsistent across Sites and time periods; Site 3 exhibited consistently higher soil nitrate under injection (Table 2.3), even when side-dress application of chemical N was high (Table 2.1). Alternatively, there were no differences between application methods when N additions were restricted to manure N at Sites 5 and 7, (Table 2.1), except for one instance at time 3 when elevated soil nitrate levels would be negligible for crop growth (Table 2.3). When treatment differences were significant, soil nitrate values were 54% higher, on average, with injection relative to surface application. When corn was ~30cm tall, PSNT numbers under injection increased by an average of 30% over surface application and were significantly higher at 2 of 7 Sites (Table 2.3). In both instances, recommendations for side-dress N would be reduced by shifting the side-dress N bracket the Site falls in from <15 mg Kg^{-1} to 15-

26 mg Kg^{-1}, potentially reducing side-dress chemical N applications (Virginia Department of Conservation and Recreation Division of Soil and Water Conservation, 2014; Maguire et al., 2019). Soil N responses to manure injection in field studies are varied: a similar Saskatchewan study reported mixed soil NO_3-N response to year over year application of injected and surface broadcast/incorporated swine slurry (Mooleki et al., 2002). Conversely, a study in Minnesota showed higher soil nitrate at corn stages V1, V4, and post-harvest under an injected application of manure, relative to surface application; however no manure was applied 2 years prior to the study, reducing potentially mineralizable N (Schmitt et al., 1995).

Crop Yield & Forage Quality

There were no significant differences between surface and injected applications of dairy slurry on crop yield (Figure 2.1) or estimated milk production (Figure 2.2). At Sites harvested as corn silage, (i.e., Sites 1,2,3,6), yields varied due to differences in location, management, and weather; Site 6 was under pivot irrigation, partially contributing to higher yields. Additionally, corn at Site 3 was planted with a 38cm row spacing while the other Sites used a 76cm spacing. Yield did not differ between application methods at Sites 3 & 6 (Figure 2.1) despite significantly higher PSNT values for the injection application (Table 2.3). Data for estimated milk production follows the same trend as dry matter yield and was highly correlated, (R^2=0.72; Figure 2.2). Forage quality parameters used in the Milk 2006 program (crude protein, neutral detergent fiber, Starch, Ash, and Fat) varied by Site but not manure application method (data not presented). In several cases yield responses were unlikely due to external factors. In Sites 1 and 2, post-harvest soil NO_3-N was high, (>20 mg kg^{-1}; Table 2.3) providing evidence that total N application was probably not limiting crop growth. Further, Sites 4, 5, and 7 were shallowly disked to prepare a seedbed after manure application, potentially reducing ammoniacal-N losses associated with surface applications of manure. Nevertheless, under the injected application yield and estimated milk production means were always centered at or above the surface application (Figure 2.1 and Figure 2.2). Inconsistent yield response to injected applications were reported by Rahman et al (2001), where alfalfa yield only increased when manure application rate was high. Similar to the present study, Jokela et al., (2014), reported no difference in corn silage yields between pre-plant surface broadcast incorporation and side-dressed injection applications of dairy slurry.

Biological Soil Health

A significant effect of year ($P<0.001$) was observed for both carbon mineralization (C-min) and substrate induced respiration (SIR). The C-min values were greater in 2016 (mean=0.36 mg-C g dry wt. soil^{-1} day^{-1}) than 2017 (mean=0.11 mg-C g dry wt. soil^{-1} day^{-1}) and 2018 (mean=0.10 mg-C g dry wt. soil^{-1} day^{-1}; Figure 2.3). This difference was likely due to the higher soil organic matter of 2016 Sites relative to 2017 and 2018 (Table 2.1). Further, a regression was fit between Site C-min means and soil organic matter content that resulted in a strong correlation ($R^2=0.88$) between parameters. Higher soil organic matter should increase basal respiration rates which are relevant in the 30-day incubations performed (Cheng et al., 2013; Phillips and Nickerson, 2015). It was expected that C-min may increase under greater manure applications through high quality carbon substrates triggering decomposition of soil C (Fierer et al., 2005; Strickland et al., 2015), or providing a nitrogen source to drive decomposition of more recalcitrant carbon sources however, this was not observed as manure application rate and total N application were poor predictors of site average C-min $R^2=.04$ and $R^2=.03$, respectively. For SIR, all years were significantly different ($P<0.0001$) with means: 2016 =0.70 ug g dry wt. soil^{-1} hr^{-1}, 2017=0.11 ug g dry wt. soil^{-1} hr^{-1}, and 2018=0.33 ug g dry wt. soil^{-1}

hr^{-1} (Figure 2.4). A regression was fit between Site average SIR and soil organic matter content which also resulted in a strong fit (R^2=0.74). Another study using SIR reported a strong correlation (R^2= -0.96) to alkylic soil carbon compounds, however, the relationship to total carbon was unclear (Beyer, 1995).

In the present study, sampling time had a significant effect on both C-min (P=0.0007) and SIR (P=0.0159), indicating the need to identify a sampling window or protocol for when biological testing should occur. Chang and Trofymow (1996), reported that SIR values differed by sampling date when studying the age of forest stands. Sampling time likely affects microbial tests due to substrate availability that is partially regulated by dynamic conditions, i.e. temperature, moisture, and carbon/nitrogen additions. Several studies reported a significant portion of variation in active microbial biomass is due to variation in soil moisture, and that active microbial biomass declines during consecutive wet-dry cycles (Wardle and Parkinson, 1990; Bottner, 1985; McGill et al., 1986). Our study did not measure soil moisture at sampling times, which represents a likely a source of variability. Both manure application methods had similar C-min patterns during the progression of the growing season (Figure 2.3). The large spike at Time 2 in Site 1 is likely a response to drying after a period of extended saturation early in the season. Substrate induced respiration was more variable than C-min and did not vary consistently between application methods (Figure 2.4). In 2016, Site 3 had 29% higher SIR under injection when measured ~1 month after manure was applied. In 2017, SIR of injected plots were lower than surface plots, possibly due to the preparation of a seedbed through shallow disking at Sites 4 & 5 after manure application (Figure 2.4); surface applied manure was incorporated to a shallow depth while the majority of injected manure was undisturbed. In 2018, Site 7 had 34% lower SIR under injection ~1 month after application relative to surface application (Figure 2.4).

Although variation was high among both metrics of soil health, SIR was positively correlated to C-min with a moderate degree of dependency (R^2=.64 and Pearson's correlation (r)=.80), suggesting some degree of multicollinearity between the biological metrics used in this study. Our results fall in-line with those obtained by Cheng et al. (2013) who reported a positive correlation (r=0.77) between microbial biomass C and basal respiration, albeit using the chloroform fumigation-extraction method to obtain microbial biomass C. Inverse responses of SIR and basal respiration have also been reported (Menyailo et al., 2002) so it is likely that this relationship, referred to as the metabolic quotient, qCO_2, will depend on type and availability of substrates in each study.

Variation of SIR & C-min in space is also high in other studies; Bruckner et al., (1999), assessed the spatial variability of SIR in a relatively small area, 18m x 18m, and reported a moderately high C.V. (~26%) relative to the quantity of samples taken, n~150. Similarly, Broos et al., (2007), conducted a power analysis after observing high variability in microbial biomass which indicated up to 93 replicates were necessary to detect a difference of 20%. Elsewhere, Cernohlavkova et al., (2009) studied the variability of microbial analyses and reported SIR and basal respiration C.V. of ~20% for arable soils, recommending 6-8 pooled subsamples per sample for proper representation. For comparison, by Site, this study observed a C.V. of ~31% for SIR and 24% for C-min with all $n \geq 6$, however, sampling time was significant and pooled subsamples were not utilized.

Logistically, C-min analysis was the most time-intensive metric in the study due to the 30 day incubation period. When compared to soil NO_3-N and SIR analysis, time invested per sample was nearly 20 times greater. The variability and logistical limitations of these soil health tests may limit their application. In our study tests differentiated between Sites at every sampling time (P<0.001), but were not able to reliably indicate differences between our treatments which represent nitrogen application rates. To this end, nutrient recommendations made by labs utilizing soil health scores may be premature, and further independent calibration is needed (Moebius-Clune et al., 2017; Roper et al., 2017; Haney et al., 2018). The observed logistics and variability of soil biological health tests suggest they should be avoided in production fields especially if only limited interpretations can be provided to producers. Otherwise, tests should be proposed on a case by case basis and adapted to meet producer's needs, e.g. potentially mineralizable nitrogen to better predict N availability.

Conclusion

The present study recommends an ideal equi-spaced sampling technique for fields injected with manure when the direction of injector travel is known; however, a standard method proved adequate and both methods proved superior to a labor intensive weighted method. Additionally, the injection application had the potential to decrease side-dress N applications by elevating soil NO_3-N at PSNT time but was not consistent across Sites, potentially limiting producer adoption of the practice. Seasonal soil nitrate was tied to manure application rate, chemical N additions, mineralizable N, and weather patterns. Crop yield and forage quality were not different between manure application methods; however, N availability, the primary difference between application methods, may not have been limiting to crop growth. Two biological soil health measurements did not respond consistently to manure application method and were instead related to other factors intrinsic to the Sites i.e., soil type and management history. The carbon-mineralization (C-min) biological test proved to be logistically intensive and provided little useful information regarding short term differences in management. The substrate induced respiration (SIR) test was less logistically demanding but was unable to consistently differentiate between manure application methods and should not be recommended to producers until practical interpretations of the test are clear.

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Equations

Equation 2.1: Haney Soil Health Test = $\frac{\text{1-day } CO_2\text{-C burst}}{10} + \frac{WEOC}{50} + \frac{WEON}{10}$

Equation 2.2: Weighted method soil NO_3-N = $(0.33 \times \text{across band}) + (0.66 \times \text{between bands})$

Tables

Table 2.1: Basic soil properties and Nitrogen (N) additions among research Sites. Manure added N displayed with the Virginia availability coefficients: 35% of organic-N, 95% of ammonical-N with injection, and 25% of ammonical-N with surface. n/a indicates no application due to cropping system, 0 indicates no application due to management decision.

Year/Site	Manure Organic-PAN	Manure Ammonical-PAN		Starter N	Side dress N	Total PAN		Soil Textural Class	Organic Matter	Soil pH
		Injection	Surface			Injection	Surface			
2016				(kg ha^{-1})					(g kg^{-1})	
Site 1	36	79	21	56	50	221	163	Silty clay loam	48.4	6.88
Site 2	8	18	5	56	0	82	69	Silt loam	51.2	6.95
Site 3	27	72	19	50	101	250	197	Silt loam	26.6	6.37
2017										
Site 4	16	34	9	73	84	207	182	Sandy loam	14.2	6.46
Site 5	14	39	8	n/a	n/a	53	22	Silt loam	15.5	5.90
2018										
Site 6	43	108	29	0	0	151	72	loam	13.6	6.53
Site 7	43	108	29	0	0	151	72	loam	14.4	6.92

Table 2.2: Pre-sidedress nitrate test (PSNT) results for sampling methods (equi-spaced, standard, weighted) within manure injected fields, compared to surface applied fields. n/a indicates the sampling method was not utilized. Method coefficient of variation (C.V.) was calculated as the mean C.V. across Sites. Where applicable, significance between sampling method is indicated by * ($P<0.05$), **($P<0.01$), *** ($P<0.001$).

Soil NO_3-N Sampling Methods for Injected Fields

Year/Site	Equi-spaced		Standard		Weighted		Surface Applied	
	PSNT	Std. Dev.	PSNT	Std. Dev.	PSNT	Std. Dev.	PSNT	Std. Dev.
				(mg kg^{-1})				
2016								
1	47.57	6.87	n/a	n/a	35.74	9.33	43.43	5.87
2	42.88	5.48	n/a	n/a	46.91	16.06	42.85	17.88
3	20.99	2.72	n/a	n/a	18.13	3.74	13.62	3.18
2017								
4	12.34	7.90	10.67	4.90	12.75	10.06	9.78	1.44
5	6.75	2.71	11.09*	0.68	5.25	1.45	8.30	4.50
2018								
6	19.64	1.52	19.46	3.30	n/a	n/a	11.17	2.07
7	12.57	7.11	10.03	4.23	n/a	n/a	7.80	2.14
Method C.V.		30		28		37		28

Table 2.3: Soil nitrate results of fields injected or surface broadcast with dairy slurry; Time 1: ~1 month after manure application, Time 2: pre side dress nitrate test window, Time 3: ~4 months after application*, Time 4: ~6 months after application. n/a indicates no measurement was taken. Where applicable, manure application methods at each Site and time period are indicated by * ($P<0.05$), **($P<0.01$), *** ($P<0.001$). °Time 3 in 2016 is post-harvest.

	Soil NO_3-N							
Year/Site	Time 1		Time 2		Time 3		Time 4	
	Injection	Surface	Injection	Surface	Injection	Surface	Injection	Surface
2016			(mg/Kg)					
1	15.5	20.7	47.6	43.4	23.7	16.6	n/a	n/a
2	19.7	22.2	42.9	42.9	3.9*	1.5	n/a	n/a
3	14.7*	8.4	21.0*	13.6	11.7*	4.8	n/a	n/a
2017								
4	14.4	13.7	12.3	9.8	4.9	8.9	6.1	8.3
5	6.5	7.5	6.8	8.3	4.7*	3.6	5.5	7.1
2018								
6	22.8*	16.6	19.6***	11.2	4.5	2.4	11.1	9.6
7	23.7	12.1	12.6	7.8	5.6	5.8	7.8	9.5

Figures

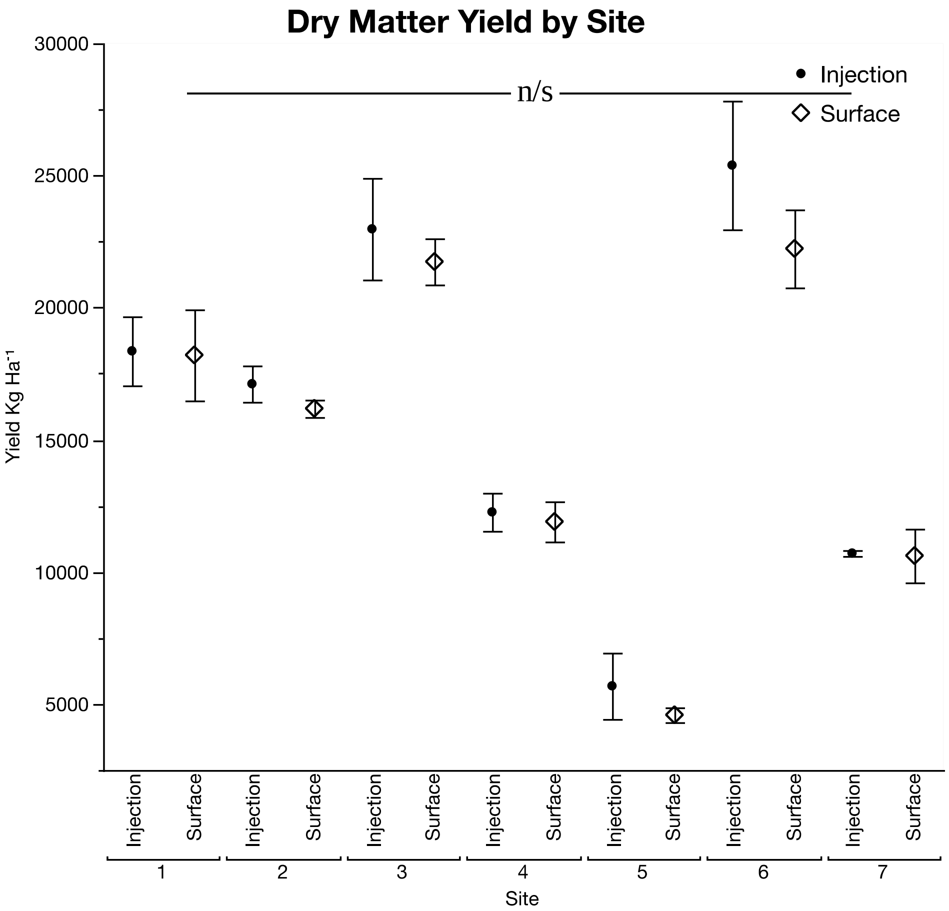

Figure 2.1: Dry matter yield of injected and surface applications of dairy slurry by Site. Sites 1,2,3 & 6 were harvested as corn silage, Sites 4 & 7 were in corn harvested for grain, and Site 5 had harvested soybean. There were no significant differences between application methods. Error bars represent standard deviations of the means.

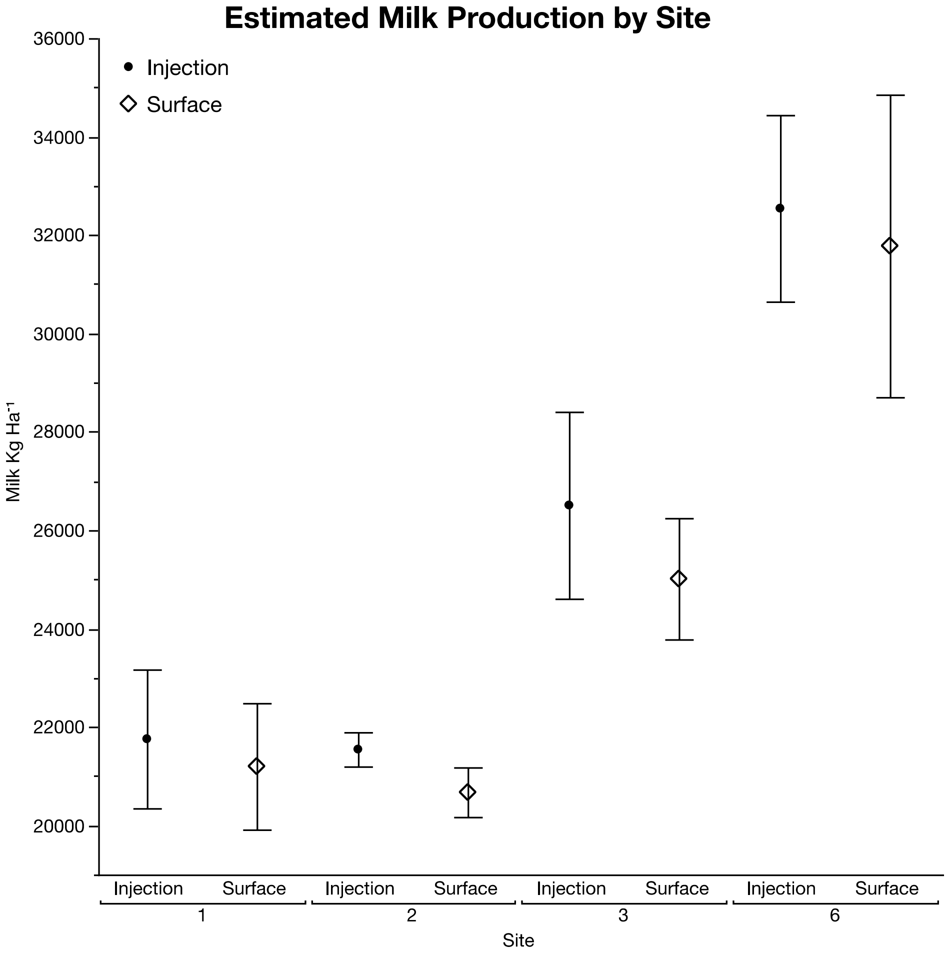

Figure 2.2: Estimated milk production of plots with injected and surface applications of dairy slurry. Estimations are based on corn silage yield and forage quality parameters using the Milk 2006 program. There were no significant differences between application methods. Error bars represent standard deviations of the means.

Carbon Mineralization by Time

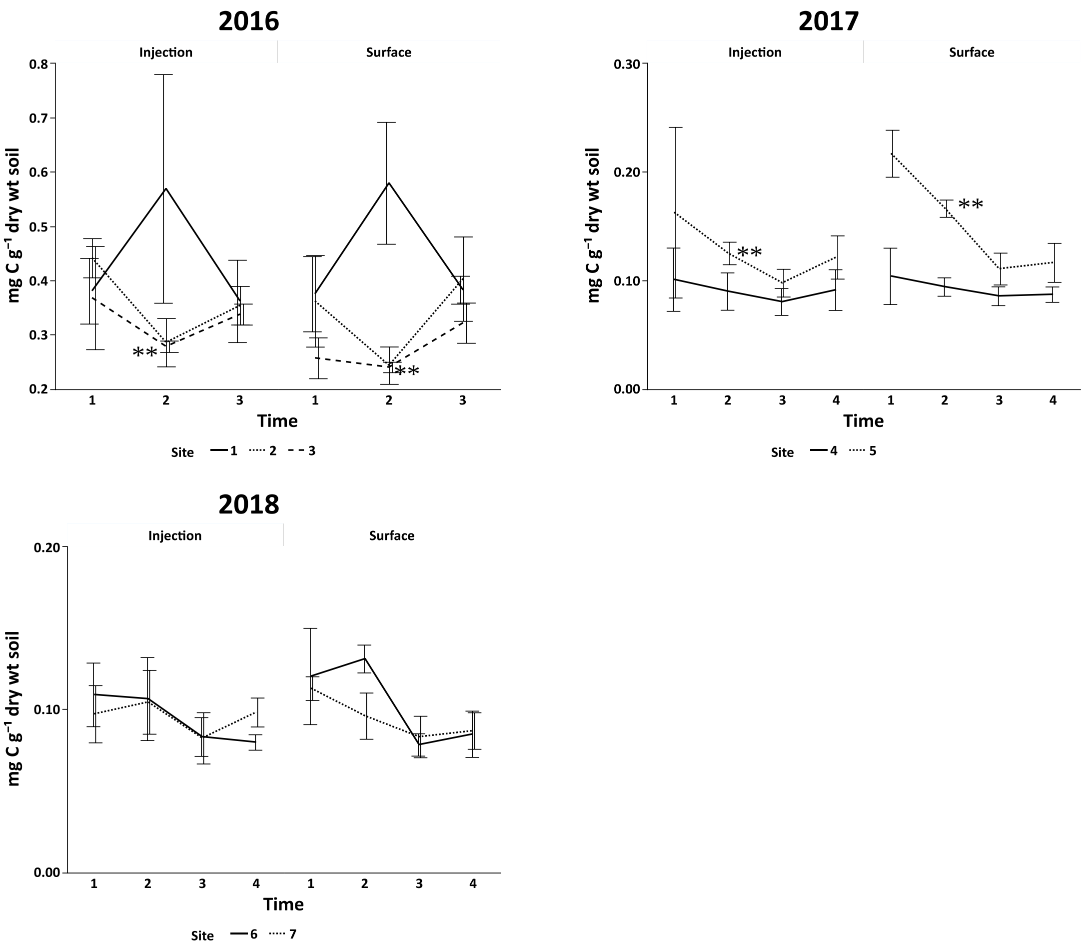

Figure 2.3: Carbon mineralized from 60-day laboratory incubations by site (trendline) and time (x-axis). Where applicable, significant differences between manure application method at each site and time period are indicated by * ($P<0.05$), **($P<0.01$), *** ($P<0.001$). Error bars represent standard deviations of the means.

Substrate Induced Respiration by Time

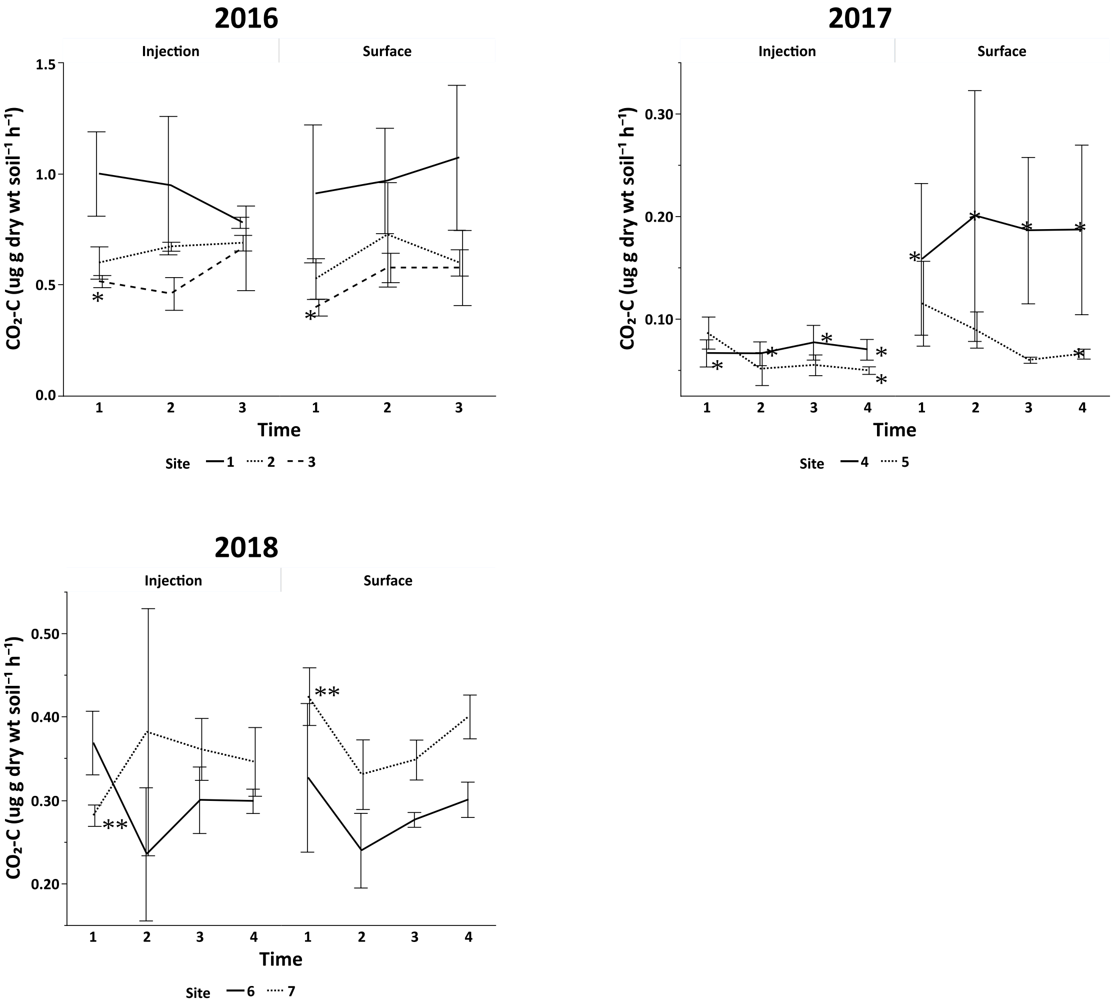

Figure 2.4: Substrate induced respiration by site (trendline) and time (x-axis) utilizing autolyzed yeast broth as a substrate. Where applicable, significant differences between manure application method at each site and time period are indicated by * ($P<0.05$), **($P<0.01$), *** ($P<0.001$). Error bars represent standard deviations of the means.

Chapter 3: Manure injection alters nitrate distribution and soil health.

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Abstract

Methods of handling and application of animal manures likely changes dynamics of nutrient cycling and should be assessed in each production system. This study contrasted nitrogen cycling, corn yield, carbon mineralization (C-min), and microbial biomass (MBC) between surface and injected applications of dairy slurry compared to a no-manure control. Additionally, the spatial distribution of parameters was measured around an injection band (In-band, 10cm, 20cm, 36cm). Soil nitrate was measured colorimetrically, bioavailable carbon measured through 60-day laboratory incubations, and microbial biomass estimated through chloroform fumigation extraction. All analysis were considered significant at the $\propto=0.05$ level. Injection had greater soil nitrate values than the surface application but was concentrated In-band. Nitrate movement was detected no further than 10cm perpendicular to the injection band, but evidence of vertical nitrate leaching was observed in 2017. Both C-min and MBC were stratified by depth; C-min was elevated under manure applications and highest with injection due to In-band samples. The increase in C-min was tied to manure applied carbon substrates and was not associated with soil nitrate. MBC was higher in 15-30cm samples under injected manure application, but was not coupled with higher C-min. Injection application supplies superior amounts of plant available nitrogen while fundamentally changing its distribution; biological soil health is improved under injection but confined to the injection band.

Introduction

Agrarian production systems utilize animal wastes for land application as manures are rich in elemental nitrogen, phosphorous, and potassium. The production and use of animal wastes has garnered increased attention over increasing confined production operations and the related handling and environmental ramifications of manure storage and use (Tisdale et al., (1993); Shober and Maguire, (2005)). The long term effects of land applications of animal manure, as in the Rothamsted experiments, has revealed both the limitations and benefits of long term manure application (Jenkinson, 1991), however, research on manure application technologies is surging in an effort to identify best practices for manure storage, handling, and application (Sorensen et al., (2003); Maguire et al., (2011)). The method of manure land application is dependent on the source and handling of manure prior to application and it is likely that differences between application methods result in changes in the supply and availability of nutrients. Thus, if the dynamics of nutrient cycling are affected by the application method of animal waste, an optimal method should be identified in each production system.

In dairy and swine production systems manure is stored in pits or lagoons and land applied in the liquid phase, typically by surface broadcast. Surface broadcast applications of manure, although quick and inexpensive (Rotz et al., 2011), are vulnerable to nutrient losses from surface runoff (Kleinman et al., (2002); Kulesza et al., (2014)) and ammoniacal nitrogen losses to volatilization (Thompson and Meisinger, (2002); Bittman et al., (2005); Bierer et al., (2017)), while also exacerbating odor production (Brandt et al., (2011); Chen et al., (2014)). Incorporation of surface applied manure is often used to reduce nutrient losses and stifle odor production but is incompatible with no-till and conservation-till systems. In response, multiple methods of subsurface manure injection are used as no-till compatible alternatives to incorporation with similar or greater reductions in nutrient losses (Maguire et al., 2011). For example, Powell et al., (2011) reported total nitrogen losses of 23% and 9% for surface aeration incorporated and injected applications of dairy slurry.

Despite the benefits of this application method, injection alters the distribution of applied nutrients by placing manure in buried bands which may change the dynamics of decomposition. Organic matter decomposition depends on soil temperature (Kirschbaum, 1995) and water content (Zhang et al., 2010) thus changing considerably by depth (Wildung et al., (1971); Gill and Burke, (2002)). Few authors have assessed the decomposition of manure under various application techniques. Flessa and Beese, (2000) reported no difference in CO_2 emissions from surface applied and injected cattle slurry applications in a laboratory setting with constant temperature, 14 C°, and moisture, 67% WHC. Dendooven et al., (1998) reported no difference in CO_2 production between injected and surface applied swine slurries, also under controlled conditions. In the field, studies on nutrient cycling dynamics between application techniques are lacking (Maguire et al., 2011). One study reported no difference in organic matter accumulation with different incorporation methods, however, incorporation depth altered the distribution of organic matter correspondingly (Sommerfeldt et al., 1988).

In addition to depth, the lateral distribution of nutrients is changed under the injection method, potentially affecting crop performance. Although exact measurements depend on injector type, manure is usually concentrated in narrow parallel bands beneath the soil surface. Nutrient concentrations within an injection band can be extreme; Sawyer and Hoeft, (1990) estimated aqueous ammonia activity after injection and reported toxic concentrations up to 29 days after application within the band, which were inhibitory to corn root growth. As a result, researchers have attempted to characterize the spatial distribution of nutrients between injection bands and address impacts on crop yield (Assefa and Chen, (2007); Assefa and Chen, (2008); Amin et al., (2014)) but often exclude surface broadcast application for comparison.

The impact of manure application on soil health is usually presumed positive as continual applications result in increases in soil organic matter (AFRC, (1991); Ding et al., (2012)). What is more, there is some degree of overlap between increasing organic matter and the parameters associated with "good" soil health, i.e. CEC, bulk density, aggregation, infiltration etc. Less is known about how manure handling and application practices may impact soil health (Acosta-Martinez and Waldrip, 2014), and where inferences can be made in CO_2 emissions studies, soil health is often not discussed (Rochette et al., 2000; Agnew et al., 2010). Elsewhere, one study considered arthropod abundance and diversity as a proxy for soil health and reported greater abundance under surface application (Schuster, 2015). Another study reported no difference in active microbial biomass between application methods, but was performed under laboratory conditions (Bierer et al., 2017). Some studies have measured carbon mineralization between surface and injected applications by measuring directly over an injection band (Dendooven et al., 1998; Flessa and Beese, 2000), but doing so provides limited interpretation of the application methods at a representative scale.

Thus, this study was designed to address several research gaps regarding the application methods of liquid manure at field scale with the following objectives. 1) Identify and contrast the dynamics of nitrogen cycling between surface broadcast and injected applications of liquid dairy slurry and their impact on forage quality parameters and corn yield. 2) Assess the lateral and vertical dispersal of nitrate from an injection band with inclusion of a surface application for comparison. 3) Observe the seasonal response of two metrics of biological soil health between manure application methods on soils under their first year of application.

Methods

Site Setup and Sampling Protocol

Plots were established at the Virginia Tech Kentland research farm (37.192727, -80.577099) in May of 2017 and 2018 location was chosen based on landscape uniformity, management history, and availability. The field had been in continuous no-till corn production with barley as winter cover which was terminated with herbicide prior to planting each year. In 2017, 5000kg ha^{-1} lime was applied to adjust soil pH. Plot location was not identical year over year, but were located in the same field within close proximity. Soil samples were taken to a depth of 30cm before manure application for basic property determination; particle size analysis was performed using a modified pipette method (Day, 1965; Green, 1981), organic matter determined by Elementar CNS (Elementar Vario MAX CNS Element Analyzer; Elementar Americas Inc., Ronkonkoma, NY), and pH in 1:1 soil water mixture. Dairy manure was gathered from a stirred storage lagoon during emptying and refrigerated prior to use. Before land application, total Kjeldahl nitrogen and ammonical-N analysis were completed by the agricultural service laboratory at Clemson University, manure C:N ratio was determined by Elementar CNS (Table 3.1) (Bremner and Breitenbeck, 1983; Peters et al., 2003). Manure plant available nitrogen was determined using Virginia availability coefficients: 35% of organic N is plant available irrespective of application method, 95% of ammonical-N with injected applications, 25% of ammonical-N with surface application and no incorporation (Virginia Department of Conservation and Recreation, 2014). Plots were established from one corn row to the next with dimensions 76cm x 305cm. There were 60cm between plots to eliminate border effects of applied manure. Small plots were established to reduce the quantity of manure stored prior to application, to reduce spatial variability of samples taken, and to obtain a high resolution for parameters around an injection band. Manure application was based on surface area and used the average Virginia application rate of 56,000 L ha^{-1}, surface application was performed by evenly splashing the slurry from a weigh bucket within marked plot boundaries. There were three treatments: a no-manure control, a surface applied manure, and a injected application of manure x 4 replicates = 12 plots each year. Manure injection was performed using a single tractor mounted disk to open the soil surface to a depth of approximately 15cm between two planted corn rows. Diary slurry was poured into the opening and the manure band was loosely covered with soil to replicate band coverage by closing discs on field scale injectors. The only source of added nitrogen the plots received was from manure application, both starter and side-dress applications of fertilizer were omitted from the study to more clearly assess nitrogen availability differences between manure application methods. Plots were sampled 10 times throughout each year: 0 1 2 4 6 8 10 14 18 and 22 weeks after the date of manure application at 30cm intervals along the length of the plot, week 0 samples were taken before manure application. At each sampling time, 2.5cm diameter soil cores were taken across plot widths, 1 core along the centerline of the plot length and 2 cores 10cm, 20cm, and 36cm on both sides of the centerline, cores were separated into 0-15cm and 15-30cm depth ranges. In surface applied and no-manure plots cores of each depth range were homogenized into two representative samples per plot, one air dried for nitrate analysis, and one kept moist for microbial analysis as detailed below. In injected manure plots soil cores were separated by distance from manure band (In-band, 10cm, 20cm, 36cm) and depth (0-15cm and 15-30cm) and were processed separately. Precipitation data was collected from a weather station ~500m from the study location.

Soil Nitrate

Soil nitrogen analysis was performed on samples at each time period. Samples were spread thinly to air dry and ground to pass a 2-mm sieve. Four grams were weighed into 50mL centrifuge tubes and 40mL 2M KCl added. Tubes were shaken for 30 min and vacuum filtered through Millipore S-PAK 0.45-μm membranes. The samples were processed on a Lachat Instruments QuickChem 8500 autoanalyzer (QuickChem 8500 FIA Automated Ion Analyzer; Lachat Instruments, Hatch Company, Loveland, CO) for NH_4^+-N and NO_3^--N using QuickChem Salicylate Method 12-107-06-2-A and QuickChem Method 12-107-04-1-B, respectively (Hofer, 2001)(Knepel, 2003).

Microbial Biomass

Microbial Biomass was quantified using a simplified chloroform fumigation extraction method (Fierer, 2003) at five sampling times (0, 2, 8, 14, and 22 weeks after application). Samples were 4mm-sieved moist and refrigerated prior to analysis to minimize changes during storage and all samples were processed within 2 weeks of sampling (Cernohlavkova et al., 2009). For each sample, a fumigated and an un-fumigated control pair were processed. Seven grams dry weight of soil was weighed into 70ml glass centrifuge tubes, 40ml of 0.5M K_2SO_4 was added to all tubes. The fumigated sub-samples had 1ml of ethanol-free chloroform added to each tube to lyze the microbial cells, all tubes were capped and shaken at 180 rotations per minute on a reciprocal shaker for 4h. Afterwards, samples were filtered through Whatman No. 42 equivalent Fisher brand filter paper into 50ml centrifuge tubes. The filtrate was bubbled for 1hr under a fume hood to remove chloroform in the fumigated samples using an apparatus that housed an array of syringes, and a water trap was used in the air line. After bubbling, samples were poured into 20ml plastic scintillation vials and frozen for long-term storage at -20°C until analysis. Samples were analyzed for non-purgeable organic carbon and total nitrogen, (Shimadzu, 2017), on a Shimadzu TOC-L equipped with a TNM-L unit (Shimadzu TOC-L & TNM-L, Shimadzu North America, Columbia, MD) with a dilution ratio of 1:15 to decrease salt loading of the machine. Inorganic nitrogen was analyzed on a Lachat Instruments Quickchem 8500 autosampler for NH_4^+-N and NO_3^--N using QuickChem Salicylate Method 12-107-06-2-E and QuickChem Method 12-107-04-1-H, respectively (Smith, 2003; Ammerman, 2003). Microbial carbon and nitrogen concentrations were calculated as the difference between the fumigated and un-fumigated pairs of samples. The data presented here was not transformed using a conversion factor but is the raw extractable carbon of the soil extracts.

Carbon Mineralization

A carbon mineralization (C-min) assay was used to estimate the amount of bioavailable carbon present at the same five sampling times as microbial biomass (0, 2, 8, 14, and 22 weeks after application). The method used the 60 day incubation protocol described by Strickland et al., (2015) to assess the more labile, bioavailable carbon within the soil samples (Fierer et al., 2005). Six grams dry weight of field moist soil were weighed into 50ml centrifuge tubes and DI water was added to adjust samples to 65% field capacity, samples with moisture contents exceeding 65% field capacity received no water. Tubes were sealed with caps housing rubber septa and had their headspace flushed with CO_2-free air for 3min to remove CO_2 present and then incubated for 24hrs at 20°C. After 24hrs incubation 5ml headspace was drawn from the tubes and analyzed on a LI-COR CO_2 H_2O gas analyzer (LI-7000 CO2/H2O analyzer; LI-COR, Lincoln, NE). Tubes were uncapped and returned to the incubator for a total of 60 days with the above headspace sampling scheme performed on days 1, 5, 10, 20, 30, 40, 50, and 60 of the incubation. During the incubation samples were weighed to account for water loss and adjusted to 65% field capacity as needed. An emission curve was drawn for each sample and the area underneath was used to estimate the total amount of carbon mineralized during the incubation.

Yield and Quality

Corn grain was collected by harvesting one row of corn on each side of a 152cm measuring stick within each plot, corn ears were shelled by hand. Yield was adjusted to 15% moisture content for comparison. Grain quality parameters (ash, crude fiber, fat, moisture, protein, and starch) were analyzed using near infra-red reflectance spectroscopy (NIR) on a FOSS XDS Rapid Content Analyzer (XM-1100 series; FOSS, Eden Prairie, MN). In 2018, two control plots were damaged by tractor passes which likely affected yields. The additional sunlight from a reduction in cropping density could have slightly increased yields of the control treatment in 2018.

Statistics

All analyses were conducted using JMP Pro 14 software (SAS Institute Inc., 2018). Prior to analysis, data were screened to select and remove outliers using the Quantile tail method where Q=3 and a tail=0.1. Due to the separation of soil samples into spacing categories of the injection treatment (In-band, 10cm, 20cm, and 36cm), analyses were completed using two sets of data to sanction a spatial resolution of soil properties around an injection band, allow an additional study to model soil nitrate transport from an injection band, and to better satisfy the equal variance assumption. The set of data for treatment level analysis averaged the spacing categories for the injection plots and is referred to as “plot level” analysis. The data set including the distances from the injection band is referred to as “spacing level”. In both cases, global analysis of variance was conducted to identify significant effects from: year, days or weeks after manure application, sampling depth, and manure application method or injection-spacing. When applicable, from the significant main effects, additional anova were performed sequentially in the logical hierarchy: Year⇒Depth⇒Days/Weeks⇒Treatment, and means were separated using the Tukey-Kramer honestly significant difference test. All analyses were conducted and considered significant at the ∝=0.05 level.

Results & Discussion

Plot level Soil Nitrate

Global analysis of soil nitrate identified significant effects of year, depth, days/weeks after manure application, and treatment (no-manure control, surface, injection). Analysis within each year resulted in depth, days after application, and treatment effects remaining significant and when considering year and depth, both days after application and treatment were still significant effects. In 2018, soil nitrate was 143% higher than in 2017, due to ~50% higher PAN from manure application in 2018 despite using the same source year over year (Table 3.1) and greater mineralization on a field scale indicated by higher Week 0 values in 2018. Soil nitrate was 123% and 178% higher in the 0-15cm depth relative to the 15-30cm depth in 2017 and 2018, respectively. Seasonal fluxes in soil nitrate followed a predictable pattern of increasing after manure application, peaking prior to the period of greatest crop uptake, and declining with crop uptake to background levels.

2017 Plot level Nitrate

In 2017 treatments were significantly different in the 0-15cm depth range 1 Week after the application of manure with means injection 13.51 mg kg^{-1} > surface 8.10 mg kg^{-1} > control 3.96 mg kg^{-1} (Figure 3.1 *A*). The second Week after manure application the injection treatment, 17.09 mg kg^{-1}, remained higher than both the surface and control; however, the surface application was no longer different from the control. The surface treatment was statistically the same as the control for the remainder of the season. This was likely due to surface runoff of manure nitrogen from the surface application during several days of rainfall between Weeks 1 and 2 of measurement; injection of manure has been shown reduce surface runoff of nitrogen and phosphorous (Watts et al., 2011; Kulesza et al., 2014). Leaching was also observed during this period of rainfall, with a 130% rise in soil nitrate in the 15-30cm depth under injection, 7.27 mg kg^{-1}, relative to surface application, 3.16 mg kg^{-1}, (Figure 3.1 *B)*. This agrees with results reported by Ball-Coelho et al., (2006), who observed greater nitrogen in tile drains below injected than surface applied plots when total nitrogen application rates of swine slurry were ≥60 kg ha^{-1}. However, Weslien et al., (1998) reported no difference in nitrate leaching between injection and other swine slurry application methods (trenching, surface banded/harrowing, surface banded).

In our study, Week 4 was closest to the corn pre-sidedress nitrate test (PSNT) time that indicates corn nitrate needs for the remainder of the season (Evanylo and Alley, 1998; Maguire et al., 2019). At Week 4, the injection application, 18.80 mg kg^{-1}, was below the Virginia recommended sufficiency (≥26 mg kg^{-1}) but substantially greater than surface application, 5.79 mg kg^{-1}, and control, 3.08 mg kg^{-1}, treatments at the 0-15cm depth. At the 15-30cm depth injection, 8.07 mg kg^{-1}, remained greater than the control, 4.11 mg kg^{-1}, but not the surface application, p=0.0753 (Figure 3.1 *A and B*). Nitrate in the injection treatment was greater than the surface application, probably due to the prevention of ammonia volatilization (Bierer et al., 2017). Russelle et al., (2008) also reported greater nitrogen recovery from injected than surface applied manures and attributed this to loss of ammoniacal nitrogen from the surface application. Ball-Coelho et al., (2006) reported 18% greater nitrogen recovery from injected applications of swine slurry relative to surface application, also due to less ammonia volatilization. Weeks 6 to

14 was during a period of high corn nitrogen uptake and caused soil nitrate in the manured treatments to decline to control levels where they remained so for the remainder of the season.

2018 Plot level Nitrate

In 2018 soil nitrate followed a similar pattern with higher averages than 2017, due to greater PAN application and greater mineralization on a field scale as indicated by higher Week 0 values in 2018 (Figure 3.1). In Week 1, soil nitrate of both manure applied treatments increased at the 0-15cm depth; however, only the injection application, 23.41 mg kg^{-1}, was significantly different from the control, 13.51 mg kg^{-1}. In the 15-30cm depth, there were no significant differences between treatments during the season except for one instance in Week 18 where both injection, 5.92 mg kg^{-1}, and surface, 7.00 mg kg^{-1} were elevated over the control, 2.71 mg kg^{-1}. This was possibly due to a period of prolonged rainfall that initiated substantive mineralization of manure residues while crop uptake was declining (Figure 3.1 *D*). There was no evidence of vertical nitrate transport from either manure application in 2018, potentially because 55% less rainfall occurred in the 2 weeks after manure application than what occurred in 2017. By 2 weeks after application soil nitrate in the surface treatment had begun to decline and was not significantly different from the control, while the injection application was greater than both surface and control. This was probably due to ammoniacal nitrogen losses in the surface application. Week 4 was PSNT time, where 26 mg kg^{-1} is the cutoff above which no sidedress nitrogen is recommended. When considering the standard 0-30cm sampling depth, the injection treatment approached this at 23.6 mg kg^{-1}. The surface application, 10.59 mg kg^{-1} , and control, 7.90 mg kg^{-1}, were far below this cutoff and additional sidedress applications of nitrogen would have been recommended (Virginia Department of Conservation and Recreation, 2014; Maguire et al., 2019). The extra nitrate in the injection plots would be an economic benefit to farmers, helping cover the extra cost associated with injection relative to surface application (Maguire et al., 2011). After Week 4, nitrate means of the surface application declined to control levels, while the means of the injection application remained significantly greater than the other treatments at Weeks 6, 10, and 14.

Spacing level Soil Nitrate

Global analysis of soil nitrate indicated year, depth, days/weeks after manure application, and spacing (In-band, 10cm, 20cm, and 36cm from band) were significant effects. Within year, all effects remained significant, and further analysis by year and depth indicated days/weeks after manure application and spacing were significant effects. This analysis is relevant to samples taken at distances from the injection band, hereafter called “spacing(s)”, and is the focus of the following discussion.

2017 Spacing level Soil Nitrate

In 2017 at the 0-15cm depth in Week 1, nitrate in the In-band samples, 33.39 mg kg^{-1}, was significantly greater than all other spacings (Figure 3.2 *A*). Interestingly, there were no significant differences at the 15-30cm depth in Week 1, p=0.1060, despite higher means of both the In-band and 10cm spacings (Figure 3.2 *B*). In Week 2 at 0-15cm the In-band soil nitrate peaked at 54.33 mg kg^{-1} and remained greater than all other spacings (Figure 3.2 *A*). The mean of the 10cm spacing increased in Week 2, 12.71 mg kg^{-1}, and Week 4, 18.03 mg kg^{-1}, but remained statistically the same as the 20cm and 36cm spacings due to variability of the 10cm samples. Evidence of nitrate leaching was observed at the 15-30cm depth in Week 2, the In-band soil nitrate, 13.54 mg kg^{-1}, was greater than the 20cm and 36cm, but not the 10cm spacing, 9.66 mg kg^{-1}, (Figure 3.2 *B*). The detection of horizontal nitrate movement in the 15-30cm depth but not at 0-15cm could be explained by increased leaching after the rainfall event between Weeks 1 and

2. In Week 4 at the 0-15cm depth, In-band soil nitrate, 50.62 mg kg^{-1}, was significantly greater than all other spacings. At Week 6, the 10cm spacing, 13.40 mg kg^{-1}, was not different from the In-band mean, 43.00 mg kg^{-1}, due to variability of In-band samples, SD=32.48 mg kg^{-1}, (Figure 3.2 *A*). At the 15-30cm depth in Week 4, the In-band nitrate peaked at 16.57 mg kg^{-1}, and remained greater than the 20cm and 36cm spacings (Figure 3.2 *B*). After this, all spacings declined to control levels and there were no further differences at the 15-30cm depth in 2017 except for one instance in Week 22. Similar to our own study, Assefa and Chen, (2007) plotted soil nitrate against distance from an injected band of swine slurry as a second degree polynomial 3, 6, and 19 weeks after application. Our own data also fit best to second degree polynomials at all depths and sampling times after application, 0-15cm samples had r^2>0.80 at Weeks 1, 2, and 4 each year. On Week 8 at the 0-15cm depth, soil nitrate declined and there were no significant differences between spacings, p=0.0576, despite elevated means of In-band and 10cm samples, 13.49 mg kg^{-1} and 12.15 mg kg^{-1}. (Figure 3.2 *A*). Differences appeared following a 1cm rainfall event in Week 10 where the In-band mean, 14.37 mg kg^{-1}, was significantly higher than both the 20cm and 36cm but indifferent from 10cm, 7.48 mg kg^{-1}, p=0.1271 . The mean of the In-band samples continued to decline over the period of study but remained significantly different from other spacing means in Week 14, and the 36cm mean in Week 18. This contrasts results observed by Assefa and Chen, (2007) which were unable to detect differences in spacings 9 weeks after manure application under similar application rates of swine slurry.

2018 Spacing level Soil Nitrate

The trends in soil nitrate were similar in 2018; in Week 1 at the 0-15cm depth In-band samples, 46.27 mg kg^{-1}, were significantly greater than all other spacings. The mean of 10cm samples, 26.38 mg kg^{-1}, was centered higher than means of 20cm, 12.41 mg kg^{-1}, and 36cm, 8.59

mg kg^{-1}, samples but was insignificant due to variability in the In-band and 10cm samples (Figure 3.2 *C*). A similar pattern was observed in a study comparing crop performance at distance from an injected band of swine slurry (Chen et al., 2010); considerably higher means were observed at 15cm from the manure band than 30cm, but means were sometimes not significantly different. In Week 2 of the present study, soil nitrate reached a peak of 111.22 mg kg^{-1} with In-band samples remaining greater than all other spacings. At the 15-30cm depth in Week 2, the In-band mean, 15.71 mg kg^{-1}, was significantly greater than the 36cm mean, 7.11 mg kg^{-1}, (Figure 3.2 *D*). At Week 4, the In-band mean, 11.68 mg kg^{-1}, remained greater than the 20cm and 36cm spacings, and the 10cm mean, 9.26 mg kg^{-1}, was greater than the 36cm spacing. At Week 6, the In-band nitrate, 4.88 mg kg^{-1}, was greater than the 36cm spacing; after which there were no significant differences in soil nitrate at the 15-30cm depth. In-band nitrogen was elevated longer in a comparable study by Westerschulte et al., (2015); soil monoliths were removed ~9 weeks after swine slurry injection where 50% of monoliths had >100 mg kg^{-1} inorganic nitrogen at 25-40cm. This could be due to increased vertical transport in the coarse soil texture (90% sand) used, and possibly the monolith sampling method. At Week 4 in the 0-15cm depth, In-band nitrate declined 9.7% from Week 2 but remained different from all other spacings; the mean of 10cm samples increased by 31% but was statistically the same as 20cm and 36cm spacings (Figure 3.2 *C*). Nitrate declined at all spacings from Week 4 to 6 as crop uptake was greatest through this time (Maguire et al., 2019), yet the In-band mean remained different from all spacings at both Week 6, and Week 8. At Week 10 both the In-band and 10cm means were more than 200% greater than the 20cm and 36cm means, but due to large standard deviations the only significant difference was between the In-band, 37.05 mg kg^{-1}, and 36cm spacing. By Week 14 soil nitrate had returned to pre application levels, nevertheless, the In-band mean, 3.99 mg kg^{-1}, remained greater than all other spacings.

Corn Yield and Quality

Global analysis of corn yield indicated higher yields in 2018, 10.62 Mg ha^{-1}, than in 2017, 4.76 Mg ha^{-1}, which was predicted because of higher nitrogen application (Table 3.1) and more favorable weather conditions (Figure 3.1 *A and C*). In 2017, corn yields were significantly higher in the injection application, 5.92 Mg ha^{-1}, than in the surface, 4.43 Mg ha^{-1}, or control 3.95 Mg ha^{-1} treatments (Figure 3.3) due to much higher soil nitrate availability in the majority of the season (Figure 3.1 *A*). In 2018, corn yields were not significantly different, p=0.1212, possibly due to crop damage encountered in two control plots early in the season. After blocking yield by year, injection was significantly higher than both the surface and control applications, while the surface application was still indistinguishable from the control. Global analysis of corn quality indicators (ash, crude protein, fat, moisture, crude fiber, and starch) indicated no significant differences between treatments. However, the parameters of ash content, crude fiber, moisture content, and protein content were significantly different in 2018 than in 2017.

Plot level Carbon Mineralization

Global analysis of C-min indicated depth and weeks after application were significant effects while both year and treatment were insignificant. When the analysis was performed by depth, treatment was a significant effect at 0-15cm, but not 15-30cm; week after application was a significant effect at both depths.

The C-min of samples was stratified by depth with means 0-15cm= 0.28 mg C g^{-1} dry weight soil, and 15-30cm= 0.13 mg C g^{-1} dry weight soil (Figure 3.4), probably due to greater biological activity at shallower depths. Depth stratification is consistently reported by other researchers performing similar C-min estimates (Franzluebbers et al., 1994; Rudrappa et al., 2006; Curiel Yuste et al., 2007; Franzluebbers and Stuedemann, 2015). Our results emphasize the importance of depth selection in C-min tests and re-asserts the need of standardizing a sampling depth. Interestingly, the variability of 0-15cm samples, SD= 0.072 mg C g^{-1} dry weight soil, was much higher than 15-30cm samples, SD=0.025 mg C g^{-1} dry weight soil. The spread of data at each sampling time was 3x at the 0-15cm depth and appeared to be due to both treatment differences and temporal variation. In contrast, the samples taken at 15-30cm were far more stable within each sampling date and differed only between time of sampling. Thus, a shallower depth range would be more appropriate when discerning between short term C-min changes. Regardless, sampling time and depth need to be consistent when treatments are compared in studies and when pooled for metanalyses.

At the 0-15cm depth C-min means of manure applied treatments increased 35% from Week 0 to Week 2 after application. The control samples increased 3% while the injection and surface treatments increased 61% and 43% respectively, potentially due to high quality carbon sources within the manure. Similar hypotheses were made in other studies measuring C-min from manure applied and fertilized soils (Rochette et al., 2000; Rudrappa et al., 2006). From Week 2 to 22, C-min from both manure applied treatments decreased as manure added C was presumably depleted. During the same period, mineralization was relatively stable in the no-manure control; semi-stable seasonal conditions have been reported in previous work on carbon cycling under control conditions (Ellis, 1974). Treatment differences in C-min were significant in Week 2 where injection, 0.36 mg C g^{-1} dry weight soil, was greater than the no-manure control, 0.24 mg C g^{-1} dry weight soil, but the same as the surface application, 0.34 mg C g^{-1} dry weight soil (Figure 3.5). The mean of the surface application was 41% greater than the control but was not separable, p=0.0773. At Week 8 the same pattern was present, greater mineralization from the injection application, 0.35 mg C g^{-1} dry weight soil, relative to the control, 0.28 mg C g^{-1} dry weight soil, and the surface application in-between. At Week 14 the mean C-min of the injection application had declined to 0.31 mg C g^{-1} dry weight soil and there were no significant differences for the remainder of the season.

Spacing level Carbon Mineralization (+ surface and control)

The control and surface treatment data were included solely in this analysis to aid comparisons. Global analysis indicated significant effects of depth and weeks after application and spacing. For both depths, weeks after application was significant; spacing was only significant in the 0-15cm samples, and year was only significant in 15-30cm samples. There was no significant difference in Week 0 C-min between all injection spacings and treatments (Figure 3.6). After manure was applied the C-min of the In-band samples sharply increased 150% to 0.57 mg C g^{-1} dry weight soil, in Week 2 which was attributed to the addition of high quality substrates within the manure. The same effect was not seen under the surface application, probably due to a smaller proportion of manure added carbon within each soil sample. Soil in this study was high in organic matter, 36 g kg^{-1}, (Table 3.1) and could be partially responsible for the minute response in the surface application. As explained by Stewart et al., (2007), as a soil approaches its theoretic carbon saturation point, the lower any response to carbon inputs will be. Carbon mineralization from the In-band samples remained greater than all spacings and treatments at Week 8 and Week 14. By Week 22, C-min of the In-band samples had decreased to 0.35 mg C g^{-1} dry weight soil but was still greater than samples from 20cm and 36cm spacings as well as the surface application.

The increase in bioavailable carbon in the injection band persisted for the duration of the experiment and contrasted nitrogen levels which declined to pre application levels by Week 14 each year (Figure 3.2 *A and C*). Additionally, soil nitrate was elevated In-band at Weeks 2 and 4 in the 15-30cm depth (Figure 3.2 *B and D*) and there were no differences in C-min between spacings at the same depth, p=0.2641. This suggests that the increase in bioavailable carbon did not follow nitrate concentrations, i.e. added nitrogen did not mobilize soil carbon, but may be tied to carbon sources within the manure. The proportion of the mineralized carbon originating from manure and soil cannot be determined in this experiment by subtracting the control as manure added carbon may be priming the mineralization of soil carbon (Bradford et al., 2008). Nevertheless, it is clear that bioavailable carbon increased within the injection band. Previous research indicates that long-term manure application results in net increases of total soil carbon (Edmeades, 2003) and its various fractions (Hai et al., 2010), thus it is likely that the measured increase in bioavailable carbon is primarily from the consumption of manure added substrates.

Plot level Microbial Biomass

Global analysis of microbial biomass indicated sampling depth was the only significant effect, with means of 50.53 ug C g^{-1} dry weight soil and 28.27 ug C g^{-1} dry weight soil in 0-15cm and 15-30cm samples (Figure 3.7). Stratification by depth has been reported in other studies (Franzluebbers et al., 1994; Franzluebbers and Stuedemann, 2015), and our results support decreasing biomass by depth. Microbial biomass was highly variable even after the outlier screening was applied, from a low of 3.79 ug C g^{-1} dry weight soil to a high of 105.69 ug C g^{-1} dry weight soil. Nevertheless, the values of extractable microbial C in our study are within the range of reported values (Kallenbach and Grandy, 2011) and similar to those reported by Bradford et al., (2008). Bradford's coefficient of variation for 0-10cm deep soil cores receiving sucrose additions was ~20%, n=9, while in the present study, 27%, 36%, and 20% was observed for control, injection, and surface plots, n=4. Another study reported ~15% coefficient of variation and ~250% greater microbial C in soils annually applied with manure after 3 years (Bouzaiane et al., 2007). The present study represents the first year following manure application; more definitive results may be obtained following successive years of application. A meta-analysis performed by Kallenbach and Grandy, (2011), reported that organic amendments increased microbial biomass C by an average of 36%, n=223, and years of application was a significant effect.

Within the 0-15cm depth, year was a significant effect with means of 45.41 ug C g^{-1} dry weight soil and 55.12 ug C g^{-1} dry weight soil for 2017 and 2018. Higher average values in 2018 were potentially due to ~7% higher monthly temperatures than in 2017 (Weatherstem, 2019); however, we would have expected the effect of temperature to be apparent within sampling time. In contrast, at the 15-30cm depth, year was not significant, but manure application method was. Here, the mean of the injection application, 33.47 ug C g^{-1} dry weight soil, was greater than both the surface application, 25.07 ug C g^{-1} dry weight soil, and no manure control, 26.05 ug C g^{-1} dry weight soil, (Figure 3.8). This suggests injection application can increase microbial biomass at lower soil depths. Injection placed the applied substrates deeper in the soil profile and should theoretically promote biological activity at depth. However, the increase of microbial biomass at 15-30cm was not coupled with an increase in C-min, thus, the mechanism of the increase in biomass is unclear.

Conclusion

Soil nitrate was considerably higher under the injection application much longer after application than surface application and wasn't lost as easily after rainfall. This should help cover the increased cost of injection by reducing reliance on chemical fertilizer. Although some leaching from the injection band was detected, surface nitrate was also lost and is probably more detrimental to water quality. Nitrate remained concentrated within an injection band throughout the growing season and horizontal movement was limited, however, as indicated by higher yields, corn roots were able to find it. There wasn't an extensive effect of manure application on the microbial tests conducted, except for when taken directly from the injection band, but sampling depth was especially important. C-min estimates were varied by sampling time, potentially limiting adoption as a soil health indicator.

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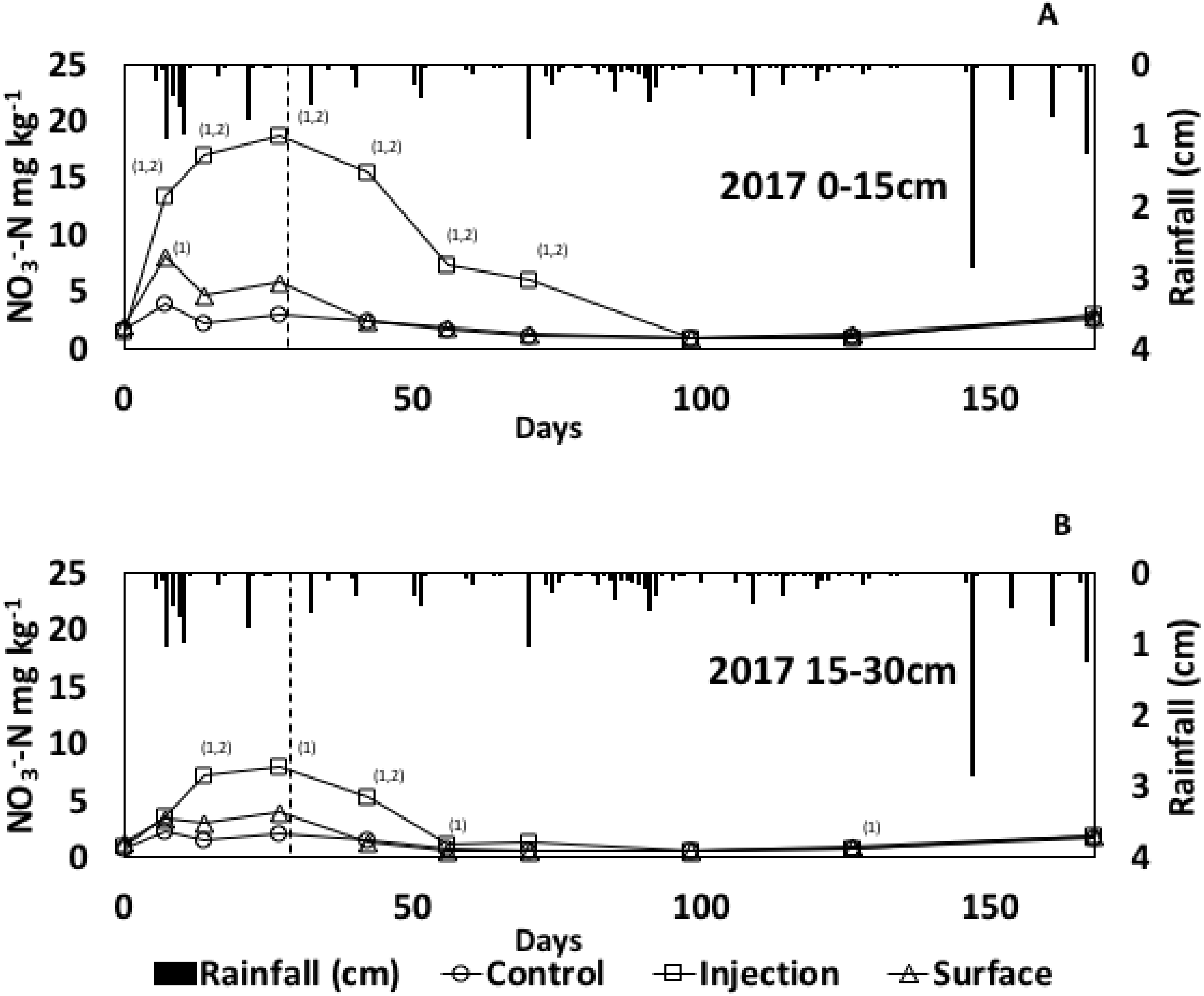
Tables

Table 3.1: Basic soil properties and Nitrogen (N) additions for each year of study. Manure added N displayed with the Virginia availability coefficients: 35% of organic-N, 95% of ammonical-N with injection, and 25% of ammonical-N with surface application, no incorporation. Nm indicates the parameter was not measured.

	Manure Organic-N	Manure Ammonical-PAN		Total PAN		Manure C:N	Sand	Clay	Soil Organic Matter	Soil pH
Year		Injection	Surface	Injection	Surface					
	kg ha^{-1}						g kg^{-1}			
2017	23.5	51.1	13.5	74.7	37.0	Nm	354	417	35.8	6.01
2018	32.2	81.0	21.3	113.2	53.5	10	384	400	36.1	5.71

Figures


A
2017 0-15cm
B
2017 15-30cm
NO$_3^-$-N mg kg^{-1}
Rainfall (cm)
Days
0
5
10
15
20
25
0
1
2
3
4
0
50
100
150
(1,2)
(1)
Rainfall (cm)
Control
Injection
Surface

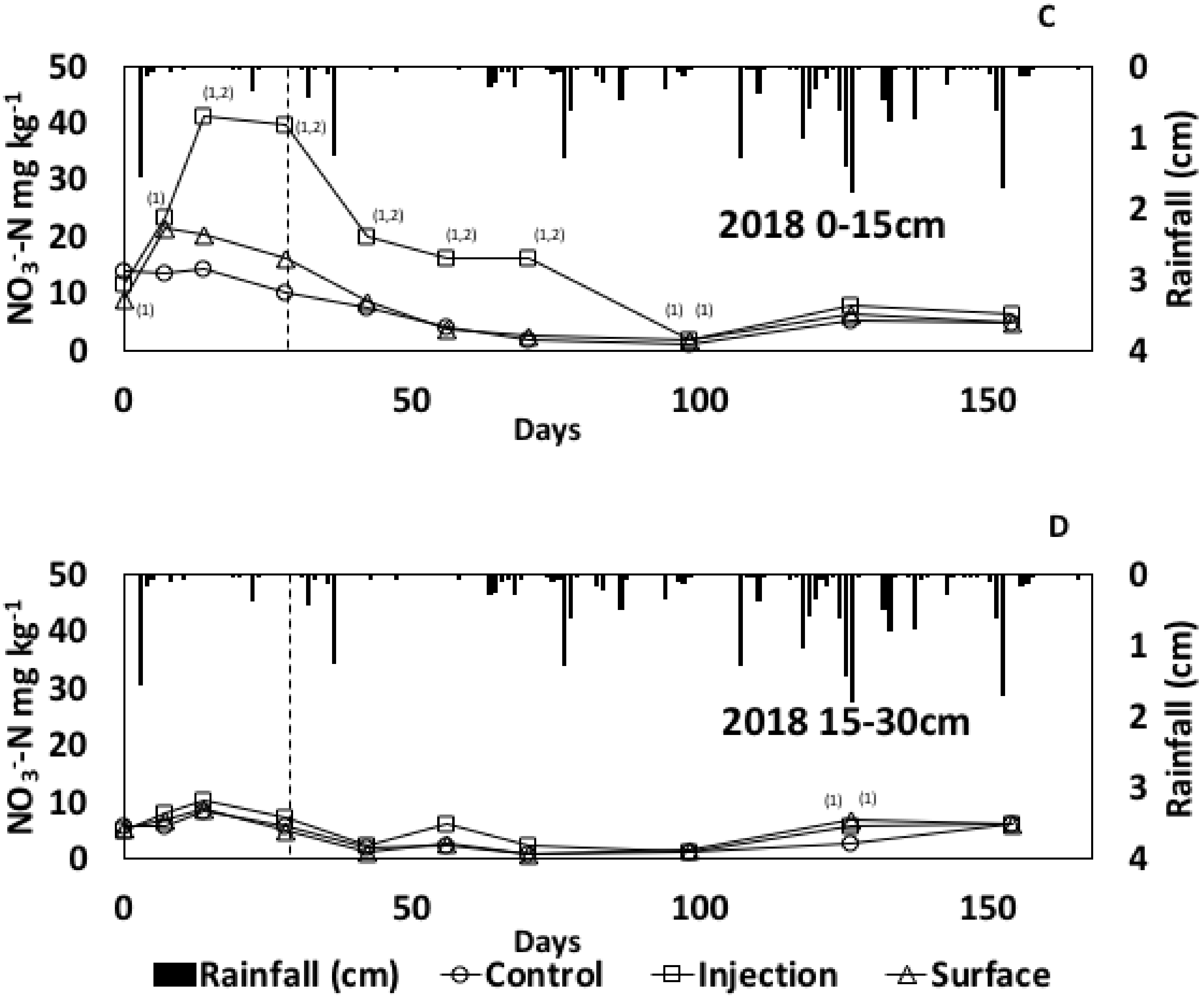
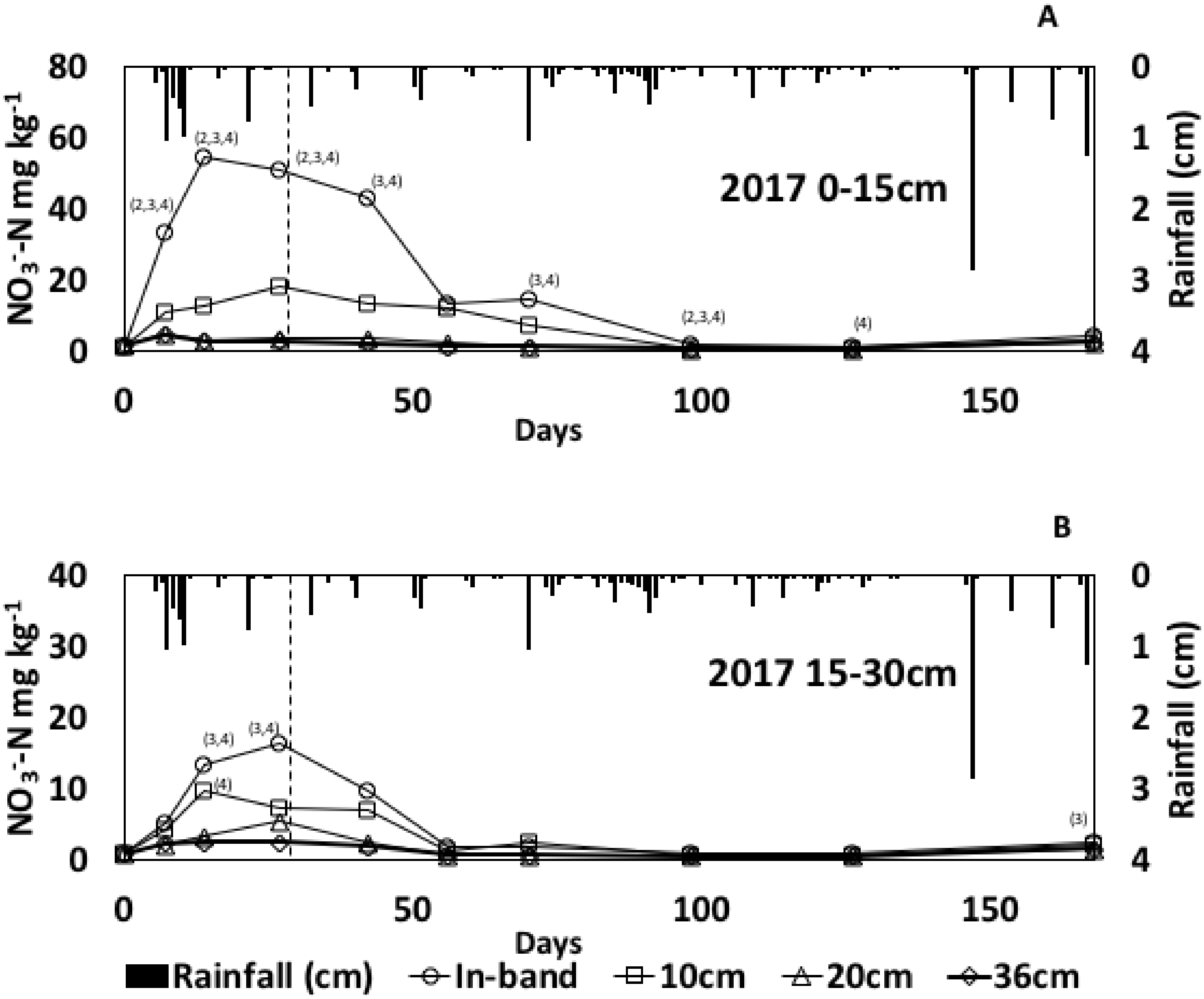


Figure 3.1: Seasonal soil nitrate trends amongst manure application methods and a no manure control, A= 2017 0-15cm, B= 2017 15-30cm, C=2018 0-15cm, D=2018 15-30cm. Days represent time after manure application, the inverted bars indicate precipitation, and the vertical dashed line represents time of pre-sidedress nitrate testing for corn. Treatment means on each sampling date were separated using the Tukey-Kramer honestly significant difference test and considered significant at the $\propto$=0.05 level. Where applicable, significant differences from the no-manure control are indicated by $^{(1)}$ while a significant difference between application methods is indicated by $^{(2)}$.


A
80
60
40
20
0
NO3--N mg kg-1
0
1
2
3
4
Rainfall (cm)
2017 0-15cm
(2,3,4)
(2,3,4)
(2,3,4)
(3,4)
(3,4)
(2,3,4)
(4)
0
50
100
150
Days
B
40
30
20
10
0
NO3--N mg kg-1
0
1
2
3
4
Rainfall (cm)
2017 15-30cm
(3,4)
(3,4)
(4)
(3)
0
50
100
150
Days
Rainfall (cm)
In-band
10cm
20cm
36cm

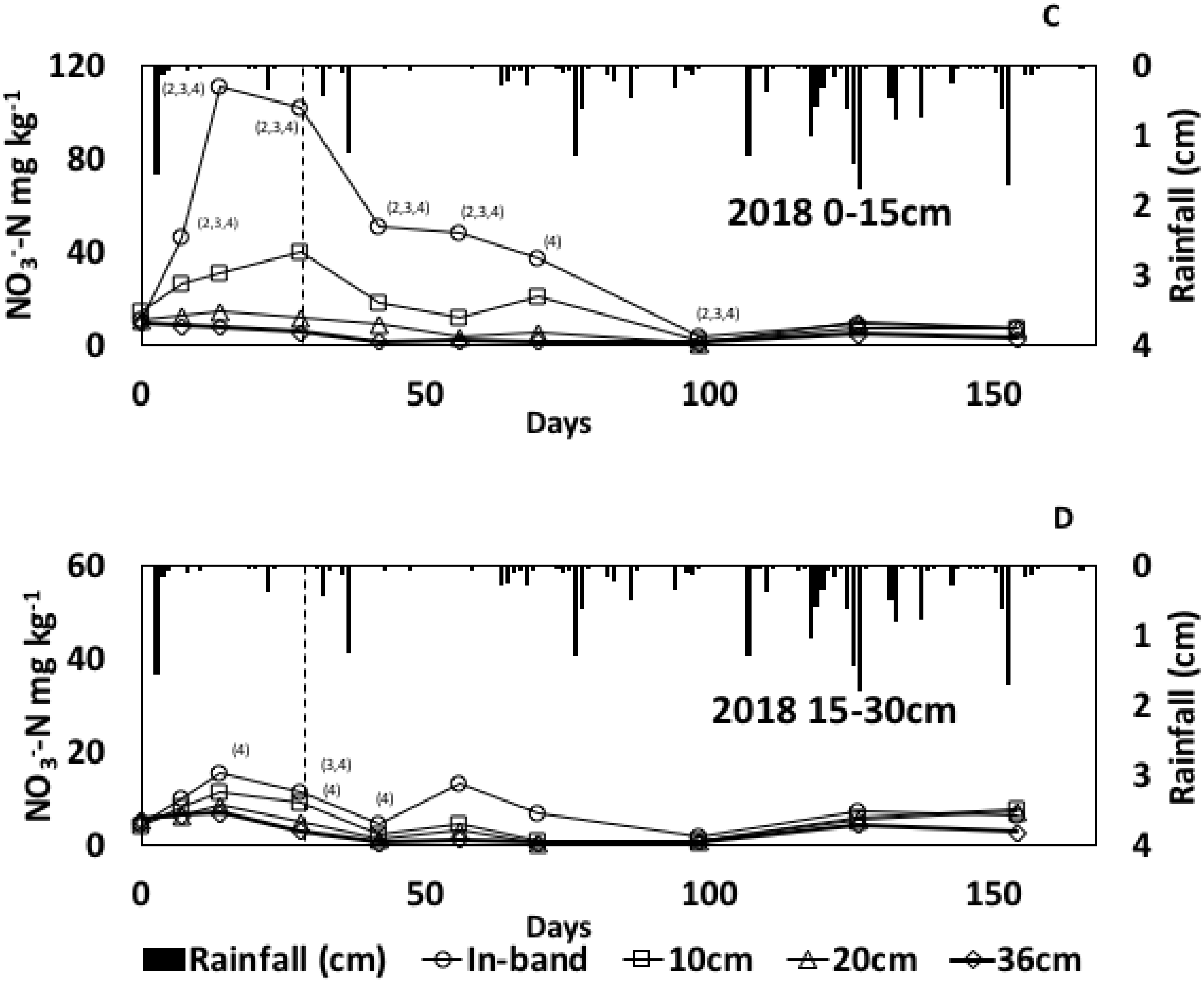


Figure 3.2: Soil nitrate manure injection spacing data; measured in-band, 10, 20, and 36cm away from the manure injection band, A= 2017 0-15cm, B= 2017 15-30cm, C=2018 0-15cm, D=2018 15-30cm. Days represent time after manure application, the inverted bars indicate precipitation, and the vertical dashed line represents time of pre-sidedress nitrate testing for corn. Spacing means on each sampling date and depth of measurement were separated using the Tukey-Kramer honestly significant difference test and considered significant at the ∝=0.05 level. Where applicable, a significant difference from In-band= (1), difference from 10cm=(2), difference from 20cm=(3), difference from 36cm=(4).

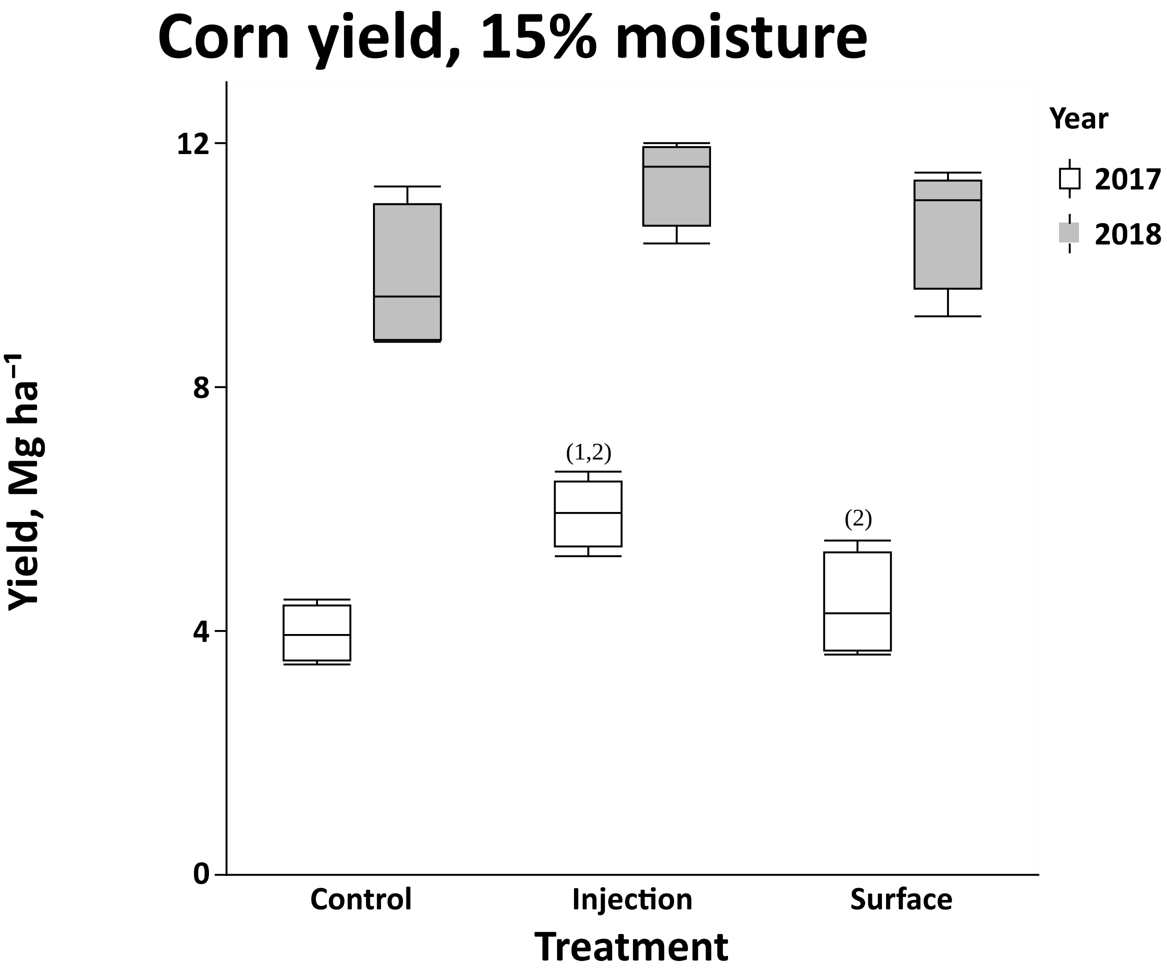


Figure 3.3: Quantile plots of corn grain yield adjusted to 15% moisture content. Treatment means within each year were separated using the Tukey-Kramer honestly significant difference test and considered significant at the ∝=0.05 level. Where applicable, a significant difference from no-manure control= (1) and a difference between manure application methods=(2).

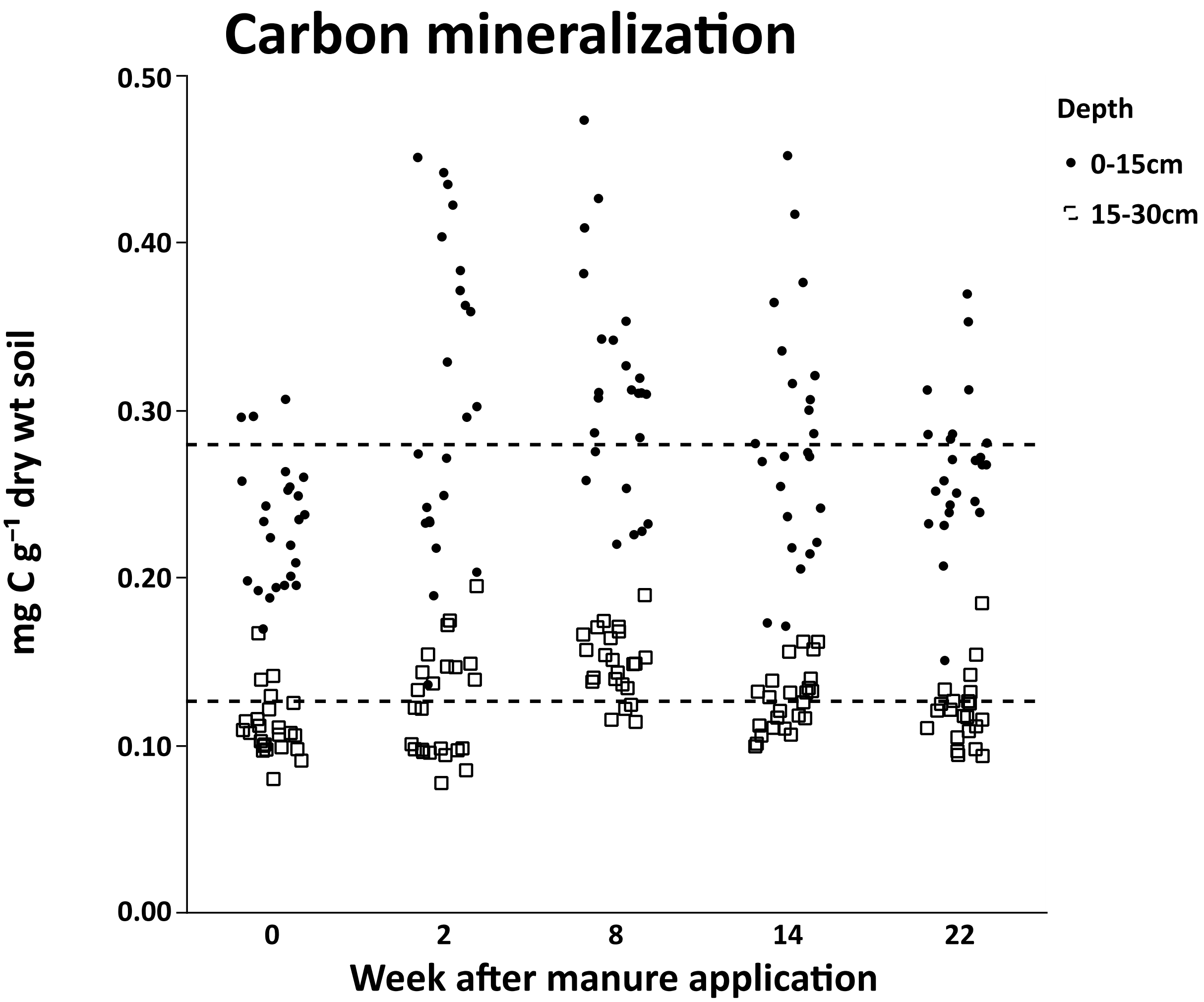


Figure 3.4: Carbon mineralized during 60-day laboratory incubations by sampling depth and weeks after manure application. Means of each depth are indicated by fit lines.

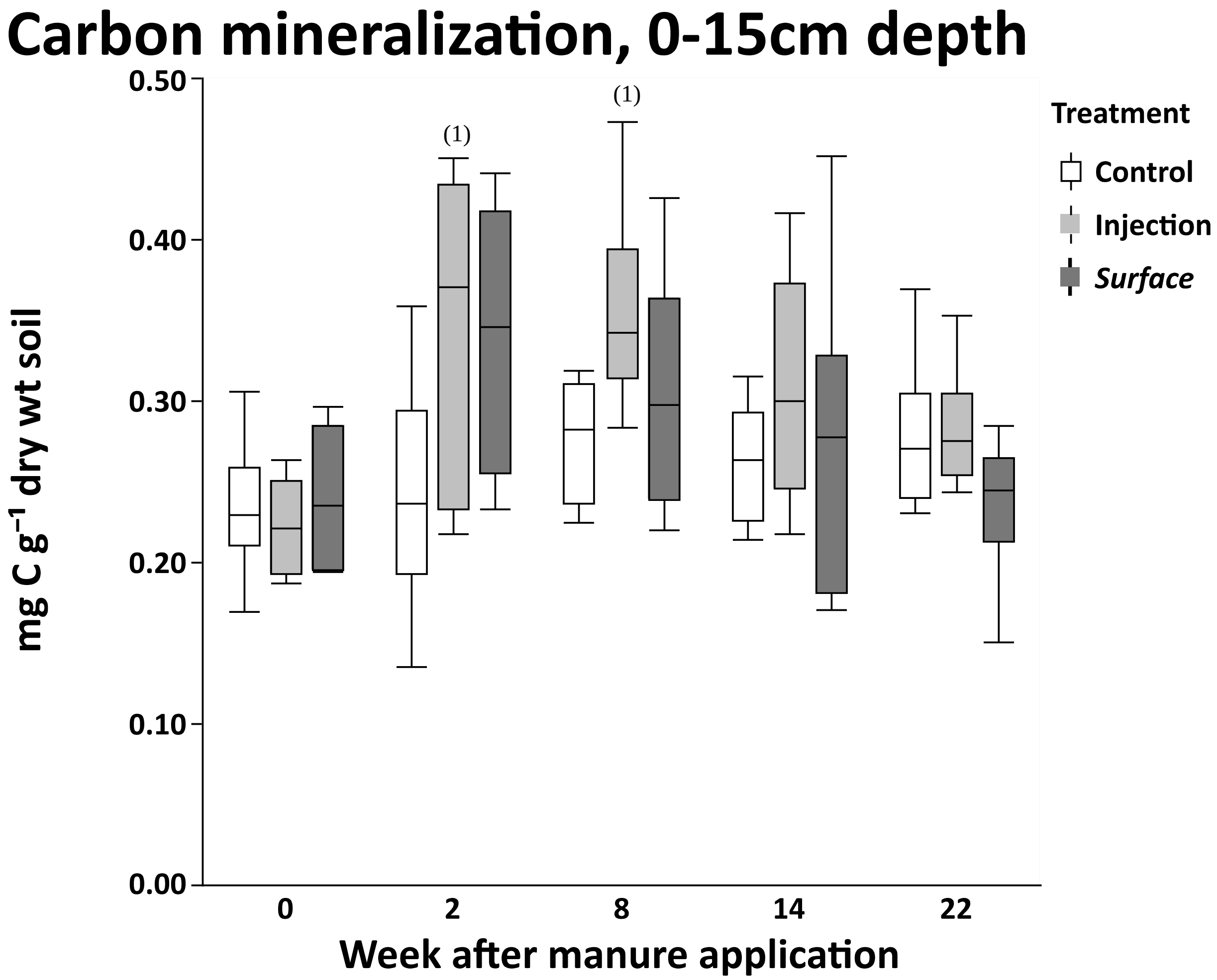


Figure 3.5: Quantile plots of carbon mineralized during 60-day laboratory incubations by treatment, 0-15cm samples. Treatment means within each sampling period were separated using the Tukey-Kramer honestly significant difference test and considered significant at the ∝=0.05 level. Where applicable, a significant difference from the no-manure control= (1) and a difference between manure application methods=(2).

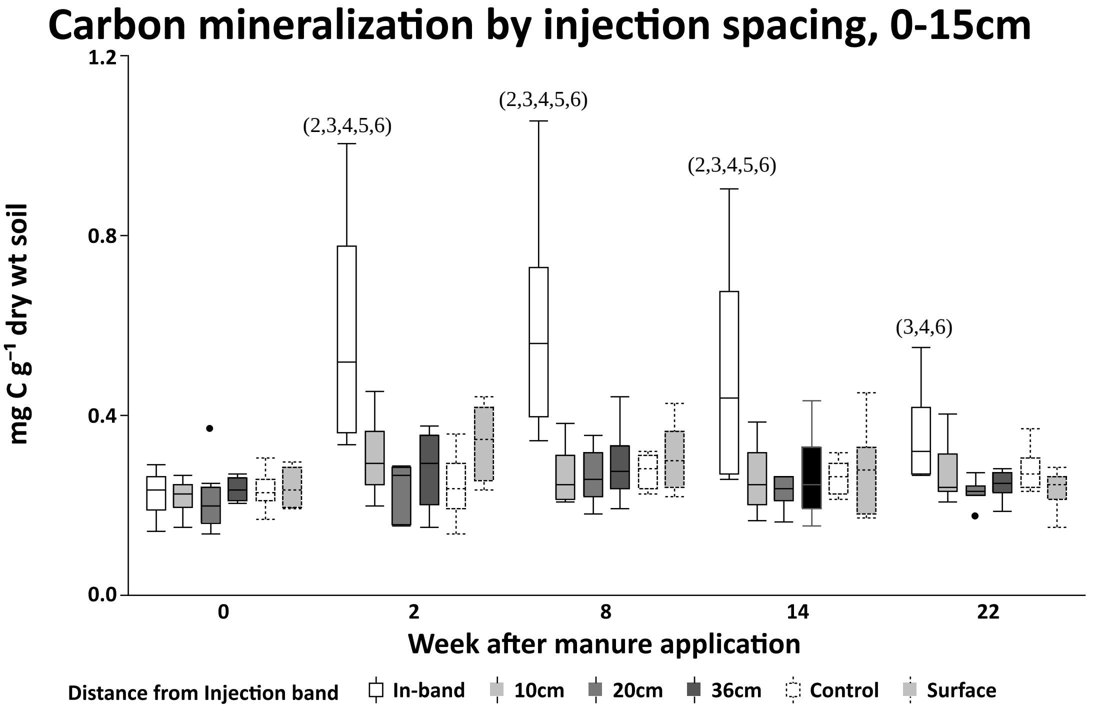


Figure 3.6: Quantile plots of carbon mineralized during 60-day laboratory incubations of 0-15cm samples taken at varied distances from manure injection band. Means within sampling period were separated using the Tukey-Kramer honestly significant difference test and considered significant at the $\propto$=0.05 level. Where applicable, a difference from in-band=(1) 10cm=(2), a difference from 20cm=(3), a difference from 36cm=(4), a difference from control=(5), and a difference from surface=(6).

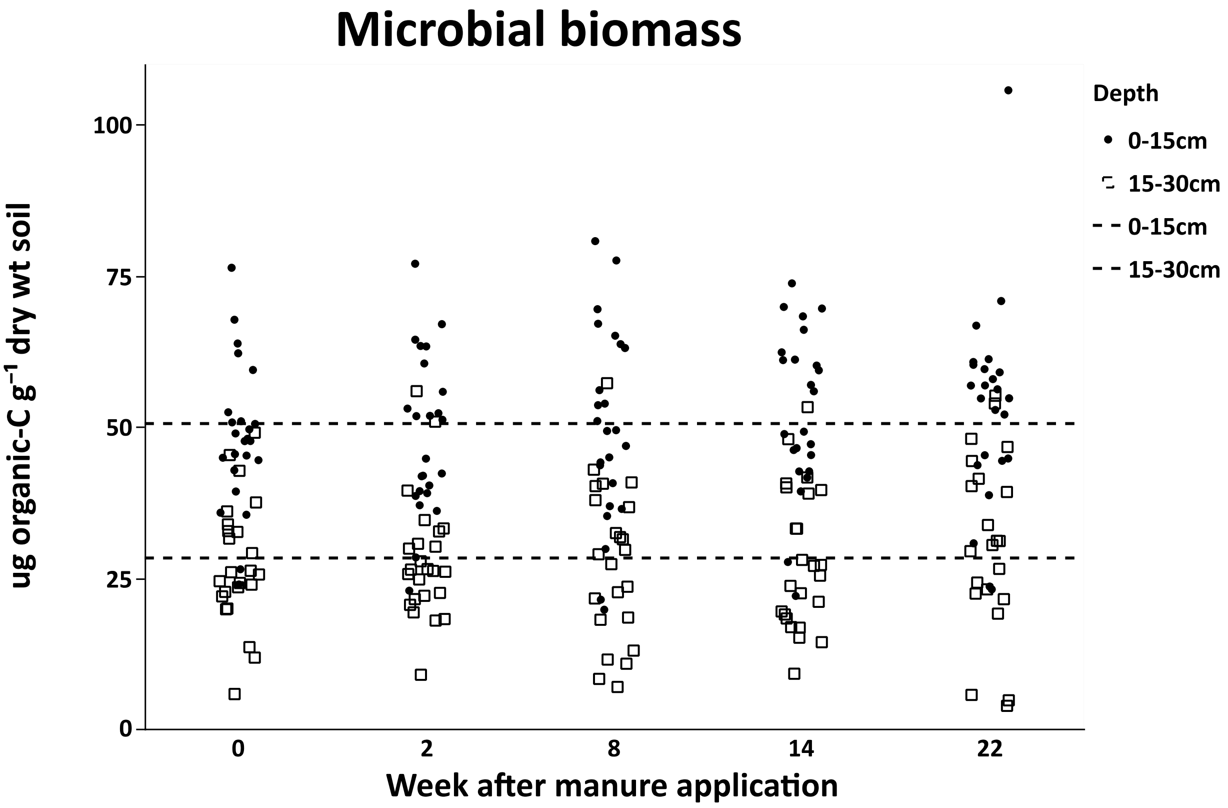


Figure 3.7: Microbial biomass by sampling depth for the two years of study estimated via chloroform fumigation extraction using 0.5M K_2SO_4. Means of each depth are indicated by fit lines.

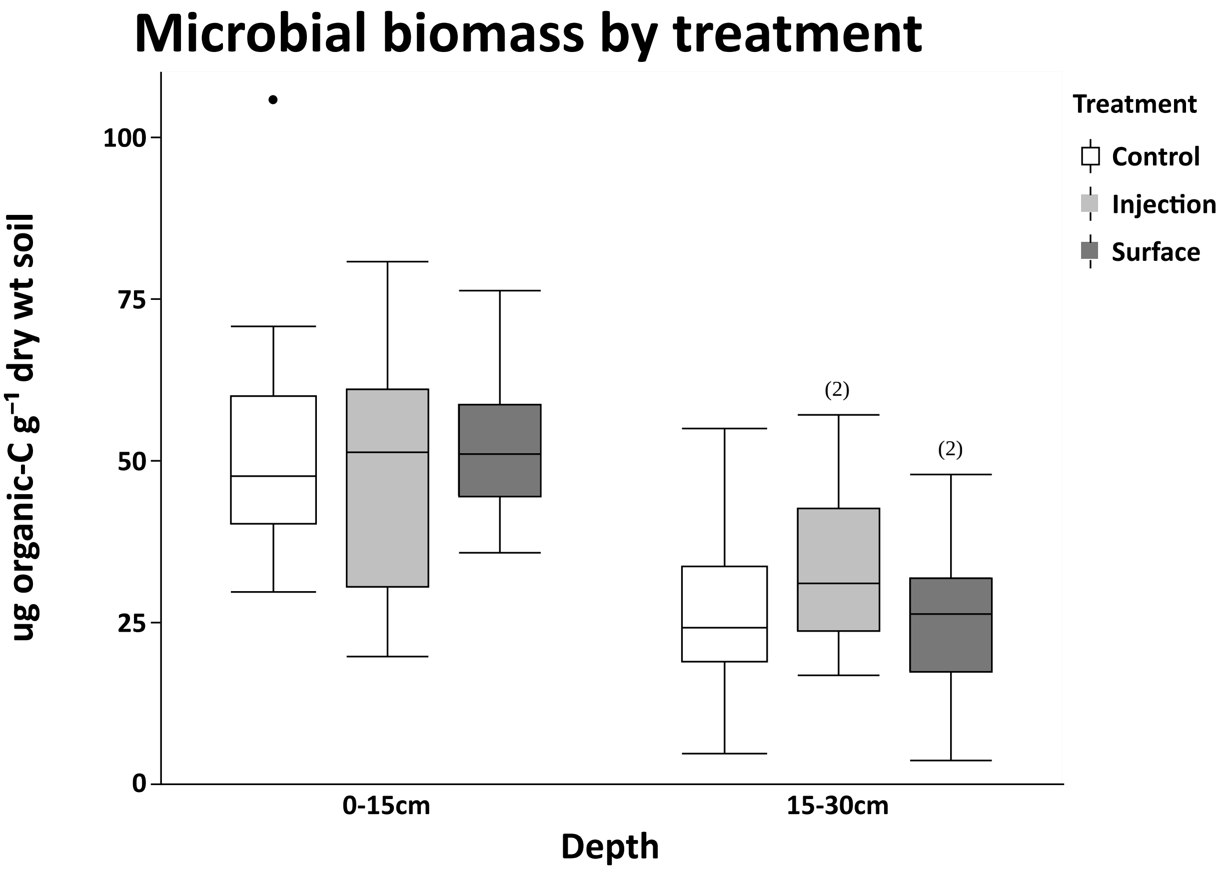


Figure 3.8: Quantile plots of microbial biomass as estimated via chloroform fumigation extraction using 0.5M K_2SO_4. Treatment means at each depth were separated using the Tukey-Kramer honestly significant difference test and considered significant at $\propto$=0.05 level. Where applicable, a significant difference from the no-manure control= (1) and a difference between manure application methods=(2).

Conclusions

Animal manures are applied to soils to supply essential plant nutrients and reduce chemical fertilizer use. Swine and dairy manures are stored in the liquid phase, surface broadcast for application, and subsequently incorporated in the soil to reduce nutrient loss. This is not feasible in no-till and conservation-till managed systems so subsurface manure injection is utilized as a no-till compatible alternative. Comparisons of subsurface injection and surface broadcast without incorporation have varied, but injection likely alters the dynamics of nutrient cycling from manure. This dissertation focused on nitrogen cycling differences and distribution, crop yield, and soil biological response to surface and injected applications of dairy manure application through three chapters of research.

In the first chapter, ammoniacal nitrogen losses were quantified in surface and injected applications to soils of different textures. It was shown that injection drastically reduces ammoniacal nitrogen losses compared to surface application, and is most prevalent in fine textured soil. Nitrogen recovery was also assessed; as a result of decreasing ammoniacal nitrogen loss, additional soil nitrate was recovered and contributed additional plant available nitrogen over the surface application. Biological response to the applications was also measured through two indicators. Active microbial biomass was estimated through substrate induced respiration (SIR) but was indifferent between both manure applications and the no-manure control, let alone application technique. Carbon mineralization (C-min) estimated the quantity of bioavailable carbon in the soil; C-min was significantly increased by manure applications and higher with surface application in the coarse textured soil. This article was published in Soil Science Journal in May 2017 and presented at the 2016 ASA, CSSA, & SSSA Annual Meeting in Phoenix AZ.

There are several recommendations that can be made from this experiment. Specifically, I recommend the use of manure injection where equipment is available to increase the nitrogen use efficiency of applied manure. Utilizing injection will increase capture of ammoniacal nitrogen and could provide substantial economic benefit, especially when chemical fertilizer nitrogen prices are high. Next, I have recommendations for future research that will be conducted similarly. The C-min and SIR estimates were made after 14-days of forced air-flow incubation for capturing ammoniacal nitrogen. This incubation time likely stimulated microbial activity and allowed active microbial biomass to increase before our estimate was performed. Second, I do not advocate measuring carbon respiration from the different manure application methods in a small scale lab setting. Due to scale lab studies are conducted on, they are most likely not representative of these application methods at the field scale. One example would be that when applying manure by surface area, e.g. L/ha, you theoretically are taking into account the inter-band space between injection bands. But, practically this is a measurement taken directly over an injection band, complicating interpretation. It should also be noted that in the field, temperature and moisture differences exist between the soil surface and the typical depth of manure injection, ~15cm, and in laboratory incubations temperature and moisture are typically kept constant across treatments.

The second chapter evaluated soil sampling procedures for fields using manure injection as it inherently changes the distribution of applied nutrients. It was found that standard random sampling was just as representative as taking several cores distributed over a manure band. Soil nitrogen recovery was also assessed and it was found that injection sometimes increased plant available nitrogen in the form of nitrate, but this was not found at all sites or times of sampling. Crop yields were never significantly higher under injection, however the means of the injected plots were always centered higher. Inferences on soil health were made using two biological indicators, SIR and C-min. Carbon mineralization was not consistently different across treatments in all sites/periods of study and appeared especially tied to site location and year. The time required to run a C-min incubation, normally 30 or 60 days, was much greater than both SIR and soil nitrogen. Substrate induced respiration also appeared to be tied to site and year; in one site-year, surface applied manure resulted in consistently higher means relative to injected manure. Overall SIR was highly variable even when run in duplicate, complicating interpretation. This manuscript has been submitted to the Journal of Soil and Water Conservation for publication and was presented at the 2017 ASA, CSSA, & SSSA Annual Meeting in Tampa, FL.

Recommendations can be made following the results of this chapter. I suggest that using random sampling in injected fields is adequate in most scenarios. When using very high application rates or if concerned about obtaining a representative sample one can utilize the "equi-spaced" sampling method as it had similar repeatability to random sampling and added little labor. It is my observation that because we are not consistently measuring elevated nitrate under injected plots, nitrogen losses from field scale manure applications may be higher than we anticipate and availability coefficients may need re-evaluated. Additionally, on working farms the chemical nitrogen applications at starter and side-dress probably make treatment differences harder to detect. I would not recommend using the respiration based metrics we used to evaluate soil health until interpretations of the tests are more clear, especially if the goal is to provide useful information to producers. Instead, I would advocate more practical biological tests, e.g. assessing potentially mineralizable nitrogen.

In the third chapter the seasonal dynamics of soil nitrate were compared when nitrogen application was limited to injected or surface applied manure and the distribution of nitrate was determined around an injection band. Injected applications recovered more nitrogen and resulted in greater soil nitrate relative to surface application for the majority of the growing season. As a result, corn grain yields were higher with injected manure. Soil nitrate was less mobile than anticipated and was detected only 10cm adjacent to the injection band, although vertical leaching was encountered in one year of study. Two biologic metrics of soil health (C-min and microbial biomass) were assessed and both metrics were highly stratified by depth. Carbon mineralization was greater with injection at shallower soil depths resulting from high C-min within the injection band. Microbial biomass was greater with injection at the deeper sampling depth but was not coupled with increased C-min. This manuscript will be submitted to the Soil Science Society of America Journal and preliminary data presented at the 2018 ASA, CSSA, & SSSA Annual Meeting in Baltimore, MD.

Recommendations following this work pertain mostly to future research on manure application method. First, when comparing this study to other field comparisons of manure application method, it was undoubtedly helpful to restrict other nitrogen applications. This provided a much clearer interpretation of the dynamics of nitrogen cycling between application methods. Second, I recommend that when C-min estimates are going to be compared between sites or years that they must be taken at the same point in time and from the same depth; further I strongly recommend a standardized sampling depth be adopted by the Soil Health Institute. Third, I recommend further research into carbon sequestration potential of manure application methods and suggest the quantification of mineral associated carbon fractions between long term surface and injected applications as a place to start. Fourth, this study will allow for the creation of a model for nitrate transport from an injection band. I recommend that such a model be refined and expanded to showcase the beneficial aspects of injection and improve estimates of nutrient losses from manure applied fields.