Evaluating dairy manure application method on soil health and nitrate

Andrew M. Bierer

Rory O. Maguire

Michael S. Strickland

Ryan D. Stewart

Wade E. Thomason

Andrew M. Bierer, Graduate Research Assistant, Ph.D. Candidate, Virginia Tech, Blacksburg, Virginia

Rory O. Maguire, Professor, School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, Virginia

Michael S. Strickland, Assistant Professor, Department of Soil and Water Systems, University of Idaho

Ryan D. Stewart, Assistant Professor, School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, Virginia

Wade E. Thomason, Professor/Extension Grains Specialist, School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, Virginia

Title

Evaluating dairy manure application method on soil health and nitrate

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, 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 Sidedress 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 greater in 7 of 25 measurements under subsurface injection and 30% higher under injection on average during the corn PSNT. 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 sidedress N applications, and either the standard method of PSNT soil sampling or an equi-spaced method can be used in injected fields.

Keywords: Corn yield—manure injection—pre sidedress nitrate test—soil health—soil nitrate

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 (N) 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 phosphorous 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 grass-dominated hay 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 15-30 cm (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 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 0.5 M K_2SO_4 (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 2015). 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 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. The study was conducted using a generalized randomized block design with treatments at all sites replicated $\geq$ 3 times. In 2016, Sites 1, 2, and 3 were planted in corn and harvested as silage; in 2017, Site 4 was corn harvested as grain and Site 5 was in soybean (Table 2). In 2018, Site 6 was planted in corn harvested as silage, and Site 7 was planted in corn harvested as grain; no location was repeated for a second year. All sites used a 76 cm row spacing except for Site 3 which used a 38 cm spacing, and Site 2 which was planted in twin rows 86 cm for outside and 57 cm for inside rows. For surface application treatments the injection equipment was raised from the soil with the pump still running, resulting in an even broadcast application without banding. Performing manure application in this manor ensured symmetric application rates between treatments. Manure application rate was decided by the land owner and is reported in Table 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 injection applied ammonical N

(Equation 2). In 2016, a shallow disc injector (Vertical Till Injector, Washington, IA) was used at all Sites with a band spacing of 76 cm and injection depth of ~15 cm. 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 61 cm and depth of ~20 cm. 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 (~9 m per pass) in the area selected for study. Soil sampling was conducted within 76 m lengths of the treatment passes and sampled a minimum of 1.5 m away from plot edges to prevent border effects.

Pre Sidedress Nitrate Test and Soil Nitrate Sampling

The routine PSNT must be conducted when the corn is ~30 cm 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 and planting but closely adhered to the following schedule: time 1= 1-month after manure application, time 2= routine PSNT when corn was ~30 cm tall, time 3= 4-months after application, and time 4= post-harvest (Table 2). In 2016, Sites were only sampled 3 times, with the third sampling date post-harvest. In 2017, Site 5 was in soybean and sampled on the same days as Site 4 due to their proximity and date of manure application (Table 2). Three soil sampling methods were compared, which we called the standard, weighted, and equi-spaced methods. The standard method used current recommendations for 10, 30 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 10 (2016) or 8 (2017) 30 cm deep soil cores, based on band spacing, centered across the injection band and the inter-band space in 2.5 cm increments with 4 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 3). The equi-spaced method (Meinen and Beegle 2015), used 5 (2016) or 4 (2017 and 2018) 30 cm deep soil cores, based on band spacing, taken 15 cm apart and perpendicular to the direction of injector travel, 4 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 as its labor requirements made it improbable for adoption. 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 50 mL centrifuge tubes and 40 mL 2 M 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 unavailable, hand harvest was performed by harvesting one row of plants on both sides of a 3 m measuring stick. 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 (Table 2). 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-day incubation. Six grams of dry weight soil were weighed into 50 ml centrifuge tubes and maintained at 65% water-holding capacity and 20 °C for the duration of the 30-day incubation. Respiration was determined across this time period on days 1, 5, 10, 20, and 30 on an infrared gas analyzer (IRGA; Model LI-7000, LiCor Biosciences, Lincoln, NE, USA). Total C-min 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 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 sampling 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 HSD test. All further soil analyses on injected plots were conducted using samples from the equi-spaced method. Soil NO_3^--N and biological soil health were analyzed by manure application method at each Site and sampling time, with means separated using the Tukey-Kramer HSD test. Crop yield and milk production were analyzed by manure application method at each Site, with means separated using the Tukey-Kramer HSD test. All analyses were considered significant at the α=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 ~30 cm 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 3). 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 ammonical-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. Bierer et al. (2017) quantified NH_3-N volatilization from injected and surface applications of dairy slurry and reported that captured ammonical N was greater in finer textured soil. Paul (2007) suggests precipitation and soil texture are regulators of mineralizable N as nitrification is conducted by obligate aerobes, thus dependent on water-filled pore space. Additionally, Sharifi et al. (2014) compared soils having a history of manure application to a no-manure application control and found that mineralizable N was elevated up to 355% in soils with previous manure applications. All Sites in the present study have an extensive history of manure application except for Site 3. Soil textures varied from Site to Site, which likely influenced N losses (Table 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 (Tables 1 and 3). 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 sidedress N applications. Virginia PSNT guidelines, revised in 2019, use 3 brackets for additional sidedress N applications; <15 mg kg^{-1} apply full rate, 15-26 mg kg^{-1} apply 50-75% of full rate, >26 mg 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 3). 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-spaced and standard methods, which elevated the coefficient of variation (C.V.) of the weighted method to 37% (Table 3). 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. Goderya et al. (1996) measured soil NO_3^--N in the top 30 cm of three large fields and reported a C.V. of 45% while a similar study assessed soil NO_3^--N in smaller 90 m x 40 m plots and reported a C.V. of 16% (Długosz and Piotrowska-Długosz 2016). Directly comparable to the present study, Zebarth et al. (1999) assessed soil N after sidedress applications of N using systematically spaced cores and random sampling. Zebarth reported similar C.V. between methods, however, increasing sidedress N rate raised the C.V. of the random sampling method. Also 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 3) 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, although the standard random sampling method proved adequate.

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 at Site 1 (Table 4). 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 in 2016, and higher than average precipitation in 2018 (Table 1). When corn was ~30 cm tall, PSNT was >40 mg kg^{-1} at Sites 1 and 2, (Table 4), 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 sidedress N applications, however, Virginia uses the corn stalk nitrate test to assess N application suitability (Sullivan and Cogger 2003).

Nevertheless, if N was not yield limiting it would be unlikely to detect yield differences between application methods which are representative of N rates (Table 1).

In 2017 and 2018, soil NO_3^--N generally declined from 1-month after application to PSNT time, likely due to crop uptake; Site 5 was planted in soybean and reported a marginal but insignificant increase in soil NO_3^--N from 1-month after application to PSNT time (time 2), potentially due to N fixation supplementing crop N uptake (Table 4). Soil NO_3^--N increased from 4-months after application to post-harvest, 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 sampling times; Site 3 exhibited consistently higher soil NO_3^--N under injection (Table 4), even when sidedress application of chemical N was high (Table 1). Sites 5 and 7 had N additions restricted to manure application (Table 1); nevertheless, treatment differences were only apparent in one instance at Site 5, 4-months after manure application when soil NO_3^--N would be inconsequential to crop growth (Table 4). When treatment differences were significant, soil NO_3^--N values were 54% higher, on average, with injection relative to surface application. When corn was ~30 cm tall, PSNT numbers under injection increased by an average of 30% over surface application and were significantly higher at 2 of 7 Sites (Table 4). In both instances, recommendations for sidedress N would be reduced by shifting the sidedress N recommendation bracket the Site falls in from <15 mg kg^{-1} to 15-26 mg kg^{-1}, potentially reducing sidedress 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 study conducted in Saskatchewan 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 NO_3^--N 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 and Forage Quality

There were no significant differences between surface and injected applications of dairy slurry on crop yield (Figure 1) or estimated milk production (Figure 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 38 cm row spacing while the other Sites used a 76 cm spacing. Yield did not differ between application methods at Sites 3 and 6 (Figure 1) despite significantly higher PSNT values for the injection application (Table 4). Data for estimated milk production follows the same trend as dry matter yield and was highly correlated, (R^2=0.72; Figure 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, such as luxury consumption of soil N and management choices made by the landowner. In Sites 1 and 2, post-harvest soil NO_3^--N was high, (>20 mg kg^{-1}; Table 4) providing evidence that N was likely not limiting crop growth. Further, Sites 4, 5, and 7 were shallowly disked to prepare a seedbed after manure application, potentially reducing ammonical-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 1 and

Figure 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 sidedressed 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 3). This difference was likely due to the higher soil organic matter of 2016 Sites relative to 2017 and 2018 (Table 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 application rates through the decomposition of high quality carbon substrates, possibly increasing decomposition of soil C through priming (Fierer et al. 2005; Strickland et al. 2015). Additionally, providing a nitrogen source to drive decomposition of more recalcitrant carbon sources could increase C-min, 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-C g dry wt. soil^{-1} hr^{-1}, 2017=0.11 ug-C g dry wt. soil^{-1} hr^{-1}, and 2018=0.33 ug-C g dry wt. soil^{-1} hr^{-1} (Figure 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 estimated soil water content at time of sampling by determining sample water content. A regression fit between sample water content and SIR, R^2=0.14, explained little, possibly because of autolyzed yeast broth addition in the SIR protocol. Both manure application methods had similar C-min patterns during the progression of the growing season (Figure 3). The large spike at PSNT time 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 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 (Figure 4), possibly due to the preparation of a seedbed through shallow disking at Sites 4 and 5 after manure application that incorporated surface applied manure 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 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, depends on the type and availability of substrates.

Variation of SIR and 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, 18 m x 18 m, 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 a significant effect 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 for assessing short term changes. 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 has been suggested (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 adapted to meet producer's needs, e.g. potentially mineralizable N 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 sidedress 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 NO_3^--N was tied to manure application rate, chemical N additions, mineralizable N, and weather patterns. Crop yield and forage quality were not affected by manure application method; 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.

References

Assefa, B., and Y. Chen. 2007. A protocol for soil nutrient sampling after liquid manure injection. Canadian Biosystems Engineering. 49: 2.7-2.13.

Beyer, L. 1995. Soil microbial biomass and organic matter composition in soils under cultivation. Biology and Fertility of Soils. 19: 197–202.

Bierer, A.M., R.O. Maguire, M.S. Strickland, W.E. Thomason, and R.D. Stewart. 2017. Effects of dairy slurry injection on carbon and nitrogen cycling. Soil Science Journal. 182(5): 181–187.

Bottner, P. 1985. Response of microbial biomass to alternate moist and dry conditions in a soil incubated with 14c- and 15n-labelled plant material. Soil Biology and Biochemistry. 17(3): 329–337.

Brandt, R.C., H.A. Elliott, M.A. Adviento-Borbe, E.F. Wheeler, P.J. Kleinman, and D.B. Beegle. 2011. Field olfactometry assessment of dairy manure land application methods. Journal of Environmental Quality. 40(2): 431–437.

Bremner, J.M., and G.A. Breitenbeck. 1983. A simple method for determination of ammonium in semimicro-Kjeldahl analysis of soils and plant materials using a block digester. Communications in Soil Science and Plant Analysis. 14(10): 905–913.

Broos, K., L.M. Macdonald, M. St, J. Warne, D.A. Heemsbergen, M.B. Barnes, M. Bell, and M.J. Mclaughlin. 2007. Limitations of soil microbial biomass carbon as an indicator of soil pollution in the field. Soil Biology and Biochemistry. 39: 2693–2695.

Bruckner, A., E. Kandeler, and C. Kampichler. 1999. Plot-scale spatial patterns of soil water content, pH, substrate-induced respiration and N mineralization in a temperate coniferous forest. Geoderma. 93: 207–223.

Cernohlavkova, J., J. Jarkovsky, M. Nesporova, and J. Hofman. 2009. Variability of soil microbial properties: Effects of sampling, handling and storage. Ecotoxicology and Environmental Safety. 72: 2102–2108.

Chang, S.X., and J.A. Trofymow. 1996. Microbial respiration and biomass (substrate-induced respiration) in soils of old-growth and regenerating forests on northern Vancouver Island, British Columbia. Biology and Fertility of Soils. 25: 145–152.

Chen, L., C. Gray, H. Neilbling, S. Yadanaparthi, M. Chahine, and M.E.D.H. Marti. 2014. On-farm comparison of two dairy manure application methods in terms of ammonia and odor emissions and costs. Applied Engineering in Agriculture. 30(5): 805–813.

Cheng, F., X. Peng, P. Zhao, J. Yuan, C. Zhong, Y. Cheng, and C. Cui. 2013. Soil microbial biomass, basal respiration and enzyme activity of main forest types in the Qinling Mountains. PLoS One. 8(6).

Długosz, J., and A. Piotrowska-Długosz. 2016. Spatial variability of soil nitrogen forms and the activity of N-cycle enzymes. Plant Soil Environment. 62(11): 502–507.

Fierer, N., J.M. Craine, K. Mclauchlan, and J.P. Schimel. 2005. Litter quality and the temperature sensitivity of decomposition. Ecology. 86(2): 320–326.

Fierer, N., and J.P. Schimel. 2002. Effects of drying - rewetting frequency on soil carbon and nitrogen transformations. Soil Biology and Biochemistry. 34: 777–787.

Goderya, F., M. Dahab, W. Woldt, and I. Bogardi. 1996. Spatial patterns analysis of field measured soil nitrate. Biological Systems Engineering. 514: 248–261.

Gunderson, L. 2017. Haney Test Interpretation Guide v1 . 0. Ward Laboratories, Inc. Kearney, NE. Accessed 2019. https://www.wardlab.com/haney-interpretation.php

Haney, R.L., A.J. Franzluebbers, F.M. Hons, and D.A. Zuberer. 1999. Soil C extracted with water or K2SO4: pH effect on determination of microbial biomass. Canadian Journal of Soil Science. 10: 529–533.

Haney, R.L., E.B. Haney, L.R. Hossner, J.G. Arnold. 2006. Development of a new soil extractant for simultaneous phosphorus, ammonium, and nitrate analysis. Communications in Soil Science and Plant Analysis. 37: 1511–1523.

Haney, R.L., E.B. Haney, D.R. Smith, R.D. Harmel, and M.J. White. 2018. The soil health tool — Theory and initial broad-scale application. Applied Soil Ecology. 125: 162–168.

Hofer, S. Determination of Ammonia (Salicylate) in 2M KCl soil extracts by flow injection analysis. QuickChemMethod 12-107-06-2-A Revised by K. Bogren (2003). Loveland, CO, Lachat Instruments.

Jokela, W.E., S.C. Bosworth, and J.J. Rankin. 2014. Sidedressed dairy manure effects on corn yield and residual soil nitrate. Soil Science Journal. 179(1): 37–41.

Kulesza, S.B., R.O. Maguire, W.E. Thomason, S.C. Hodges, and D.H. Pote. 2014. Effects of poultry litter injection on ammonia volatilization, nitrogen availability, and nutrient losses in runoff. Soil Science Journal. 179(4): 190–196.

Magdoff, F., and D.S. Ross. 1984. A soil test for nitrogen availability to corn. Soil Science Society of America Journal. 48: 1301–1304.

Maguire, R.O., P.J.A. Kleinman, C.J. Dell, D.B. Beegle, R.C. Brandt, J.M. McGrath, and Q.M. Ketterings. 2011. Manure application technology in reduced tillage and forage systems: A review. Journal of Environmental Quality. 40(2): 292–301.

Maguire, R.O., W.E. Thomason, G.K. Evanylo, and M.M. Alley. 2019. Nitrogen soil testing for corn in Virginia.Virginia Cooperative Extension, Publication 418-016, Virginia Tech, Blacksburg, VA.

McGill, W.B., K.R. Cannon, J.A. Robertson, and F.D. Cook. 1986. Dynamics of soil microbial biomass and water-soluble organic C in Breton L after 50 years of cropping to two rotations. Canadian Journal of Soil Science. 66(1): 1–19.

Meinen, R., and D. Beegle. 2015. Soil nitrate testing protocol development for lands recieving injected manure. Accessed March, 2018. https://lpelc.org/soil-nitrate-testing-protocol-development-for-lands-recieving-injected-manure/

Menyailo, O. V, B.A. Hungate, and W. Zech. 2002. The effect of single tree species on soil microbial activities related to C and N cycling in the Siberian artificial afforestation experiment. Plant Soil. 242: 183–196.

Misselbrook, T.H., J.A. Laws, and B.F. Pain. 1996. Surface application and shallow injection of cattle slurry on grassland: Nitrogen losses, herbage yields and nitrogen recoveries. Grass and Forage Science. 51(3): 270–277.

Moebius-Clune, B., D. Moebius-Clune, R. Schindelbeck, K. Kurtz, H. Van Es, and A. Ristow. 2017. Comprehensive assessment of soil health, the cornell framework. 3.2. Cornell University, Geneva, NY.

Mooleki, S.P., J.J. Schoenau, G. Hultgreen, G. Wen, and J.L. Charles. 2002. Effect of rate , frequency and method of liquid swine manure application on soil nitrogen availability , crop performance and N use efficiency in east-central Saskatchewan. Canadian Journal of Soil Science. 82: 457–467.

Natural Resources Conservation Service. Soil health: Healthy soil for life. Accessed

2019. https://www.nrcs.usda.gov/wps/portal/nrcs/main/soils/health/.

NOAA. 2019. Climate data online. National oceanic and atmospheric administration, national centers for environmental information. Accessed 2019. https://www.ncdc.noaa.gov/cdo-web/

Paul, E.A. 2007. Soil microbiology, ecology, and biochemistry (P Eldor, Ed.). Third. Academic Press, Boston, MA.

Peters, J., S. Combs, B. Hoskins, J. Jarman, J. Kovar, M. Watson, A. Wolf, and N. Wolf. 2003. Recommended methods of manure analysis. University of Wisconsin-Extension, Madison, WI.

Pfost, D. 2018. Odors from livestock operations: Cause and possible cures. University of Missouri. Accessed January, 2019. https://extension2.missouri.edu/g1884

Phillips, C.L., and N. Nickerson. 2015. Soil respiration. Reference Module in Earth Systems and Environmental Science. Elsevier.

Pote, D.H., W.L. Kingery, G.E. Aiken, F.X. Han, P.A. Moore Jr., and K. Buddington. 2003. Water-quality effects of incorporating poultry litter into perennial grassland soils. Journal of Environmental Quality. 32: 2392–2398.

Rahman, S., Y. Chen, Q. Zhang, S. Tessier, and S. Baidoo. 2001. Performance of a liquid manure injector in a soil bin and on establishing forages. Canadian Biosystems Engineering. 43: 2.33-2.40.

Rodhe, L., and A. Etana. 2005. Performance of slurry injectors compared with band spreading on three Swedish soils with ley. Biosystems Engineering. 92(1): 107–118.

Roper, W.R., D.L. Osmond, J.L. Heitman, M.G. Wagger, and S.C. Reberg-horton. 2017. Soil health indicators do not differentiate among agronomic management systems in North Carolina soils. Soil Science Society of America Journal. 81: 828–843.

SAS Institute Inc. 2018. JMP Pro®. Version 14. Cary, NC: SAS Institute Inc.

Sawyer, J.E., and R.G. Hoeft. 1990. Effect of injected liquid beef manure on soil chemical properties and corn root distribution. Journal of Production Agriculture. 3(1): 50–55.

Schmitt, M., S. Evans, and G. Randall. 1995. Effect of liquid manure application methods on soil nitrogen and corn grain yields. Journal of Production Agriculture. 8(2): 186–189.

Sharifi, M., B.J. Zebarth, J.J. Miller, D.L. Burton, and C.A. Grant. 2014. Soil nitrogen mineralization in a soil with long-term history of fresh and composted manure containing straw or wood-chip bedding. Nutrient Cycling in Agroecosystems. 99: 63–78.

Shaver, R. 2006. Corn Silage Evaluation: MILK 2000 challenges and opportunities with MILK 2006. Univeristy of Wisconsin, College of Agriculture and Life Sciences, Madison, WI.

Soil Health Institute. 2019. National soil health measurements to accelerate agricultural transformation. Soil Health Institute, Accessed 2019. http://soilhealthinstitute.org/national-soil-health-measurements-accelerate-agricultural-transformation/

Strickland, M.S., J.L. Devore, J.C. Maerz, and M.A. Bradford. 2010. Grass invasion of a hardwood forest is associated with declines in belowground carbon pools. Global Change Biology. 16(4): 1338–1350.

Strickland, M.S., L.Z. H., S.E. B., and B.M. A. 2015. Biofuel intercropping effects on soil carbon and microbial activity. Ecological Society of America. 25(1): 140–150.

Sullivan, D.M., and C.G. Cogger. 2003. Post-harvest soil nitrate testing for manured cropping systems west of the cascades. Oregon State University, Extension Service, Corvallis, Oregon.

Sullivan, D., and D. Granatstein. 2015. Are “ haney tests ” meaningful indicators of soil health and estimators of nitrogen fertilizer credits?. Nutrient Digest. 7(2): 1–2.

Virginia Department of Conservation and Recreation Division of Soil and Water Conservation. 2014. Commonwealth of Virginia. Virginia nutrient management standards and criteria. Richmond,VA.

Wardle, D.A., and D. Parkinson. 1990. Biology and fertility interactions between microclimatic variables and the soil microbial biomass. Biology and Fertility of Soils. 9: 273–280.

Watts, D.B., T.R. Way, and H.A. Torbert. 2011. Subsurface application of poultry litter and its influence on nutrient losses in runoff water from permanent pastures. Journal of Environmental Quality. 40(3): 421–430.

Yost, M.A., K.S. Veum, N.R. Kitchen, J.E. Sawyer, J.J. Camberato, P.R. Carter, and R.B. Ferguson. 2018. Evaluation of the haney soil health tool for corn nitrogen recommendations across eight midwest states. Journal of Soil and Water Conservation. 73(5): 587–592.

Zebarth, B.J., M.F. Younie, J.W. Paul, J.W. Hall, and G.A. Telford. 1999. Fertilizer banding influence on spatial and temporal distribution of soil inorganic nitrogen in a corn field. Soil Science Society of America Journal. 63: 1924–1933.

Table Captions / Tables

Table 1: Basic soil properties and Nitrogen (N) additions among research Sites. Starter N and sidedress N applications are assumed entirely plant available. Manure added N is displayed as plant available nitrogen (PAN) with the Virginia availability coefficients: 35% of organic-N, 95% of ammonical-N with injection, and 25% of ammonical-N with surface (Equation 2). Total PAN is equal to the sum of starter N, sidedress N, manure organic PAN, and manure ammonical PAN for injection or surface application respectively. A n/a indicates no application due to cropping system, and a 0 indicates no application due to management decision.

Table 2: Dates of manure application, planting and crop planted, harvest, and soil NO_3^--N sampling. For soil NO_3^--N sampling, 1-month and 4-month indicate the time after manure application. Sites 1, 2, and 3 were not sampled 4-months after manure application. Where applicable, ~ approximates the date.

Table 3: Pre-sidedress nitrate test (PSNT) results for sampling methods (equi-spaced, standard, weighted) within manure injected fields, compared to surface applied fields. A 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), and *** (P<0.001).

Table 4: Soil nitrate results of fields injected or surface broadcast with dairy slurry; 1- month after manure application, pre sidedress nitrate test (PSNT) window, 4-months after application, and post-harvest, a n/a indicates no measurement was taken. Where applicable, manure application methods at each Site and sampling time are indicated by * (P<0.05), **(P<0.01), and *** (P<0.001).

Table 1

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

Table 2

Site	Manure Application	Planting	Crop	Harvest	Soil NO_3^--N sampling			
					1-month	PSNT	4-month	Post-harvest
1	4/13/16	5/16/16	Corn (silage)	9/05/16	5/20/16	6/13/16	n/a	9/08/16
2	3/11/16	4/25/16	Corn (silage)	8/26/16	4/15/16	6/06/16	n/a	9/06/16
3	4/20/16	5/22/16	Corn (silage)	9/05/16	5/20/16	6/14/16	n/a	9/13/16
4	4/11/17	5/02/17	Corn (grain)	9/22/17	5/10/17	6/09/17	8/11/17	10/11/17
5	4/11/17	~5/09/17	Soybean	10/11/17	5/10/17	6/09/17	8/11/17	10/11/17
6	4/11/18	~4/25/18	Corn (silage)	8/21/18	5/11/18	6/01/18	8/14/18	8/28/18
7	4/11/18	~4/25/18	Corn (grain)	~9/03/18	5/11/18	6/01/18	8/14/18	9/11/18

Table 3

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 4

Year/Site	Soil NO_3^--N 1-Month Injection	1-Month Surface	PSNT Injection	PSNT Surface	4-Months Injection	4-Months Surface	Post-harvest Injection	Post-harvest Surface
2016	(mg kg^{-1})							
1	15.5	20.7	47.6	43.4	n/a	n/a	23.7	16.6
2	19.7	22.2	42.9	42.9	n/a	n/a	3.9*	1.5
3	14.7*	8.4	21.0*	13.6	n/a	n/a	11.7*	4.8
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

Equations

Equation 1: Haney soil health test = $\frac{\text{1-day CO}_2\text{-C burst}}{10} + \frac{WEOC}{50} + \frac{WEON}{10}$

WEOC= Water Extractable Organic Carbon
WEON= Water Extractable Organic Nitrogen

Equation 2: Manure plant available nitrogen (PAN) =

Surface applied= $(0.35 \times total\ organic\ N) + (0.25 \times total\ ammonical\ N)$

Injection applied= $(0.35 \times total\ organic\ N) + (0.95 \times total\ ammonical\ N)$

Equation 3: Weighted method soil NO_3^--N = $(0.33 \times \text{across band NO}_3) + (0.66 \times \text{between bands NO}_3)$

Figure captions / Figures

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

Figure 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.

Figure 3: Carbon mineralized during 30-day laboratory incubations by Site and sampling time (1-month, PSNT, 4-months, and Post-harvest). Carbon mineralized was estimated by integrating CO_2 production over days 1, 5, 10, 20, and 30 of the incubation. Where applicable, significant differences between manure application method at each site and time period are indicated by * (P<0.05), **(P<0.01), and *** (P<0.001). Error bars represent standard deviations of the means.

Figure 4: Substrate induced respiration during 4-hr laboratory incubations after addition of an autolyzed yeast broth substrate. Incubations were performed by Site and sampling time (1-month, PSNT, 4-months, and Post-harvest). Where applicable, significant differences between manure application method at each site and time period are indicated by * (P<0.05), **(P<0.01), and *** (P<0.001). Error bars represent standard deviations of the means.

Figure 1

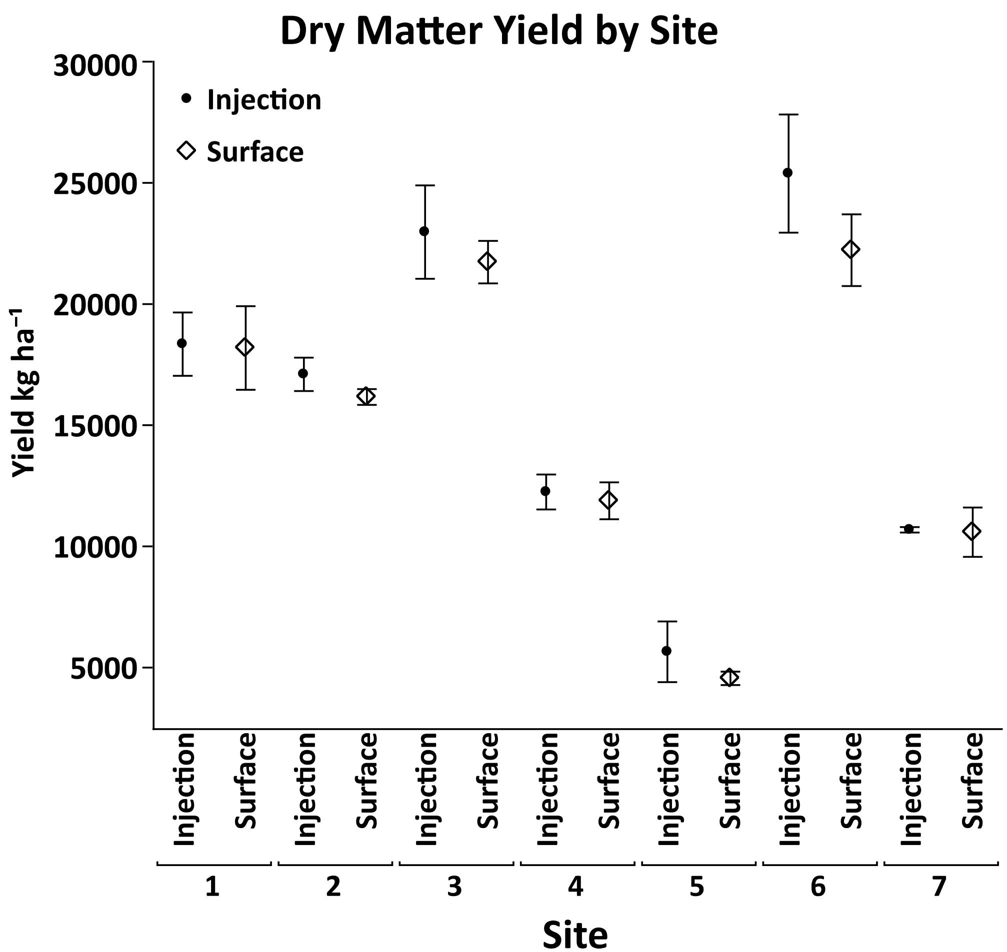
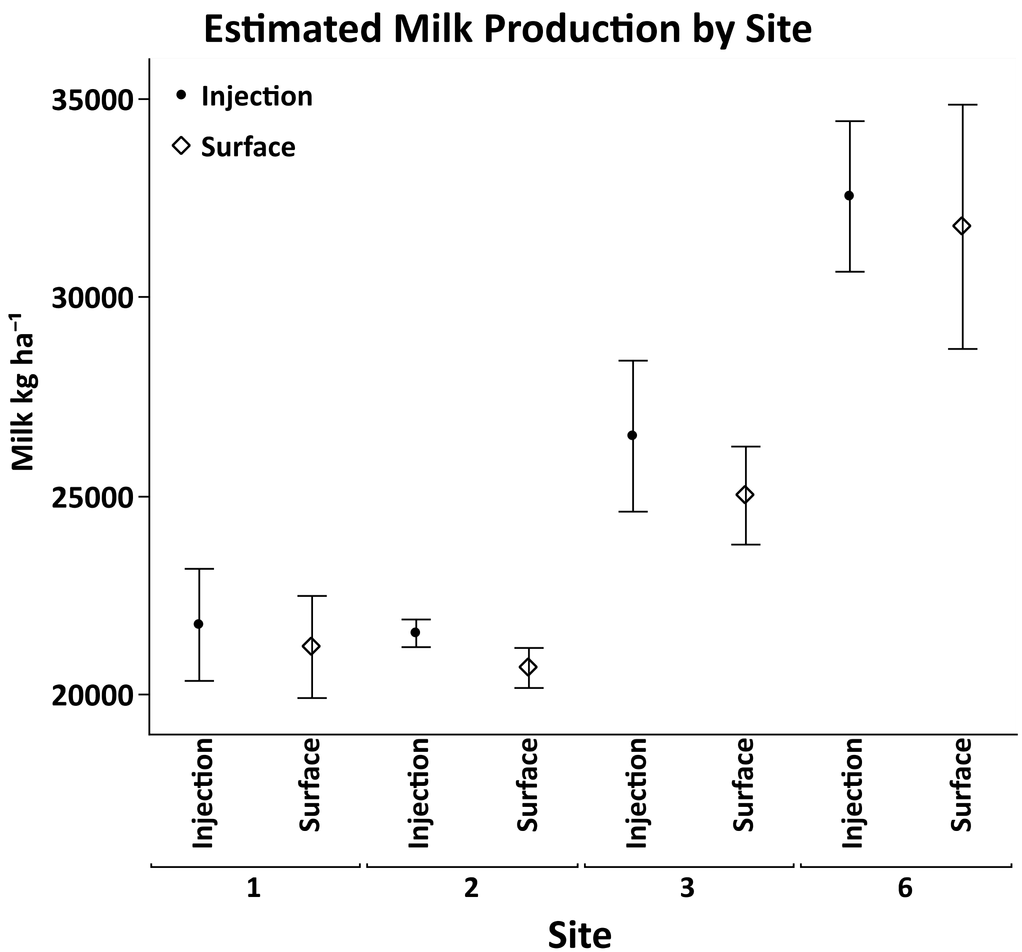

Dry Matter Yield by Site
Injection
Surface
Yield kg ha⁻¹
30000
25000
20000
15000
10000
5000
Injection
Surface
1
2
3
4
5
6
7
Site

Figure 2



Estimated Milk Production by Site
Injection
Surface
35000
30000
25000
20000
Milk kg ha⁻¹
Injection
Surface
Injection
Surface
Injection
Surface
Injection
Surface
1
2
3
6
Site

Figure 3

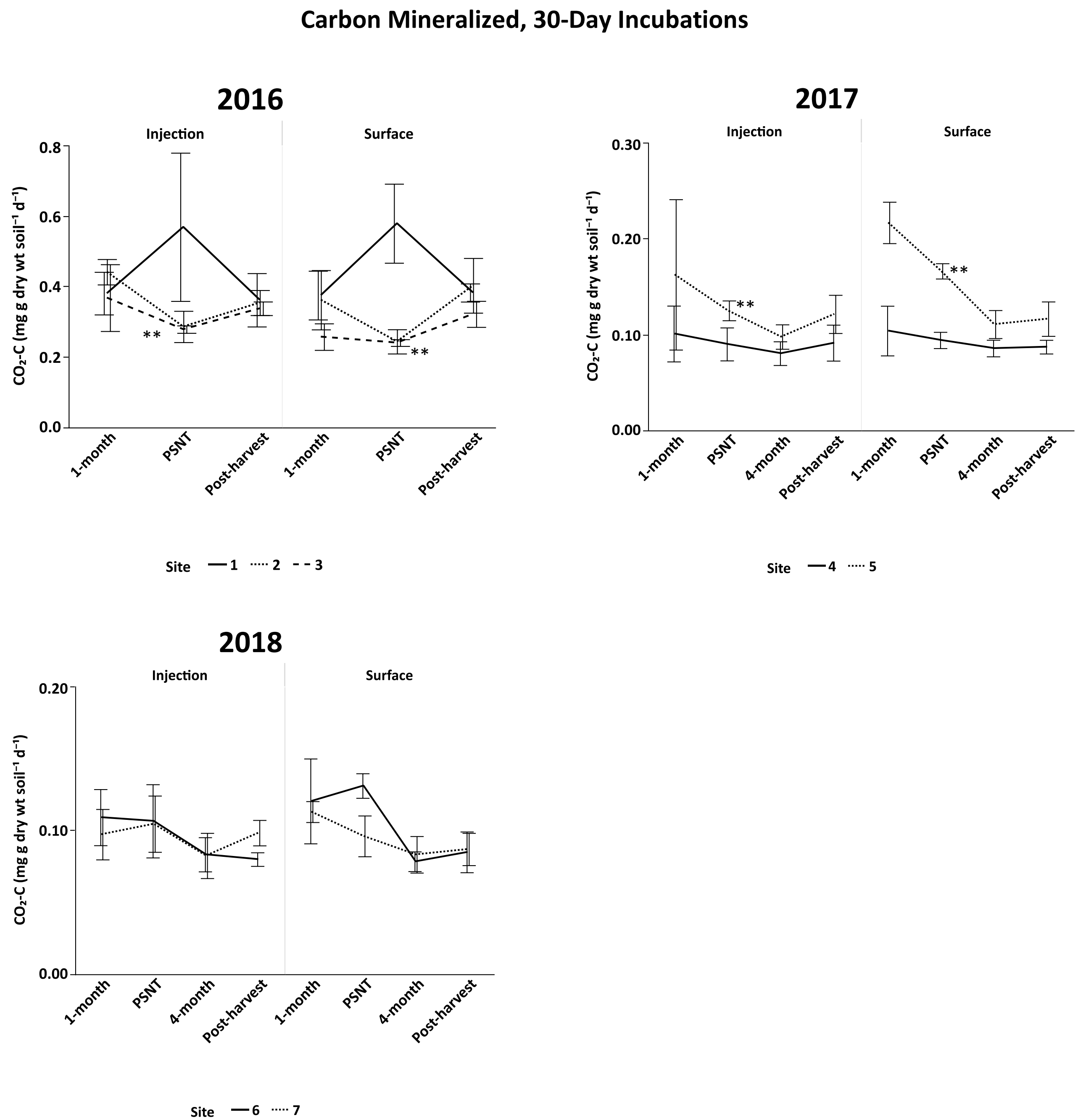
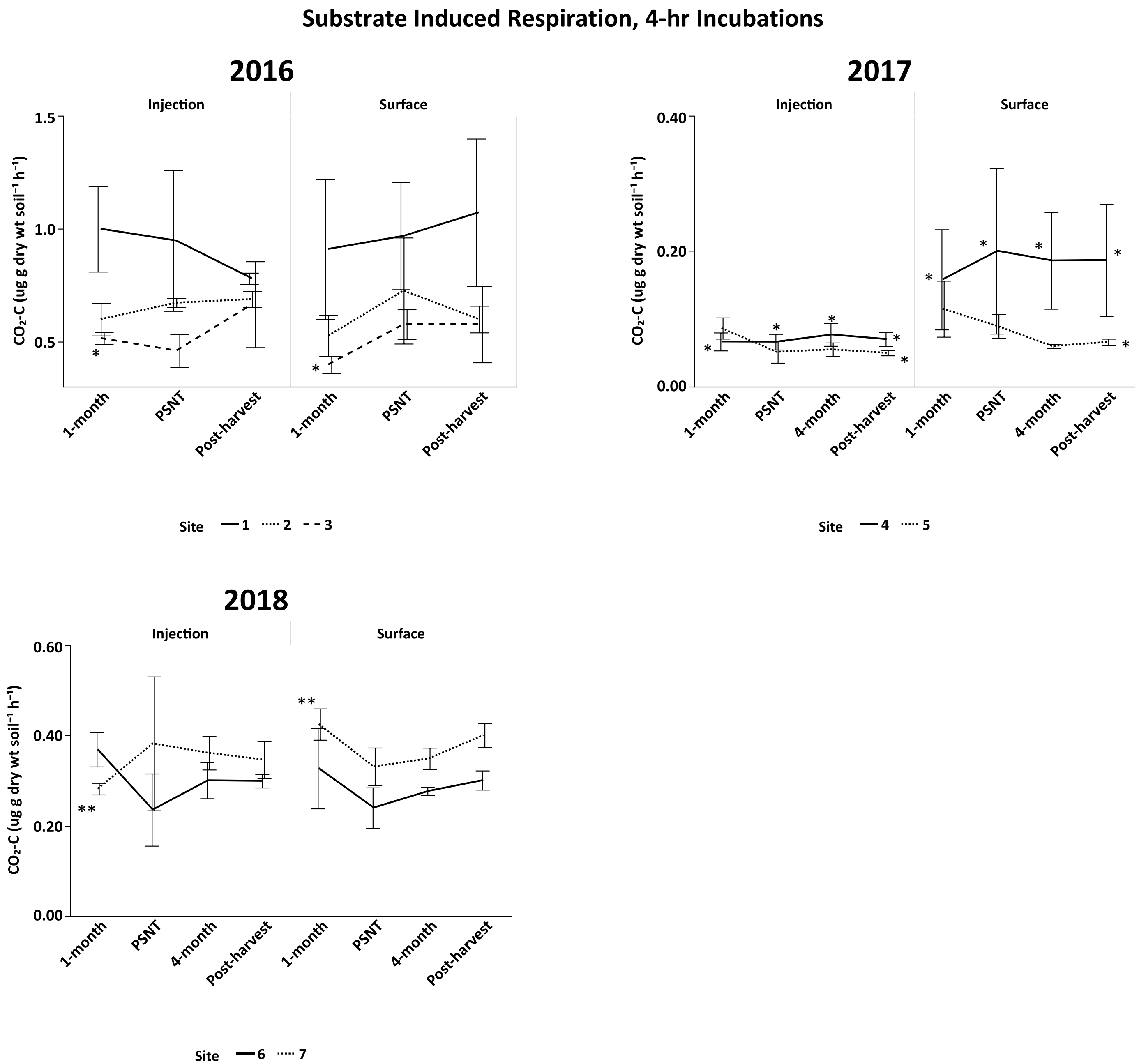

Carbon Mineralized, 30-Day Incubations
2016
Injection
Surface
0.8
0.6
0.4
0.2
0.0
CO₂-C (mg g dry wt soil⁻¹ d⁻¹)
**
**
1-month
PSNT
Post-harvest
1-month
PSNT
Post-harvest
Site 1 2 3
2017
Injection
Surface
0.30
0.20
0.10
0.00
CO₂-C (mg g dry wt soil⁻¹ d⁻¹)
**
**
1-month
PSNT
4-month
Post-harvest
1-month
PSNT
4-month
Post-harvest
Site 4 5
2018
Injection
Surface
0.20
0.10
0.00
CO₂-C (mg g dry wt soil⁻¹ d⁻¹)
1-month
PSNT
4-month
Post-harvest
1-month
PSNT
4-month
Post-harvest
Site 6 7

Figure 4


Substrate Induced Respiration, 4-hr Incubations
2016
Injection
Surface
CO2-C (ug g dry wt soil−1 h−1)
1.5
1.0
0.5
1-month
PSNT
Post-harvest
Site 1 2 3
2017
Injection
Surface
0.40
0.20
0.00
1-month
PSNT
4-month
Post-harvest
Site 4 5
2018
Injection
Surface
0.60
0.40
0.20
0.00
1-month
PSNT
4-month
Post-harvest
Site 6 7