doi:10.2489/jswc.2021.00002

# Manure injection alters the spatial distribution of soil nitrate, mineralizable carbon, and microbial biomass

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**Abstract:** Subsurface injection of liquid manure is a no-till compatible application method that may fundamentally change nutrient cycling dynamics. This study investigated subsurface injection and surface broadcast application methods on soil nitrate-nitrogen ($NO_3^-$-N) at 0, 1, 2, 4, 6, 8, 10, 14, 18, and 22 weeks after first-year applications of liquid dairy slurry in 0 to 15 cm and 15 to 30 cm sampling depths. The effect on corn (*Zea mays* L.) grain yield and quality parameters was considered. Soil health was assessed by proxy on weeks 0, 2, 8, 14, and 22 by two microbial indicators sensitive to change: carbon (C) mineralization and microbial biomass C. Soil $NO_3^-$-N and the microbial indicators were also measured at distances from an injected band of manure: In-band, 10 cm, 20 cm, and 36 cm. The injection application resulted in significantly ($p < 0.05$) higher soil $NO_3^-$-N levels relative to the surface application and no-manure control from week 2 to week 10 after manure application. Soil $NO_3^-$-N concentrations in the 15 to 30 cm sampling depth suggested $NO_3^-$-N leaching was exacerbated by the injection application method in 1 of 2 years. Soil $NO_3^-$-N decreased with increasing sampling distance from an injected manure band following quadradic plateau models at weeks 1, 2, and 4 each year. Crop yield was ~14.5% higher under injection application relative to surface application; however, grain quality parameters were not significantly ($p > 0.05$) different between treatments. Carbon mineralization was significantly ($p < 0.05$) increased under only the injection application relative to the control. The increase in C mineralization was entirely dependent on the In-band sampling distance as the 10, 20, and 36 cm sampling distances were not significantly ($p > 0.05$) different from the no-manure control. Microbial biomass C was significantly ($p < 0.05$) higher in the In-band samples in either the 15 to 30 or 0 to 15 cm sampling depths each year relative to the control, but was not appreciably affected by surface application. Findings from this study refine the understanding of nutrient concentrations around an injection band and accentuate the potential for manure N retention under surface and injected manure applications. However, as treatments were not reliably differentiated by C mineralization nor microbial biomass C, the capability of these tests to identify first-year management changes is disputable.

**Key words:** manure injection—microbial biomass—nitrogen cycling—soil health—soil nitrate

**Agricultural production systems often utilize animal wastes for land application as manures are rich in crop nutrients nitrogen (N), phosphorus (P), and potassium (K).** The use of animal wastes has gained attention as confined production operations concentrate nutrients spatially, often exacerbating the 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, have revealed both the limitations and benefits of long-term manure application (Jenkinson 1991). Research on manure application technologies is increasing 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. Differences between manure application methods result in changes in the supply and availability of nutrients, especially N (Maguire et al. 2011).

In many dairy and swine production systems, liquid manure is stored in pits or lagoons and applied in the liquid phase 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 N losses to volatilization (Thompson and Meisinger 2002; Bittman et al. 2005; Bierer et al. 2017), while also resulting in nuisance 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 compared to no incorporation, but is incompatible with no-till and conservation-till systems (Maguire et al. 2018). 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, in a comparison between aeration incorporation immediately following surface application and shank type subsurface injection, subsurface injection resulted in manure N losses of 9% while the surface application resulted in 23% of manure N lost (Powell et al. 2011).

Despite the benefits of manure injection, this application method alters the distribution of applied nutrients by placing manure in buried bands, which may change the dynamics of decomposition and availability

Received December 23, 2019; Revised July 28, 2020; Accepted August 17, 2020.

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of nutrients. 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 carbon dioxide ($CO_2$) emissions from surface applied and injected cattle slurry applications in a laboratory setting with constant temperature (14°C) and moisture (67% water holding capacity). Dendooven et al. (1998) reported no difference in $CO_2$ production between injected and surface-applied swine slurries, also under controlled conditions. Still, field studies on nutrient cycling dynamics between application techniques are needed (Maguire et al. 2011). One study reported no difference in organic matter accumulation with different incorporation methods, although incorporation depth altered the distribution of organic matter correspondingly (Sommerfeldt et al. 1988).

In addition to depth, the lateral distribution of nutrients is changed by injection, potentially affecting crop performance. Although exact measurements depend on injector type, manure is usually concentrated in narrow parallel bands beneath the soil surface. Sawyer and Hoeft (1990) reported the potential for toxic ammonia ($NH_3$) concentrations after injection; the $NH_3$ was inhibitory to corn (*Zea mays* L.) growth at the injection site up to 21 days after knife injection of manure at the agronomic N rate for corn. 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, 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 (Rothamsted 1991; Ding et al. 2012). 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 rarely discussed (Rochette et al. 2000; Agnew et al. 2010). Biological indicators of soil health are thought of as sensitive to management changes and may indicate a difference, if any, between manure application methods. One study considered arthropod abundance and diversity as a proxy for soil health and reported greater abundance under surface application of manure (Schuster 2015). Another study reported no difference in active microbial biomass between application methods under laboratory conditions (Bierer et al. 2017). Some studies have measured organic carbon (C) mineralization between application methods by measuring directly over an injection band compared to a surface-broadcast application (Dendooven et al. 1998; Flessa and Beese 2000), but doing so provides limited interpretation of the injection application method outside of the injection site.

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 N cycling between surface-broadcast and injected applications of liquid dairy slurry and their impact on corn yield and grain quality. (2) Assess the concentration of soil nitrate ($NO_3^-$) laterally and vertically around an injection band while comparing a surface application. (3) Observe the seasonal response of two indicators of biological soil health, C mineralization and microbial biomass, between manure application methods on soils under their first year of manure application.

## Materials and Methods

***Site Setup and Sampling Protocol.*** This study was established at the Virginia Tech Kentland research farm (37°11'33.81" N, 80°34'37.55" W) in May of 2017 and 2018 on predominantly Hayter Silt loams: fine-loamy, mixed, active, mesic Ultic Hapludalfs with slopes of 2% to 7%. The location of this study was chosen based on uniformity of landscape relief, management history, and availability. A rectangular segment at the end of a larger field of similar management was chosen for the study. The larger field had no recent history of manure application and had been in continuous corn/barley cover (*Hordeum vulgare* L.) rotation utilizing no-till. Barley was terminated with herbicide prior to planting each spring. In 2017, 5,000 kg ha$^{-1}$ of agricultural lime ($CaCO_3$) was applied to the larger field as a whole to increase soil pH from 5.8 to within 6.0 to 6.5 based on preplant soil test recommendations. Basic soil properties reported are for the segment of field on which this study took place. Plot location was not identical in each year but located within the same segment of field. For basic property determination, soil samples were taken to a depth of 30 cm before manure application. Particle size analysis was performed using a modified pipette method (Day 1965; Green 1981), organic matter measured by conversion of total C values determined by Elementar CNS (Elementar Vario MAX CNS Element Analyzer; Elementar Americas Inc., Ronkonkoma, New York), and pH measured in 1:1 soil water mixture. Soil texture was 369 g kg$^{-1}$ sand, 222 g kg$^{-1}$ silt, and 409 g kg$^{-1}$ clay; soil organic matter was 36 g kg$^{-1}$; and soil pH was 6.01 and 5.71 in 2017 and 2018, respectively. Although soil pH was 5.71 in 2018, no lime was applied to the larger field as a whole in 2018.

Dairy manure was gathered from a stirred storage lagoon during emptying and refrigerated at 4°C prior to use. Before land application, total Kjeldahl N and ammonical N analyses were completed by the agricultural service laboratory at Clemson University; manure C:N ratio was calculated through C and N determination by Elementar CNS (Bremner and Breitenbeck 1983; Peters et al. 2003). Manure organic-N was 60.0 and 82.1 kg ha$^{-1}$ while manure ammonical-N was 53.8 and 85.3 kg ha$^{-1}$ in 2017 and 2018, respectively. Manure C:N was not measured in 2017 and 10:1 in 2018.

Plots were established based on corn row spacing (76 cm) between two corn rows with total dimensions of 76 cm × 305 cm staged within a larger corn field. Small plots were established to reduce spatial variability of samples taken and to obtain a high resolution of parameters around an injection band. Manure application and corn planting took place on May 15 and May 17 in 2017 and May 24 and May 11 in 2018, respectively. In 2017 manure application was performed prior to planting, while in 2018 manure application was performed after planting to ease site setup. There was a minimum of 60 cm between plots to eliminate border effects of applied manure. 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 carefully splashing the slurry from a weigh bucket within plot boundaries. There were three treatments: a no-manure control, a surface-applied manure, and an injected application of manure replicated four times for a total of 12 plots each year. Manure injection was performed using a tractor mounted single disk to open the soil surface to a depth of approximately 15 cm at half the planting distance between corn rows (i.e., 76 cm ÷ 2 = 38 cm). Diary slurry was poured into the opening, and the manure band was loosely covered with soil to simulate band coverage by closing discs on field scale injectors. Manure was the only source of added N to the plots; both starter and side-dress applications of N were omitted from the study to more clearly assess N 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 30 cm intervals along the length of the plot. Week 0 samples were taken before manure application. At each sampling time, 2.5 cm diameter soil cores were taken across each plot by push probe: 1 core along the centerline of the plot length and 1 core 10 cm, 20 cm, and 36 cm on each side of the centerline to accommodate spatial interpretation of the injected manure treatment. The furthest sampling distance from manure bands was 36 cm as sampling at half the corn row spacing (38 cm) would have resulted in disruption of the growing corn crop. In injected plots, cores were separated by distance from manure band (In-band, 10 cm, 20 cm, 36 cm) and depth (0 to 15 and 15 to 30 cm) before processing. Surface-applied and no-manure control plots followed the same sampling scheme; however, each depth range was homogenized in the field by hand. All samples were separated into two for analysis: one air dried for $NO_3^-$-N analysis and one kept moist for microbial analysis as detailed below. Precipitation data were collected from a weather station approximately 500 m from the study location. Soil moisture and temperature plots are available as supplementary material (figure S1) (Weatherstem 2019).

***Soil Nitrate.*** Soil $NO_3^-$-N analysis was performed on subsamples from each time period. Samples were spread thinly to air dry, ground to pass through a 2 mm sieve, and thoroughly mixed. Four grams of soil were weighed into 50 mL centrifuge tubes and 40 mL 2 mol $L^{-1}$ KCl was added. Tubes were shaken for 30 minutes 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, Hach Company, Loveland, Colorado) for $NO_3^-$-N using QuickChem Method 12-107-04-1-B (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 passed through a 4 mm sieve moist, thoroughly mixed, and refrigerated at 4°C prior to analysis to minimize changes during storage. All microbial biomass samples were processed within two weeks of sampling as recommended by Cernohlavkova et al. (2009). For each sample, a fumigated and an unfumigated control pair were processed. Seven grams dry weight equivalent of soil was weighed into 70 mL glass centrifuge tubes; 40 mL of 0.5 mol $L^{-1}$ $K_2SO_4$ was added to all tubes. The fumigated subsamples had 1 mL of ethanol-free chloroform added to each tube to lyse the microbial cells, and all tubes were capped and shaken at 180 rotations $min^{-1}$ on a reciprocal shaker for 4 hours. Afterwards, samples were filtered through Fisher brand filter paper (Whatman No. 42 equivalent) into 50 mL centrifuge tubes. The filtrate was bubbled for 1 hour under a fume hood to remove chloroform in the fumigated samples using an apparatus that housed an array of syringes connected to lab air, and a water trap was used in the air line. After bubbling, samples were poured into 20 mL plastic scintillation vials and frozen for long-term storage at –20°C until analysis. The samples were analyzed for organic C and total N, (Shimadzu 2017), on a Shimadzu TOC-L equipped with a TNM-L unit (Shimadzu TOC-L and TNM-L, Shimadzu North America, Columbia, Maryland). Microbial biomass C was calculated as the difference between the fumigated and unfumigated pair for all samples. The data presented in this study were not transformed using a "kec-factor" but represent the raw extractable organic C of the soil extracts, where an increase in microbial biomass C will be considered beneficial to soil health.

***Carbon Mineralization.*** A C mineralization (C-min) assay was used to estimate the amount of bioavailable C present at five sampling times: 0, 2, 8, 14, and 22 weeks after manure application. A 60 day incubation protocol described by Strickland et al. (2015) was used to assess the labile, bioavailable C within the soil samples (Fierer et al. 2005). Six grams dry weight equivalent of soil were weighed into 50 mL centrifuge tubes, and deionized water was added to adjust samples to 65% of gravimetric water holding capacity determined for each sample using the protocol of Oldfield et al. (2014). Samples with moisture contents exceeding 65% water holding capacity received no water. Tubes were sealed with caps housing rubber septa and had their headspace flushed with $CO_2$-free air for 3 minutes to remove $CO_2$ present and then incubated for 24 hours at 20°C. After 24 hour incubation, 5 mL headspace was drawn from the tubes and analyzed on a LI-COR $CO_2$ $H_2O$ gas analyzer (LI-7000 $CO_2/H_2O$ analyzer; LI-COR, Lincoln, Nebraska). The 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% water holding capacity as needed. An emission curve was drawn for each sample, and the area underneath the curve was used to estimate the total amount of C mineralized during each 60 day incubation. Here, increased C-min was interpreted as an increase in general microbial activity due to the presence of labile substrates and considered beneficial to soil health.

***Yield and Quality.*** Corn grain was collected by harvesting one row of corn on each side of a 152 cm measuring stick within each plot, and corn ears were shelled by hand. Yield was adjusted to 150 g $kg^{-1}$ moisture content to standardize comparison, and grain quality parameters (ash, crude fiber, fat, moisture, protein, and starch) were analyzed using near infrared reflectance spectroscopy (NIR) on a FOSS XDS Rapid Content Analyzer (XM-1100 series; FOSS, Eden Prairie, Minnesota).

***Statistics.*** Statistical analyses were conducted using JMP Pro 14 software (SAS Institute Inc. 2018), and soil $NO_3^-$-N regressions were fit using the "easynls" package (Kaps and Lamberson 2009) in RStudio (RStudio Team 2018). Due to the separation of soil samples by sampling distance in the injection treatment (In-band, 10 cm, 20 cm, and 36 cm), 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 $NO_3^-$-N transport from an injection band, and to better satisfy the assumption of equal variance. The set of data for the comparison of control, injection, and surface treatments averaged the spacing categories for the injection plots. The surface and control treatments were excluded from analysis of soil NO$_3^-$–N by sampling distance in the injection treatment as it was only pertinent to compare sampling distance. For all analyses multiple regression models were fit with year, weeks after manure application, depth, and treatment as fixed effects. Significance of model effects were determined by ANOVA. Post-hoc testing was conducted on the highest order interaction using the Tukey-Kramer method. All analyses were conducted and considered significant at the $\alpha$ = 0.05 level. Corn yield and quality parameters were block centered by year to circumvent year to year variability. In some cases, a lower interaction effect accompanies a higher interaction effect to aid interpretation.

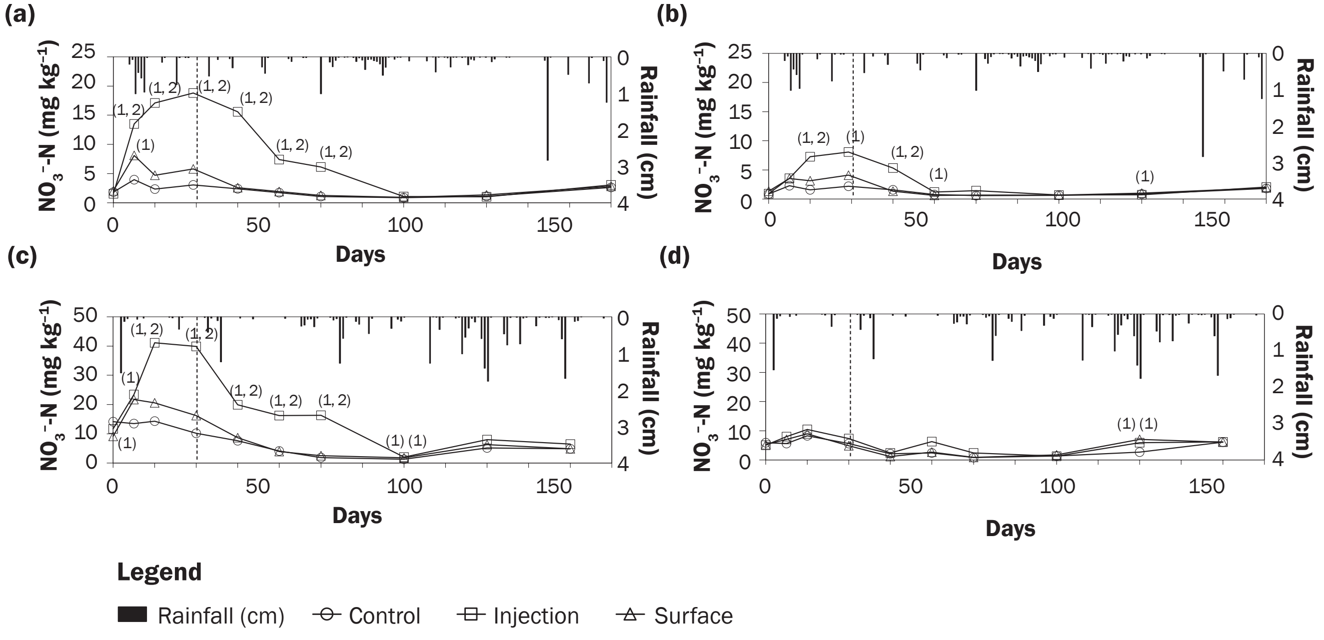
## Results and Discussion

***2017 Soil Nitrate.*** Due to the interaction of treatment effects over the year, data are presented by year. In 2017 application methods were significantly different ($p < 0.001$) in the 0 to 15 cm depth 1 week after the application of manure with soil NO$_3^-$–N means: injection, 13.5 mg kg$^{-1}$ > surface, 8.1 mg kg$^{-1}$ > control, 4.0 mg kg$^{-1}$ (figure 1a). From weeks 1 to 2, soil NO$_3^-$–N increased by 27% under injection to 17.1 mg kg$^{-1}$ while decreasing by 42% to 4.7 mg kg$^{-1}$ under surface application in the 0 to 15 cm depth. The surface application did not differ from the control for the remainder of the season, likely due to manure applied N losses via NH$_3$ volatilization and corn uptake of N. In the 15 to 30 cm depth, soil NO$_3^-$–N increased by 103% from week 1 to week 2 under injection application. The increase in NO$_3^-$–N is evidence of increased leaching relative to surface application after several days of rainfall between weeks 1 and 2 (figure 1b). Evidence that injection application can exacerbate N leaching was also reported by Ball-Coelho et al. (2006), who reported higher N concentrations in tile drains below injected manure applications relative to surface applications when total N application rates of swine slurry were ≥60 kg ha$^{-1}$.

At week 4, 0 to 15 cm soil NO$_3^-$–N of the injection application was 18.8 mg kg$^{-1}$, substantially higher than the surface application or control at 5.8 and 3.1 mg kg$^{-1}$, respectively. In the 15 to 30 cm depth, NO$_3^-$–N of the injection treatment, 8.1 mg kg$^{-1}$, remained greater than the control, 4.1 mg kg$^{-1}$, but not the surface application ($p$ = 0.075) (figures 1a and 1b). Soil NO$_3^-$–N in the injection application was consistently greater than the surface application from week 4 through week 10 in the 0 to 15 cm sampling depth, likely due to substantial preclusion of NH$_3$ volatilization, which has been shown frequently in other studies (Dell et al. 2011; Bierer et al. 2017) (figure 1a). Dell et al. (2011) aggregated 14 studies measuring NH$_3$ volatilization after surface and injected manure applications and reported reductions of at least 40% and up to 90% while injecting, while Bierer et al. (2017) showed reductions as high as 98% under laboratory conditions. In the present study, weeks 6 through week 14 followed the typical corn presidedress nitrate test (PSNT) time. This period of growth is the

**Figure 1**

**Seasonal soil nitrate-nitrogen (NO$_3^-$-N) trends amongst manure application methods and a no manure control: (a) 2017, 0 to 15 cm; (b) 2017, 15 to 30 cm; (c) 2018, 0 to 15 cm; and (d) 2018, 15 to 30 cm. Days represent time after manure application, the inverted bars indicate precipitation, and the vertical dashed line represents time of presidedress NO$_3^-$ testing for corn. Days are presented as precipitation totals are on a daily time step, and *x*-axis tick marks denote two weeks. Treatment means on each sampling date were separated using the Tukey-Kramer honestly significant difference test and considered significant at the $\alpha$ = 0.05 level. A significant difference from the no-manure control = (1), and a significant difference between application methods = (2).**



highest period of N uptake by corn (Aldrich 1984; Walsh 1997; Licht and Al-Kaisi 2005), presumably causing soil $NO_3^-$–N in the manured treatments to decline to control levels where they remained for the remainder of the season (figures 1a and 1b).

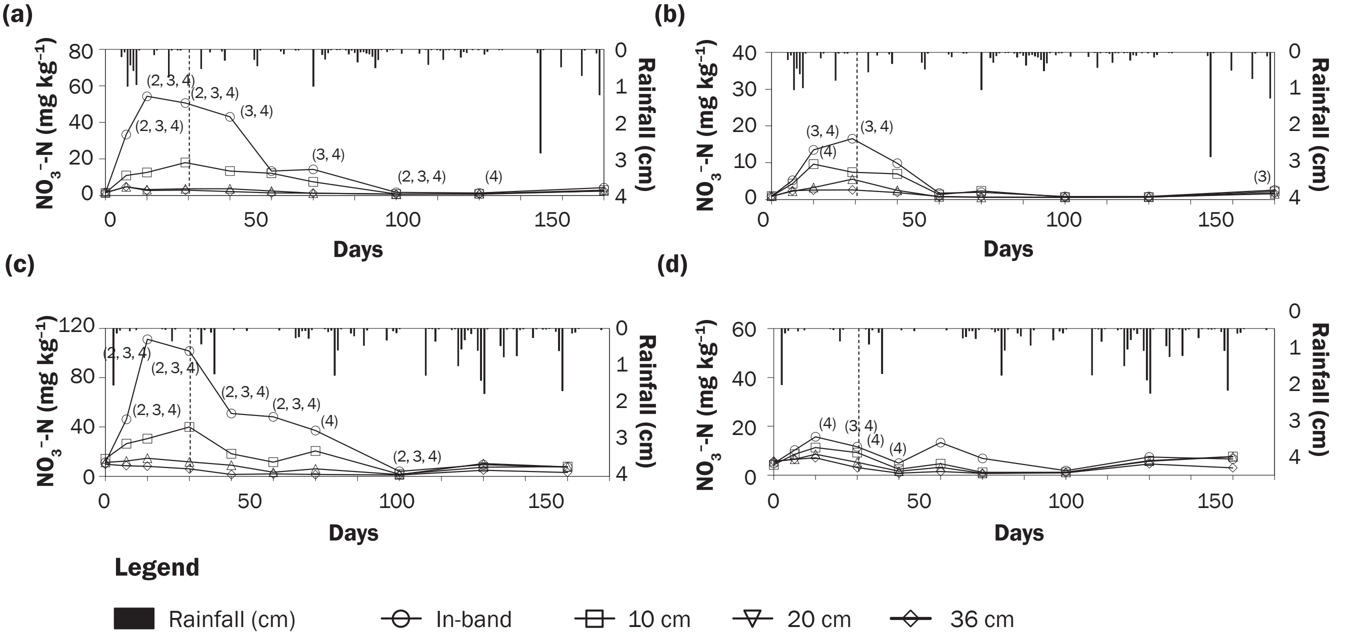
***2018 Soil Nitrate.*** In 2018, soil $NO_3^-$–N followed a similar pattern but with higher means than 2017 as total applied manure N was 47% higher in 2018 than in 2017 at 167.4 and 113.8 kg N $ha^{-1}$, respectively. Additionally, higher background N mineralization was suggested by numerically higher soil $NO_3^-$–N in the control treatment in week 0 through week 4 in 2018 relative to 2017 (figure 1). At week 1, soil $NO_3^-$–N of both manure applications numerically increased in the 0 to 15 cm depth, but only the injection application was significantly different ($p < 0.050$) than the control, 23.4 and 13.5 mg $kg^{-1}$, respectively (figure 1c). In the 15 to 30 cm depth, there were no significant differences ($p > 0.050$) between treatments during the season except for one instance at week 18 where both injection and surface applications were greater than the control: 5.9, 7.0, and 2.7 mg $kg^{-1}$, respectively (figure 1d). There was no evidence of vertical $NO_3^-$–N transport from either manure application in 2018, probably as 55% as much rainfall occurred in the first 2 weeks after manure application in 2018 (1.82 cm) relative to 2017 (3.30 cm). Two weeks after manure application in the 0 to 15 cm depth, soil $NO_3^-$–N in the surface treatment had begun to decline and was not significantly different ($p > 0.050$) from the control, while the injection application was greater than both surface and control ($p < 0.001$) (figure 1c). This was probably due to greater ammoniacal N losses under the surface application of manure.

Week 4 was closest to the corn PSNT time that would estimate corn N needs for the remainder of the season (Magdoff 1991). As an exercise, 0 to 15 cm and 15 to 30 cm soil $NO_3^-$–N means were averaged for each treatment to simulate the typical PSNT sampling depth (0 to 30 cm). The resulting soil $NO_3^-$–N means for the injection, surface, and control treatments were 23.6, 10.6, and 7.9 mg $kg^{-1}$, respectively. Under this exercise, soil $NO_3^-$–N of the injection treatment approached the Virginia recommended sufficiency level of 26 mg $kg^{-1}$, while the surface and control treatments were further below this value and additional sidedress applications of N would be recommended (Maguire et al. 2019). The extra soil $NO_3^-$–N in the injected application would be an economic benefit to farmers, helping to reduce the extra cost associated with injection relative to surface application (Maguire et al. 2011). After week 4, soil $NO_3^-$–N means of the surface application declined to control levels, while the means of the injection application remained significantly greater ($p < 0.050$) than the surface and control treatments at weeks 6, 8, and 10 before reaching control levels (figure 1c).

***Soil Nitrate at Sampling Distances: 2017 Injected Application.*** In the 0 to 15 cm depth at week 1, the mean soil $NO_3^-$–N of In-band samples was significantly greater than all other sampling distances at 33.4 mg $kg^{-1}$ ($p < 0.050$) (figure 2a). In-band soil $NO_3^-$–N increased in 0 to 15 cm samples to 49.3 mg $kg^{-1}$ at week 2 and remained greater than all other sampling distances until week 6, where

**Figure 2**

**Soil nitrate-nitrogen ($NO_3^-$-N) measured at injection sampling distances (In-band, 10, 20, and 36 cm away from the manure injection band): (a) 2017, 0 to 15 cm; (b) 2017, 15 to 30 cm; (c) 2018, 0 to 15 cm; and (d) 2018, 15 to 30 cm. Days represent time after manure application, the inverted bars indicate precipitation, and the vertical dashed line represents time of presidedress $NO_3^-$ testing for corn. Days are presented as precipitation totals are on a daily time step, and *x*-axis tick marks denote two weeks. Means on each sampling date and depth of measurement were separated using the Tukey-Kramer honestly significant difference test and considered significant at the $\alpha = 0.05$ level. A significant difference from In-band = (1), difference from 10 cm = (2), difference from 20 cm = (3), and difference from 36 cm = (4).**



$NO_3^-$–N in the injection band was presumably reduced by crop uptake (figure 2a). The 10 cm sampling distance observed increases in soil $NO_3^-$–N in 0 to 15 cm samples after manure application at weeks 1, 2, and 4, but was not significantly different from 20 cm and 36 cm sampling distances at each week ($p > 0.050$). Indication that $NO_3^-$–N leaching had occurred was observed in the 15 to 30 cm depth at week 2, as In-band soil $NO_3^-$–N was no longer significantly different ($p > 0.050$) from the 10 cm sampling distance, 13.5 and 9.7 mg kg$^{-1}$, respectively (figure 2b). This could be explained by dispersion during vertical $NO_3^-$–N leaching after the rainfall events between weeks 1 and 2. In the 15 to 30 cm sampling depth, In-band soil $NO_3^-$–N peaked at 16.6 mg kg$^{-1}$ at week 4 before declining to control levels presumably due to crop uptake (figure 2b). A sharp decline of In-band soil $NO_3^-$–N was observed at week 8 in the 0 to 15 cm depth where there were no significant differences ($p = 0.058$) between sampling distances (figure 2a).

***Soil Nitrate at Sampling Distances: 2018 Injected Application.*** In 2018 the mean soil $NO_3^-$–N measured for all samples increased by 107% over observations in 2017 at 12.0 and 5.8 mg kg$^{-1}$, respectively. This was likely due to greater amounts of manure N applied in 2018 and greater background N mineralization as stated earlier. The trends in soil $NO_3^-$–N were much the same in 2018 as in 2017. One week after manure application, the mean soil $NO_3^-$–N of In-band samples was significantly greater ($p < 0.050$) than all other sampling distances at 46.3 mg kg$^{-1}$ in the 0 to 15 cm depth. The mean soil $NO_3^-$–N of the 10 cm sampling distance was numerically higher than the 20 cm and 36 cm samples at week 1 in the 0 to 15 cm sampling depth: 26.4, 12.4, and 8.6 mg kg$^{-1}$, respectively. Despite this, the 10 cm sampling distance was not statistically different from 20 cm and 36 cm distances at this time ($p > 0.050$) (figure 2c). In a comparable study, Chen et al. (2010) reported considerably higher soil $NO_3^-$–N means at a 15 cm relative to 30 cm sampling distance from an injected application of liquid swine manure; however, the means were only occasionally considered statistically different ($p < 0.01$). At week 2 of the present study, In-band soil $NO_3^-$–N peaked at 111.2 mg kg$^{-1}$ in the 0 to 15 cm sampling depth and remained significantly higher ($p < 0.050$) than all other spacings while declining during the period of highest crop N uptake until week 10 (figure 2c). At week 10, the In-band and 10 cm sampling distance soil $NO_3^-$–N means were numerically far greater than the 20 cm and 36 cm means in the 0 to 15 cm depth. Nevertheless, due to large variation within sampling distance, only the In-band, 37.1 mg kg$^{-1}$, and 36 cm sampling distance, 1.5 mg kg$^{-1}$, were statistically different ($p < 0.050$). By week 18, soil $NO_3^-$–N had returned to near control levels at all sampling distances in the 0 to 15 cm sampling depth.

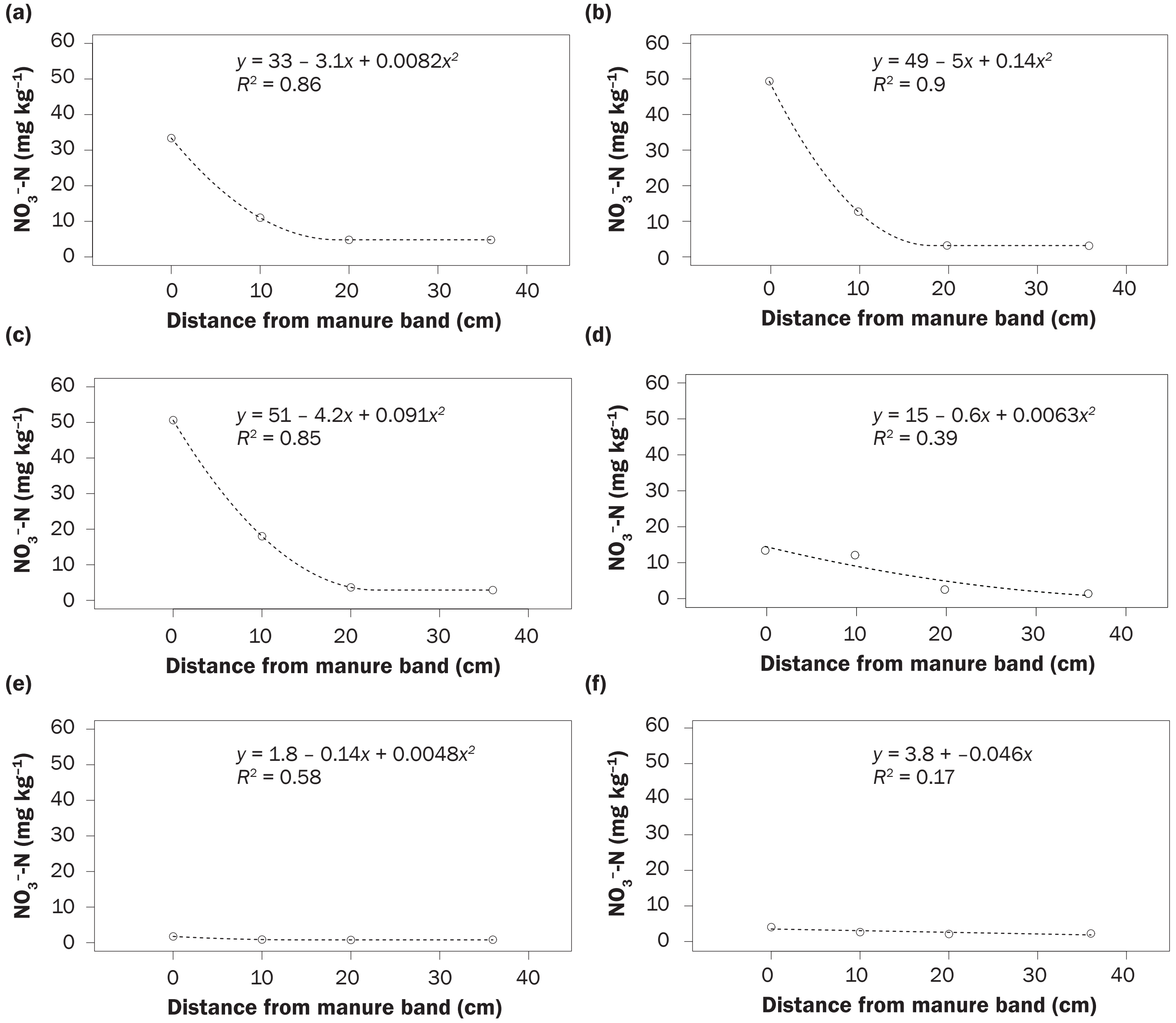
In the 15 to 30 cm depth at week 2, the In-band soil $NO_3^-$–N mean was significantly greater ($p < 0.050$) than the 36 cm sampling distance mean at 15.7 and 7.1 mg kg$^{-1}$, respectively (figure 2d). By week 6, the In-band soil $NO_3^-$–N mean was larger than the 36 cm sampling distance, 4.9 and 0.8 mg kg$^{-1}$, respectively. After week 6 there were no significant differences in soil $NO_3^-$–N in the 15 to 30 cm depth ($p > 0.050$). The results obtained in the present study can be compared with those obtained by Westerschulte et al. (2015) who removed soil monoliths approximately 9 weeks after an injected application of liquid swine slurry. Soil $NO_3^-$–N in 50% of the monoliths were reported to have >100 mg kg$^{-1}$ of inorganic N at a 25 to 40 cm sampling depth directly beneath the injection band (Westerschulte et al. 2015). This difference from the present study was attributed to exacerbated vertical N transport in the coarser soil texture (sand versus clay), higher dry matter (80 versus 64 g kg$^{-1}$), total N content of manure (7 versus 3 g kg$^{-1}$), and possibly the monolith soil sampling method.

***Effect of Sampling Distance on Nutrient Concentration.*** It was evident that soil $NO_3^-$–N concentration is highly dependent on distance from the injection band for 4 to 8 weeks after manure application and that $NO_3^-$–N concentration decreases rapidly from the manure band (figures 3 and 4). This suggests precaution should be taken in obtaining a representative soil sample from recently injected fields, as here week 4 was the time for PSNT soil sampling (figures 1 and 2). The same concern was expressed by Assefa and Chen (2007) who reported decreasing soil $NO_3^-$–N with increasing distance from an injected liquid swine manure band at 3, 6, and 19 weeks after application. Assefa and Chen (2007) fit second degree polynomials to the observed soil $NO_3^-$–N suggesting that simple random sampling may not be sufficient for injected fields. Additionally, the use of a directed paired sampling scheme was tested, which did increase accuracy over random sampling (Assefa and Chen 2007); however, recent research has suggested that random sampling may be sufficient for PSNT testing corn (Bierer et al. 2020). Nevertheless, it is likely that the suitability of random sampling increases with time after injection as the spatial variability of soil $NO_3^-$–N declines (figures 3 and 4). The present study predominantly agrees with Assefa and Chen (2007); however, quadratic plateau models were recommended for the distribution of soil $NO_3^-$–N at our sampling distances in the 0 to 15 cm depth (figures 3 and 4). These relationships illustrate how planting proximity to the injection site can alter early season $NO_3^-$–N access by plant roots. Previous research by Chen et al. (2010) reported increased corn tiller count, length, and biomass at 15 cm relative to 45 cm from an injection band at the R4 growth stage. Schroder et al. (2015) reported greater silage yields when planting ~10 cm from a typical injection band relative to injection at 20 cm spacings using the same application rate. Thus, there are supporting studies that indicate planting within 10 to 15 cm of an injection band allows for early access to N by plant roots while remaining outside the areas of highest nutrient concentration where salt damage (Hergert et al. 2012) or $NH_3$ toxicity (Sawyer and Hoeft 1990) may affect germination and root growth.

***Corn Grain Yield and Quality.*** Mean corn grain yield across all treatments was higher in 2018 than in 2017 at 10.6 and 4.8 Mg ha$^{-1}$, respectively (figure 5). This was likely a result of 47% higher manure N application in 2018 relative to 2017. In 2018, two control plots were damaged by tractor passes, which likely affected yields. The additional light exposure from a reduction in cropping density may have contributed to increased yields of the control treatment in 2018. After centering yield by year, manure applications increased yield by ~18% compared to the no-manure control. Here, only the injected application was significantly different from the control ($p < 0.001$), although the surface application was numerically higher than the control (figure 5). This was explained by observed soil $NO_3^-$–N values throughout the experiment. Clearly, the injection application resulted in greater amounts of plant available $NO_3^-$–N for about 10 weeks during the study each

**Figure 3**

Relationship between soil nitrate-nitrogen ($NO_3^-$-N) and sampling distance from an injected manure band at (a) 1, (b) 2, (c) 4, (d) 8, (e) 14, and (f) 22 weeks after manure application for 2017 samples, 0 to 15 cm. Markers represent the mean soil $NO_3^-$-N at the given position, and the dashed line and equation represent the best fit model. Quadratic plateau models have the following critical *x* values: week 1 = 18.7, week 2 = 18.3, week 4 = 22.9, and week 14 = 14.2.

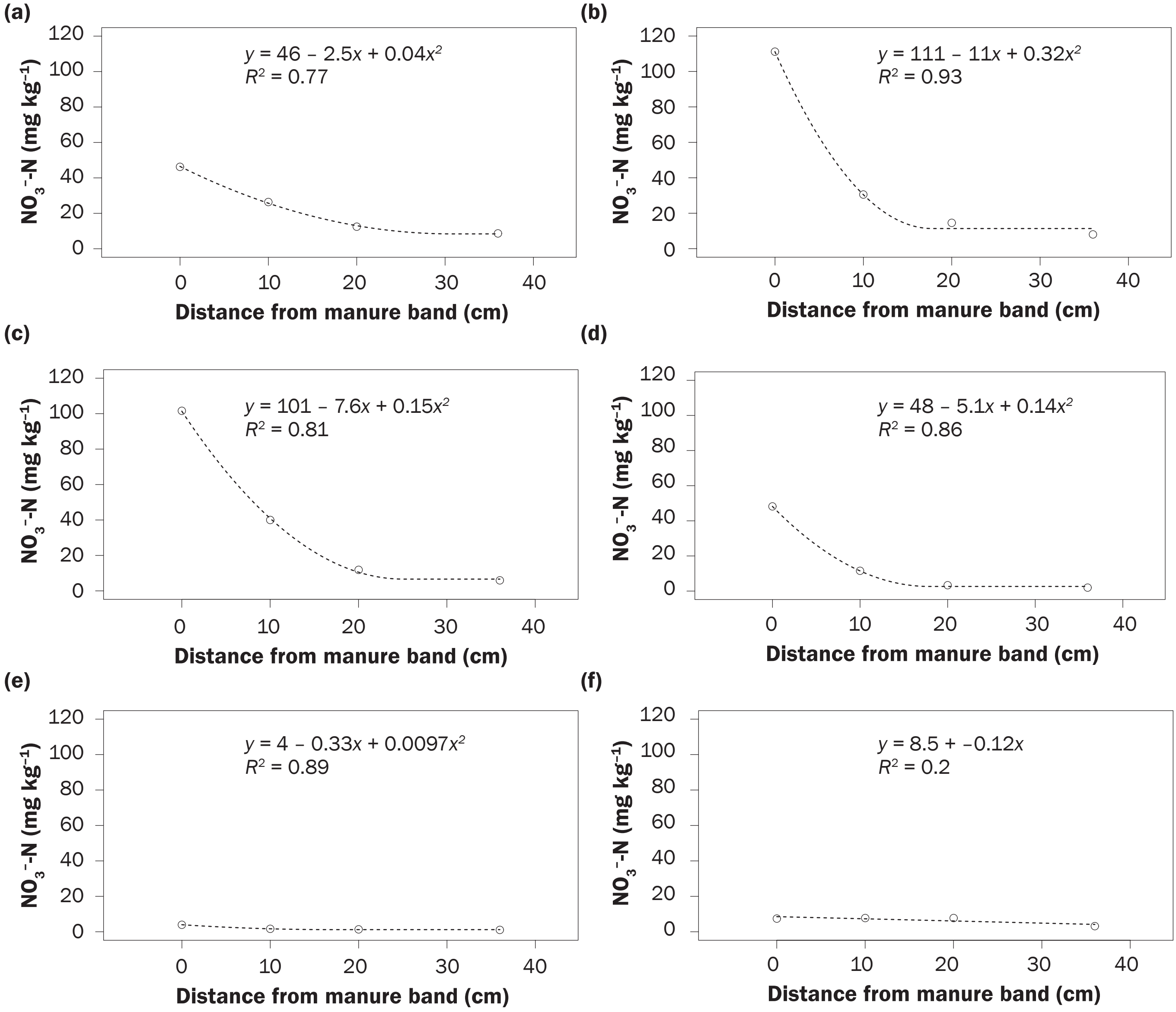


year relative to the other treatments (figure 1). The results of this study are similar to those obtained in a comparable study in which an injected application of manure increased corn grain yield an average of 11% over surface application (Schmitt et al. 1995). Yield responses reported in studies considering both surface and injected applications of manure vary according to application rate and timing, post application management (e.g., incorporation), crop studied, and degree of N limitation, but trend positive for injected application (Safley et al. 1980; Sutton et al. 1982; Rahman et al. 2001; Jokela et al. 2014). Despite a yield response, there were no significant differences in grain quality parameters (ash, crude fiber, fat, moisture, starch) across treatments ($p > 0.050$) (data not shown). Elsewhere, Sutton et al. (1982) reported significantly higher ($p < 0.050$) N content in corn leaves under injected applications of swine manure relative to surface applications. This was attributed to the subsurface location and root access to manure nutrients as total N loading met nutrient requirements of the crop under both application methods.

***Carbon Mineralization Treatment Effects.*** Analysis of C-min indicated a substantial main effect of depth across all treatments, while the absence of an interaction of treatment effects over year allowed 2017 and 2018 data to be pooled. Data are presented by week after manure application and depth due to an interaction with treatment. Carbon mineralization in the 0 to 15 cm depth was

**Figure 4**

Relationship between soil nitrate-nitrogen ($NO_3^-$-N) and sampling distance from an injected manure band at (a) 1, (b) 2, (c) 4, (d) 8, (e) 14, and (f) 22 weeks after manure application for 2018 samples, 0 to 15 cm. Markers represent the mean soil $NO_3^-$-N at each position, and the dashed line and equation represent the best fit model. Quadratic plateau models have the following critical $x$ values: week 1 = 30.7, week 2 = 17.8, week 4 = 25.1, week 8 = 17.9, and week 14 = 16.9.

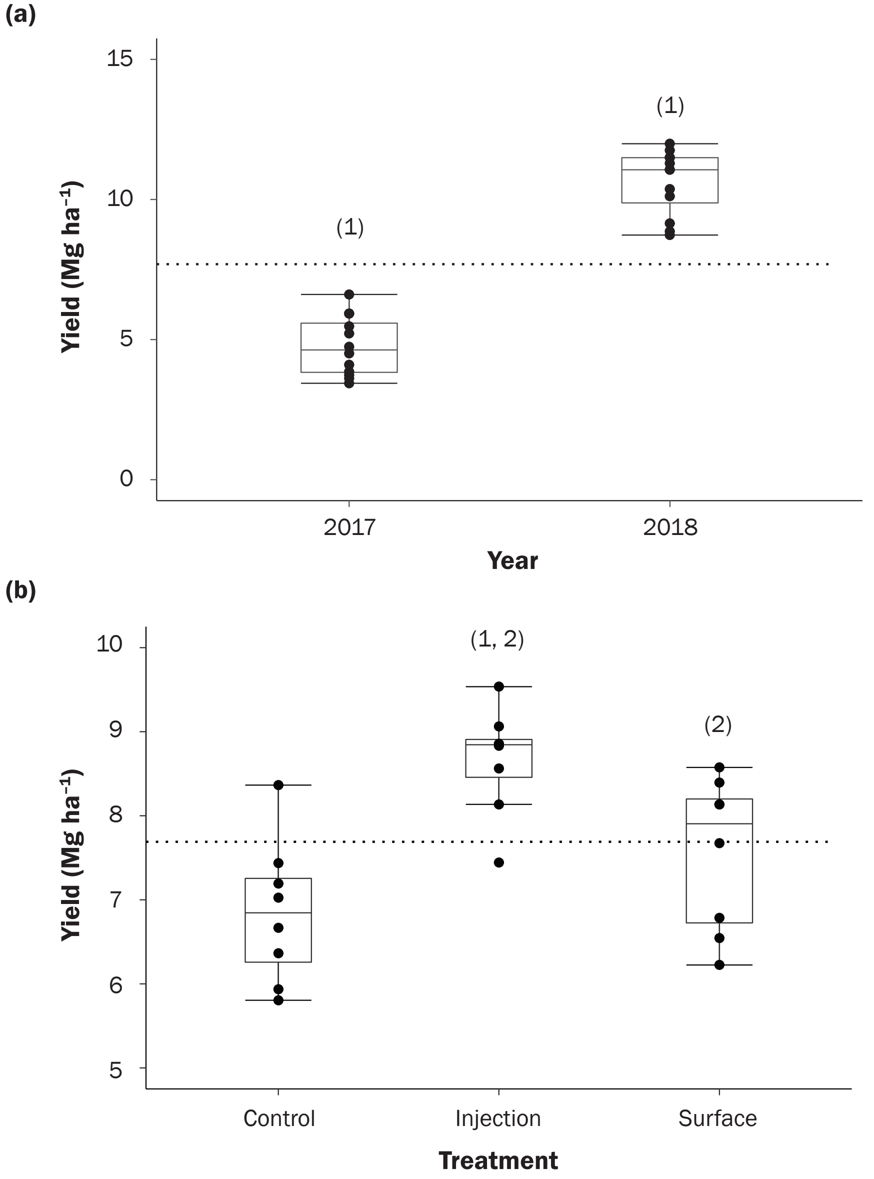


on average 123% higher than in the 15 to 30 cm depth at 0.281 and 0.126 mg C g$^{-1}$ dry weight (wt) soil, respectively. Observation of decreasing C-min with depth is commonly made amongst smaller sampling depth increments (Franzluebbers and Arshad 1997; Rey et al. 2008), especially when under no-till conditions (Alvarez et al. 1995). This study was conducted on a site with a recent history of no-till corn production; the absence of tillage probably promoted biological activity at shallower soil depths as substrate mixing was limited. There were no significant differences ($p > 0.050$) between either manure application and the control in the 15 to 30 cm sampling depth (figure 6).

In the 0 to 15 cm sampling depth, the mean C-min for all treatments numerically increased from week 1 to week 2; however, only the injected manure application was significantly different ($p < 0.050$) from the control at 0.359 and 0.242 mg C g$^{-1}$ dry wt soil, respectively (figure 6). This was possibly due to high variability encountered across the microbial tests performed, particularly in the 0 to 15 cm sampling depth. The larger relative increase in C-min from week 1 to week 2 in surface and injected treatments was presumably due to highly labile C substrates within the manure (Van Kessel et al. 2000). By week 8, C-min in the injection application remained greater than the control while in the following weeks 14 and 22 there were no significant differences ($p > 0.050$), presumably as more of the highly labile C applied had been metabolized. Carbon min-

**Figure 5**

**Quantile plots of corn grain yield adjusted to 15% moisture content. In (a) the plot between years, treatment values were pooled. When (b) comparing treatments, values were block centered by year. Means were separated using the Tukey-Kramer honestly significant difference test and considered significant at the α = 0.05 level. Where applicable, a significant difference between year = (1), a significant difference from no-manure control = (1), and a difference between manure application methods = (2).**



eralization was relatively consistent in the control treatment; mean C-min at any given week was within 10% of the treatment mean across all sampling weeks, 0.258 mg C g$^{-1}$ dry wt soil. The application of manure added bioavailable C to the soil, but no effect on C-min was detectable after week 8 for either manure application method.

***Carbon Mineralization at Injection Sampling Distance, Control and Surface Comparison.*** The mean C-min of In-band samples increased by approximately 150% from 0.228 mg C g$^{-1}$ dry wt soil at week 1 to 0.580 mg C g$^{-1}$ dry wt soil at week 2 after manure was applied (figure 7). This was significantly higher than C-min measured in the surface application, 0.339 mg C g$^{-1}$ dry wt soil ($p < 0.010$), and all other sampling distances from the injection band ($p < 0.001$). This scale of increase was not achieved by the surface application as the labile C substrates were distributed evenly over the plot. Further, C-min means from the 10, 20, and 36 cm sampling distances were not statistically different from the control at weeks 2, 8, 14, and 22 of measurement ($p > 0.050$). Consequently, the treatment effect discussed above was wholly dependent on the In-band samples in the injected manure application. Aside from the distribution of labile C substrates, soil in this study was relatively high in organic matter (36 g kg$^{-1}$) and could partly explain the insignificant response of C-min to the surface application of manure. As soils become progressively saturated with organic C, the slighter any response to additional C inputs will be (Stewart et al. 2007). This study did not measure different forms of soil C. However, it was notable that since the stimulation of C-min was local to the In-band samples, any lateral movement of manure applied C was insufficient to initiate a detectable C-min response. The mean C-min from In-band samples numerically peaked at week 8 at 0.602 mg C g$^{-1}$ dry wt soil before declining at week 14 and week 22, 0.492 and 0.348 mg C g$^{-1}$ dry wt soil, respectively (figure 7). The increase in C-min in the injection band was not always synchronized with soil $NO_3^-$-N, which had declined to pre-manure application levels by week 14 each year (figures 2a and 2c).

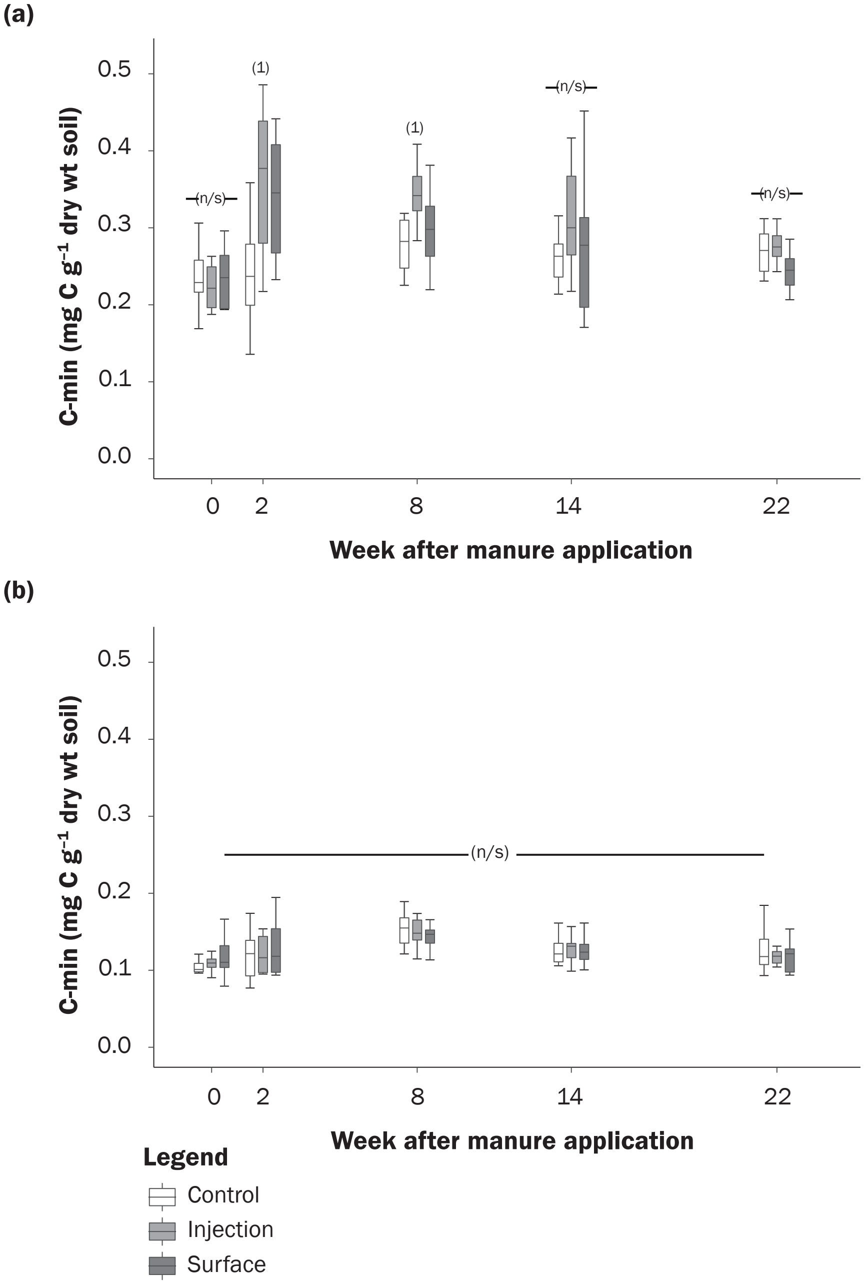
In the 15 to 30 cm sampling depth there were no significant differences between sampling distances and the control and surface treatments ($p > 0.050$). Thus, injection created distinct shallow bands of high microbial activity with a surplus of labile substrates outside of which C-min was not appreciably affected. Surface application distributed these substrates spatially resulting in a diminished response.

***Microbial Biomass Treatment Effects.*** Analysis of microbial biomass indicated a significant ($p < 0.001$) main effect of sampling depth across treatment and year, with means of 50.5 and 27.6 μg C g$^{-1}$ dry weight soil in the 0 to 15 cm and 15 to 30 cm sampling depths, respectively (figure 8). Similar to C-min discussed above, stratification of microbial biomass by depth is commonly reported in the literature (Franzluebbers et al. 1994; Franzluebbers and Stuedemann 2015). The interaction of treatment effects over year guided further analysis.

In 2017 there were no significant differences ($p > 0.050$) between treatments in the 0 to 15 cm sampling depth despite an

**Figure 6**

**Quantile plots of carbon mineralized (C-min) during 60-day laboratory incubations in the (a) 0 to 15 cm and (b) 15 to 30 cm sampling depths for control, injection, and surface treatments. Data were pooled across years. Means within each sampling period were separated using the Tukey-Kramer honestly significant difference test and considered significant at the $\alpha$ = 0.05 level. A significant difference from the control = (1), a difference between manure application methods=(2), and (n/s) = no significant differences.**

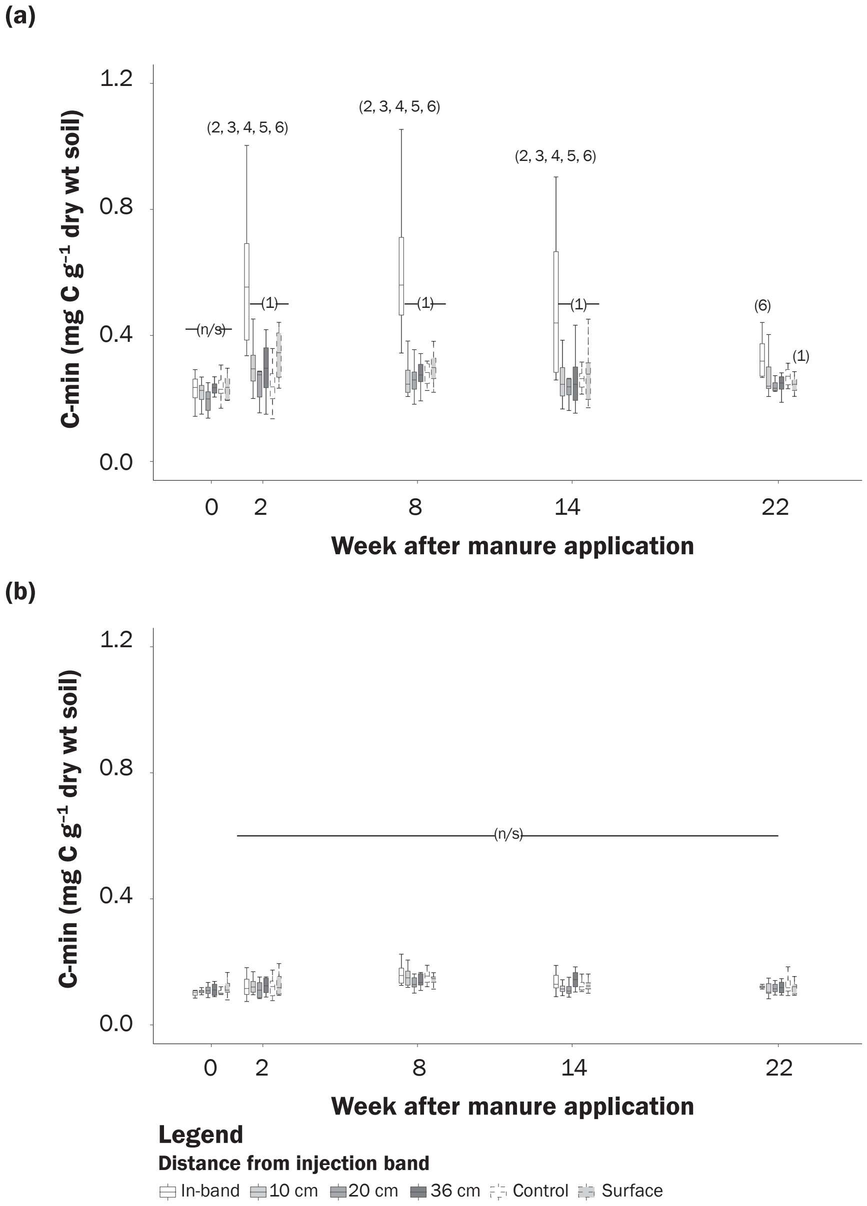


approximately 23% numerically higher mean observed under the surface application relative to injection at 49.9 and 40.4 µg C g$^{-1}$ dry wt soil, respectively (figure 8). In the 15 to 30 cm sampling depth, the inverse was true; microbial biomass under the injection application was significantly greater ($p < 0.001$) than the surface application and the no manure control at 37.4, 23.8, and 23.7 µg C g$^{-1}$ dry wt soil, respectively. This could be explained by the rainfall event shortly after manure application transporting manure constituents to this depth. However, the increased biomass observed here was not coupled with an increase in C-min and was not observed in 2018 (figures 1, 6, and 8). In 2018 there were no significant differences in either the 0 to 15 or 15 to 30 cm sampling depths ($p > 0.050$). Overall, microbial biomass was highly variable, ranging from a nondetect minimum to a maximum of 105.7 µg C g$^{-1}$ dry weight soil. Nevertheless, values of extractable microbial C in this study are low but within the range of reported values (Bradford et al. 2008; Kallenbach and Grandy 2011). Microbial biomass is usually considered to be sensitive to management changes, yet the manure application method did not affect estimates of microbial biomass consistently in this study. That being said, this study is representative of first-year manure applications, and different results have been obtained following successive years of application. A comparable study reported an increase in microbial biomass C of ~150% after three years of annual farmyard manure application relative to no amendment (Bouzaiane et al. 2007). Elsewhere, a meta-analysis reported organic amendments increased microbial biomass C by an average of 36%, $n$ = 223, and years of application was a significant effect (Kallenbach and Grandy 2011).

***Microbial Biomass at Sampling Distance, Control and Surface Comparison.*** In 2017 in the 0 to 15 cm sampling depth, there were no significant differences ($p > 0.050$) in microbial biomass C between sampling distances from the injection band or treatments (figure 9). In the 15 to 30 cm sampling depth, mean microbial biomass C of In-band and 36 cm sampling distances were greater ($p < 0.050$) than both the surface and control treatments: 40.4, 37.5, 23.8, and 23.7 µg C g$^{-1}$ dry wt soil, respectively. The increase of In-band samples could be due to the rainfall event between week 1 and week 2 as discussed above, while the increase at the 36 cm sampling distance could be attributed to its proximity to planting rows. By contrast, in 2018 there were no significant differences ($p > 0.050$) in microbial biomass C in the 15 to 30 cm sampling depth. Relatedly, Lalande et al. (2000) reported no effect of swine manure application on microbial biomass C in samples taken from 15 to 30 cm after 18 years of successive application, although a significant effect in the 0 to 15 cm depth range was observed. In 2018 of the present study, the mean microbial biomass C of In-band samples was ~36% numerically higher than the mean of surface or control samples in the 0 to 15 cm sampling

**Figure 7**

Quantile plots of carbon mineralized (C-min) during 60-day laboratory incubations in the (a) 0 to 15 cm and (b) 15 to 30 cm sampling depths for injection sampling distances, control, and surface treatments. Data were pooled across years. Means within each sampling period were separated using the Tukey-Kramer honestly significant difference test and considered significant at the $\alpha = 0.05$ level. A significant difference from In-band = (1), 10 cm = (2), 20 cm = (3), 36 cm = (4), control = (5), surface = (6), and (n/s) = no significant differences.



depth: 72.9, 53.8, and 53.3 μg C $g^{-1}$ dry wt soil, respectively. Additionally, In-band mean microbial biomass C was significantly different ($p < 0.01$) than all sampling distances except for 36 cm, likely owing to its proximity from planting rows.

Despite the inconsistent depth of effect in 2017 and 2018, the injection application appeared to promote microbial biomass in vertical alignment with the injection band. Directly comparable studies including biological measurements between manure application methods are scarce and further exploration is still needed. Elsewhere, microbial biomass estimates are typically higher under manure applications relative to control or chemically fertilized conditions, albeit after successive years of application (Kanazawa et al. 1988; Rochette and Gregorich 1998; Lalande et al. 2000; Chakraborty et al. 2011).

## Summary and Conclusions

The application method of liquid dairy slurry had a profound effect on soil $NO_3^-$-N stores and distribution while also affecting crop yield. Limiting applications of N to manure applied N revealed N dynamics typically masked by chemical N application in on-farm field studies. Surface manure application resulted in considerably less soil $NO_3^-$-N during peak crop need compared to the injected application, presenting an obvious advantage in plant available N when injecting. The additional $NO_3^-$-N found under injection was consistently concentrated at the injection site, and movement outside of the injection band was minimal. Corn responded to the additional retained N under injection with higher yields, and quadratic plateau models fit to soil $NO_3^-$-N concentration show that planting within 10 to 15 cm of the injection band would provide roots early access to $NO_3^-$-N. The interpretation of the microbial indicators of soil health were more difficult. Mineralizable C was elevated after manure application, especially within the injected manure band. Outside of the injection band, C-min was not significantly different from the no-manure control. Microbial biomass was elevated in vertical alignment with the injected manure band. Thus, injection creates linear zones of elevated microbial activity in the direction of injector travel. Despite this, the microbial indicators did not consistently identify treatment differences as the season progressed for C-min, and each year in the case of microbial biomass. Consequently, as this study was conducted on land with no recent history of manure application and our treatments were not reliably differentiated by C-min nor microbial biomass C, the ability of these tests to detect management changes under the first year of change is disputable.

## Supplemental Material

The supplementary material for this article is available in the online journal at https://doi.org/10.2489/jswc.2021.00002.

## Acknowledgements

This work was supported by the National Fish and Wildlife Foundation, the Virginia Agricultural Experiment Station (Blacksburg) and the USDA National Institute of Food and Agriculture, (Washington, DC).

**Figure 8**

Quantile plots of microbial biomass in (a, b) 0 to 15 cm and (a, c) 15 to 30 cm sampling depths by year, (a) with and (b, c) without pooling of treatments. Treatment means were separated using the Tukey-Kramer honestly significant difference test and considered significant at the $\alpha$= 0.05 level. Where applicable, a difference between sampling depths = (1), a difference from the control = (1), a difference between manure application method = (2), and (n/s) = no significant differences.

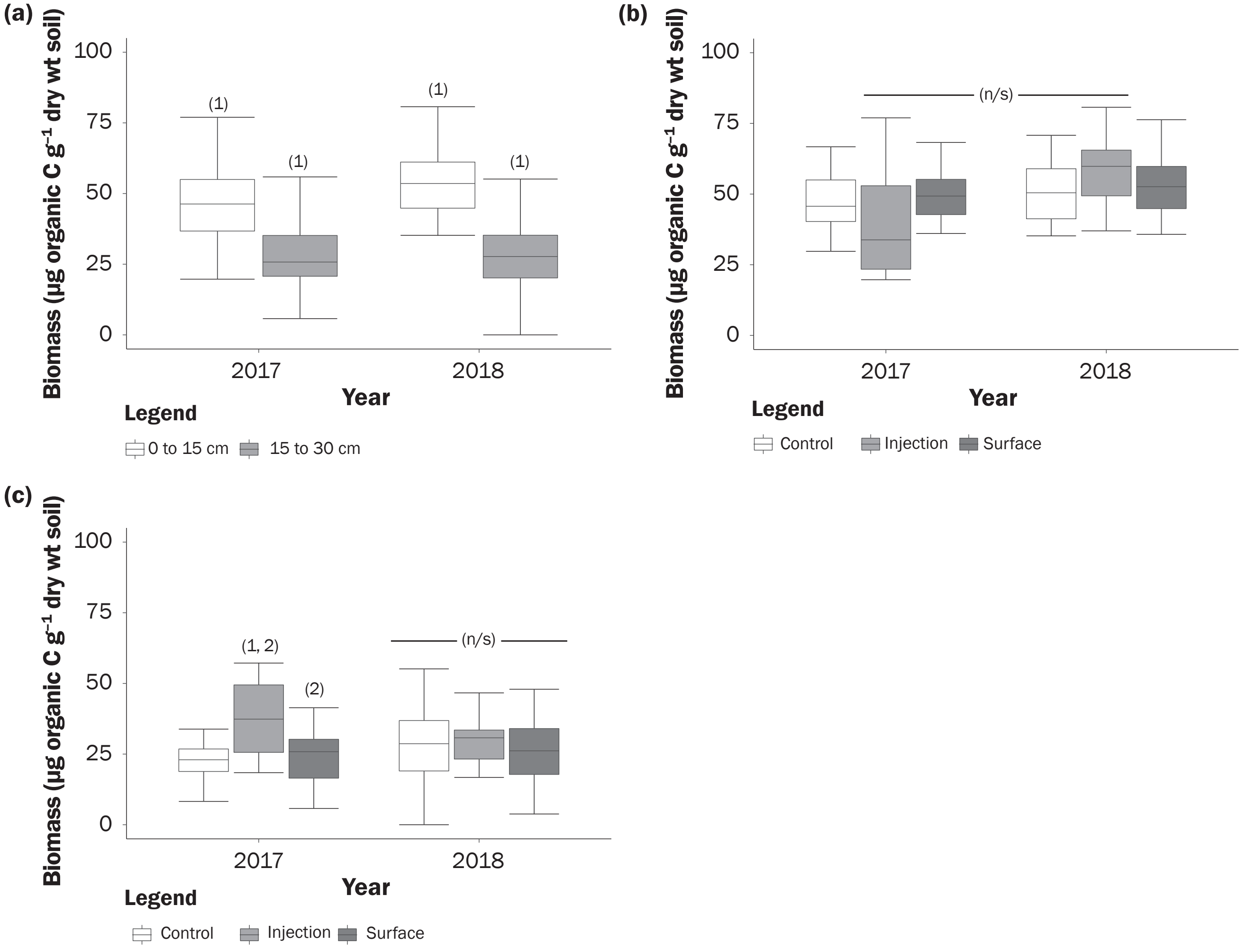


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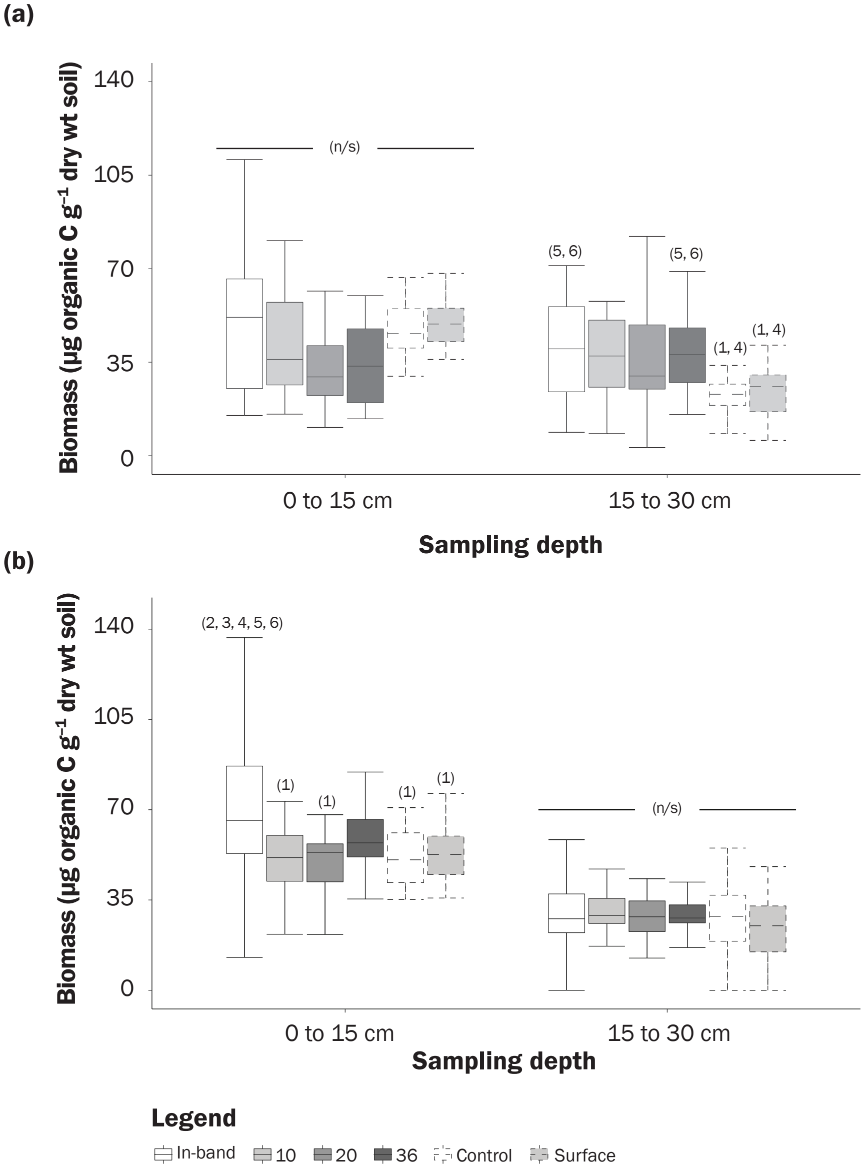
**Figure 9**

**Quantile plots of microbial biomass in the 0 to 15 cm and 15 to 30 cm sampling depths for injection sampling distances, control, and surface treatments in (a) 2017 and (b) 2018. Means within each sampling depth and year were separated using the Tukey-Kramer honestly significant difference test and considered significant at the $\alpha = 0.05$ level. At each sampling depth, a significant differences from In-band = (1), 10 cm = (2), 20 cm = (3), 36 cm = (4), control = (5), surface = (6), and (n/s) = no significant differences.**



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