

Evaluating effects of dairy manure application method on soil health and nitrate

A.M. Bierer, R.O. Maguire, M.S. Strickland, R.D. Stewart, and W.E. Thomason

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 of 2016 through fall of 2018 on seven sites to assess pre-sidedress nitrate test (PSNT) methodology, seasonal soil nitrate nitrogen (NO_3^- -N) trends, corn (*Zea mays* L.) 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 equispaced (30%) sampling methods. Soil NO_3^- -N was greater in 7 out 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 (C-min) took considerably more time than other tests. There were no significant differences in C-min 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 equispaced method can be used in injected fields.

Key words: corn yield—manure injection—pre-sidedress nitrate test—soil health—soil nitrate

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). In addition, when manure is below the soil surface, N and phosphorous (P) losses to runoff are reduced (Kulesza et al. 2014; Watts et al. 2011). Odor is reduced by preventing atmospheric contact/transport of the gases (NH_3 , hydrogen sulfide [H_2S], and volatile organic compounds [VOCs]) commonly released from manure (Pfoest 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 ammoniacal nitrogen (NH_3 -N) emissions but failed to increase grass-dominated hay (alfalfa [*Medicago sativa* L.]) 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 (*Trifolium* L.) yield despite significant reductions in NH_3 -N emissions under shallow injection.

For soils that have received manure, the corn (*Zea mays* L.) 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 to 30 cm high (Magdoff and Ross 1984; Maguire et al. 2019). However, values of soil nitrate nitrogen (NO_3^- -N) are magnitudes different when taken from manure bands and the interband 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 $>100 \text{ mg kg}^{-1}$ in measured soil N, making a reportable value difficult to obtain (Sawyer and Hoefst 1990).

Soil health is comprised of physical, chemical, and biological parameters essential for sustainable plant production (USDA NRCS 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 one day microbial respiration response to rewetting of dry soil and water extractable organic carbon (C) and N to form a composite score from 1 to 50, with values above 7 being considered healthy (Gunderson 2017).

Haney soil health test = (1)

$$\frac{1 \text{ d CO}_2\text{-C burst}}{10} + \frac{\text{WEOC}}{50} + \frac{\text{WEON}}{10},$$

Andrew M. Bierer is a graduate research assistant and PhD candidate in the Department of Crop and Soil Environmental Sciences, Virginia Tech, Blacksburg, Virginia. **Rory O. Maguire** is a professor, **Ryan D. Stewart** is an assistant professor, and **Wade E. Thomason** is a professor and extension grains specialist at the School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, Virginia. **Michael S. Strickland** is an assistant professor in the Department of Soil and Water Systems, University of Idaho, Moscow, Idaho.

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where WEOC is water extractable organic C, WEON is water extractable organic N, and CO₂-C is carbon dioxide C.

The development of the Haney soil health test originated from work concluding that water can be used as an extractant for microbial C in lieu of 0.5 M potassium sulfate (K₂SO₄) (Haney et al. 1999). Subsequent study on inorganic N extractants, ultimately resulting in Haney's H³A extract, reported high correlations ($R^2 > 0.90$) between water, potassium chloride (KCl), and H³A 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 CO₂ 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. 2016). 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 C and N mineralization and soil organic C 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 of 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 NO₃⁻-N, crop yield, milk production, and biological soil health.

Materials and Methods

Site Setup and Properties. Research plots were established on working dairy oper-

ations in spring of 2016, 2017, and 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. In all cases, manure was gathered from a stirred slurry storage lagoon during emptying. Manure total Kjeldahl N and NH₃-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 or soybean (*Glycine max* L.) 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 ≥ 3 times. In 2016, sites 1, 2, and 3 were planted in corn and harvested as silage. In 2017, site 4 was planted in corn and harvested as grain and site 5 was planted in soybean (table 2). In 2018, site 6 was planted in corn and harvested as silage, and site 7 was planted in corn and harvested as grain. No location was repeated for a second year. All sites used a 76 cm row spacing, except for site 3 that used a 38 cm spacing and site 2 that 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 band-

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 ammoniacal nitrogen (NH₃-N) with injection application, and 25% of NH₃-N with surface application (equations 2 and 3). Total PAN is equal to the sum of starter N, sidedress N, manure organic PAN, and manure ammoniacal PAN for injection or surface application, respectively. An n/a indicates no application due to cropping system, and o indicates no application due to management decision.

Year/site	Starter N (kg ha ⁻¹)	Sidedress N (kg ha ⁻¹)	Manure organic PAN (kg ha ⁻¹)	Manure ammoniacal PAN (kg ha ⁻¹)		Total PAN (kg ha ⁻¹)		Soil textural class	Organic matter (g kg ⁻¹)	Soil pH
				Injection	Surface	Injection	Surface			
2016										
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

Dates of manure application, planting, crop planted, harvest, and soil nitrate nitrogen (NO_3^- -N) sampling. For soil NO_3^- -N sampling, one month and four months indicate the time after manure application. Sites 1, 2, and 3 were not sampled four months after manure application.

Site	Manure application	Planting	Crop	Harvest	Soil NO_3^- -N sampling			
					One month	PSNT	Four months	Postharvest
1	Apr. 13, 2016	May 16, 2016	Corn (silage)	Sept. 5, 2016	May 20, 2016	June 13, 2016	n/a	Sept. 8, 2016
2	Mar. 11, 2016	Apr. 25, 2016	Corn (silage)	Aug. 26, 2016	Apr. 15, 2016	June 6, 2016	n/a	Sept. 6, 2016
3	Apr. 20, 2016	May 22, 2016	Corn (silage)	Sept. 5, 2016	May 20, 2016	June 14, 2016	n/a	Sept. 13, 2016
4	Apr. 11, 2017	May 2, 2017	Corn (grain)	Sept. 22, 2017	May 10, 2017	June 9, 2017	Aug. 11, 2017	Oct. 11, 2017
5	Apr. 11, 2017	~May 9, 2017	Soybean	Oct. 11, 2017	May 10, 2017	June 9, 2017	Aug. 11, 2017	Oct. 11, 2017
6	Apr. 11, 2018	~Apr. 25, 2018	Corn (silage)	Aug. 21, 2018	May 11, 2018	June 1, 2018	Aug. 14, 2018	Aug. 28, 2018
7	Apr. 11, 2018	~Apr. 25, 2018	Corn (grain)	~Sept. 3, 2018	May 11, 2018	June 1, 2018	Aug. 14, 2018	Sept. 11, 2018

Notes: PSNT = pre-sidedress nitrate test. Where applicable, ~ approximates the date.

ing. Performing manure application in this manner ensured symmetric application rates between treatments. Manure application rate was decided by the land owner and is reported in table 1. Manure plant-available N (PAN) was calculated using Virginia availability coefficients: 35% of total organic N, 25% of surface-applied NH_3 -N, and 95% of injection applied NH_3 -N (equations 2 and 3):

$$\text{Surface-applied PAN} = (0.35 \times \text{total organic N}) + (0.25 \times \text{total ammoniacal N}), \text{ and} \quad (2)$$

$$\text{Injection applied PAN} = (0.35 \times \text{total organic N}) + (0.95 \times \text{total ammoniacal N}). \quad (3)$$

In 2016, a shallow disc injector (Vertical Till Injector, Washington, Iowa) 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 and manure was pumped in, followed by slit closure with two angled discs. In 2017 and 2018, a Dietrich footed shank injector (DSI Inc., Goodfield, Illinois) was used at a band spacing of 61 cm and depth of ~20 cm. Sequentially, a disc cut surface residue and 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 one or two 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 four 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 = one month after manure application, time 2 = routine PSNT when corn was ~30 cm tall, time 3 = four months after application, and time 4 = postharvest (table 2). In 2016, sites were only sampled three times, with the third sampling date postharvest. In 2017, site 5 was planted 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 equispaced 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 (in 2016) or 8 (in 2017) 30 cm deep soil cores based on band spacing, centered across the injection band and the interband space in 2.5 cm increments with four subsamples per plot. The resulting soil NO_3^- -N concentrations from across band and interband samples were combined based on the area they were hypothesized to represent (equation 4):

$$\text{Weighted method soil } \text{NO}_3^- \text{-N} = (0.33 \times \text{across band } \text{NO}_3^-) + (0.66 \times \text{between bands } \text{NO}_3^-). \quad (4)$$

The equispaced method (Meinen and Beegle 2015) used five 30 cm deep soil cores in 2016 or four 30 cm deep soil cores in 2017 and 2018, based on band spacing, taken 15 cm apart and perpendicular to the direction of injector travel, four subsamples per plot. In 2016, the equispaced 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 of 2 M KCl were 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 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, sites 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, Minnesota). 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 met-

Table 3

Pre-sidedress nitrate test (PSNT) results for sampling methods (equispaced, standard, and weighted) within manure-injected fields, compared to surface-applied fields. An n/a indicates the sampling method was not utilized. Method coefficient of variation (CV) was calculated as the mean CV across sites.

Year/site	Equispaced (mg kg ⁻¹)		Standard (mg kg ⁻¹)		Weighted (mg kg ⁻¹)		Surface applied (mg kg ⁻¹)	
	PSNT	Std. dev.	PSNT	Std. dev.	PSNT	Std. dev.	PSNT	Std. dev.
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 CV	30		28		37		28	

Note: Where applicable, significance between sampling methods is indicated by * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

rics 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 CO₂ 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, Nebraska). Total C-min was estimated by integrating CO₂ 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 one hour preincubation with shaking, the soil slurries (i.e., soil and solution combinations) were incubated for four hours 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. 2018). Analysis of variance was performed by site and sampling time if applicable; treatment means were separated using the Tukey-Kramer honestly significant difference (HSD) 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 equispaced method. Soil NO₃⁻-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 $\alpha = 0.05$ level; error bars in figures are the standard deviations of the means.

Results and Discussion

Pre-Sidedress Nitrogen in the Injected Plots.

PSNT numbers when corn reached a height of ~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 in 2017 and 2018, and year had 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 NH₃-N plus mineralized soil and manure organic-N minus NO₃⁻-N lost to leaching, plant uptake, and denitrification. These factors are greatly affected by weather, soil properties, and management history that influenced the PSNT values observed in this study. Bierer et al. (2017) quantified NH₃-N volatilization from injected and sur-

face applications of dairy slurry and reported that captured NH₃-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. In addition, 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 three brackets for additional sidedress N applications: <15 mg kg⁻¹ apply full rate, 15 to 26 mg kg⁻¹ apply 50% to 75% of full rate, >26 mg kg⁻¹ N sufficient (Virginia DCR SWC 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 equispaced 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 equispaced and standard methods, which elevated the coefficient of variation (CV) of the weighted method to 37% (table 3). Both the equispaced and standard methods resulted in similar CV values to those obtained in the surface-applied plots. All methods had acceptable CV values when compared to other studies that examined general grid sampling of soil N in fields. Goderya et al. (1996) measured soil NO₃⁻-N in the top 30 cm of three large fields and reported a CV of 45%, while a similar study assessed soil NO₃⁻-N in smaller 90 × 40 m plots and reported a CV of 16% (Dlugosz and Piotrowska-Dlugosz 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 CV between methods; however, increasing sidedress N rate raised the CV of the random sampling method. Similar to the present study, Assefa and Chen (2007) reported localized elevated soil NO₃⁻-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 CV of the standard and equispaced methods was low (table 3), and the labor of sampling was not greatly increased using the equispaced method. The present study, in addition to prior research, would recommend using the equispaced 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 NO₃⁻-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 postharvest to a high of 47.57 mg kg⁻¹ at PSNT at site 1 (table 4). Year had a significant effect on soil NO₃⁻-N at all sample times (*p* < 0.01); however, no sites were repeated year to year. Soil NO₃⁻-N was higher one 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 NO₃⁻-N remained elevated in the postharvest 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 postharvest soil NO₃⁻-N tests >20 mg kg⁻¹, fields are under consideration for reductions in manure or sidedress N applications; however, Virginia uses the corn stalk NO₃⁻ 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 that are representative of N rates (table 1).

In 2017 and 2018, soil NO₃⁻-N generally declined from one 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 NO₃⁻-N from one month after application to PSNT time (time 2), potentially due to N fixation supplementing crop N uptake (table 4). Soil NO₃⁻-N increased from four months after application to postharvest, 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 NO₃⁻-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 four months after manure application when soil NO₃⁻-N would be inconsequential to crop growth (table 4). When treatment dif-

Table 4
Soil nitrate (NO₃⁻) results of fields injected or surface broadcast with dairy slurry: one month after manure application, pre-sidedress nitrate test (PSNT) window, four months after application, and postharvest.

Year/site	One month (mg kg ⁻¹)		PSNT (mg kg ⁻¹)		Four months (mg kg ⁻¹)		Postharvest (mg kg ⁻¹)	
	Injection	Surface	Injection	Surface	Injection	Surface	Injection	Surface
2016								
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

Notes: An 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.

ferences 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 two of seven 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 $<15 \text{ mg kg}^{-1}$ to 15 to 26 mg kg^{-1} , potentially reducing sidedress chemical N applications (Virginia DCR SWC 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 postharvest under an injected application of manure, relative to surface application; however no manure was applied two 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, and 6), yields varied due to differences in location, management, and weather; site 6 was under pivot irrigation, partially contributing to higher yields. In addition, 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 follow the same trend as dry matter yield and were 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, postharvest soil NO_3^- -N was high ($>20 \text{ mg kg}^{-1}$; table 4), providing evidence that N was likely not limiting crop growth. Further, sites 4, 5, and

Figure 1

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

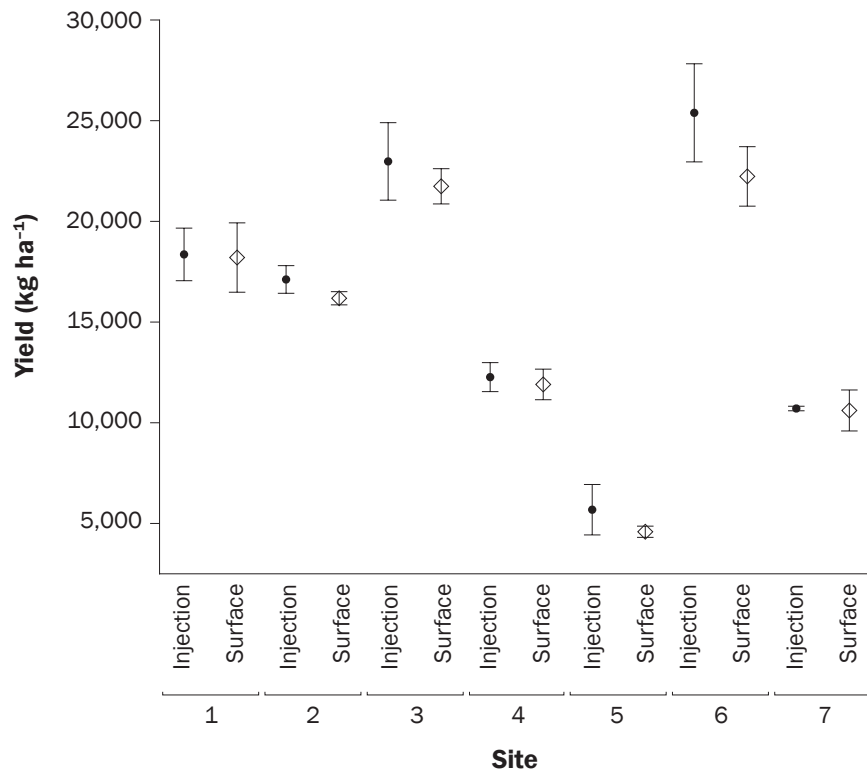
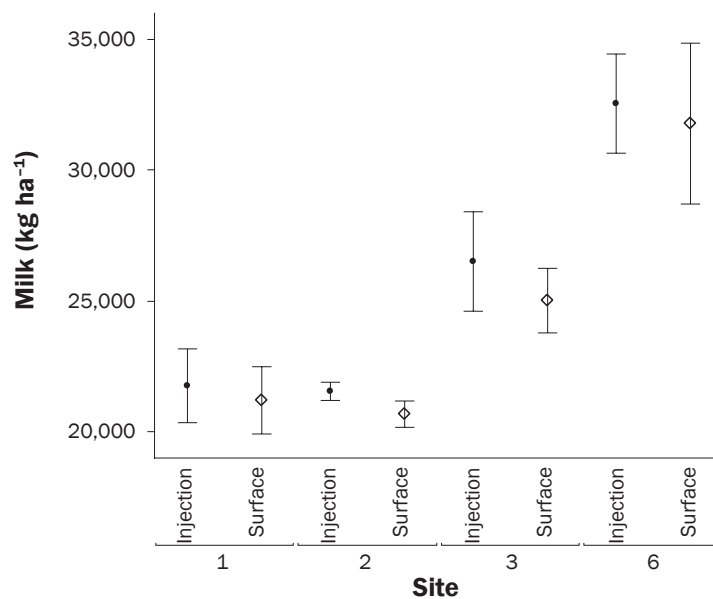


Figure 2

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.

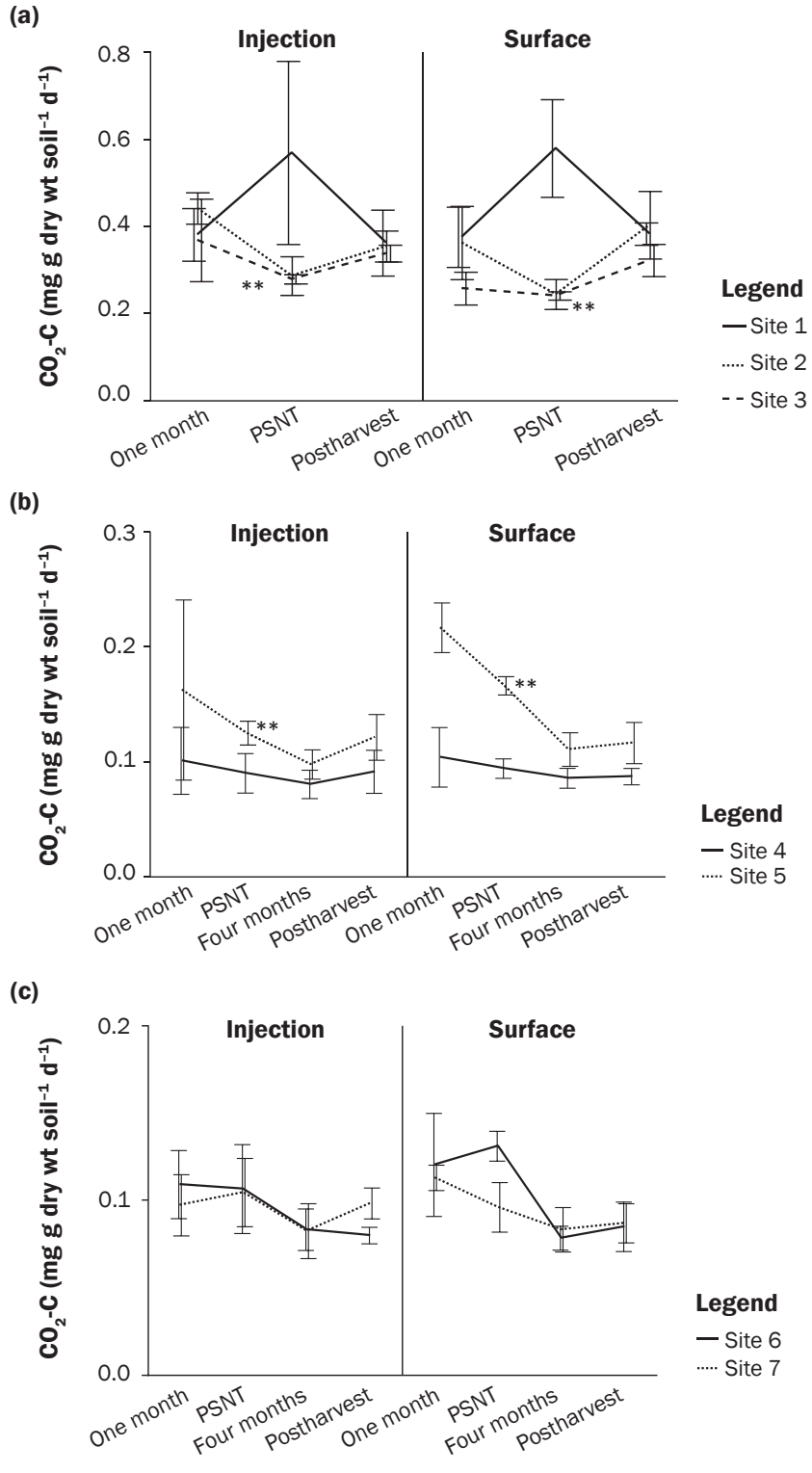


7 were shallowly disked to prepare a seedbed after manure application, potentially reducing $\text{NH}_3\text{-N}$ losses associated with surface applications of manure. Nevertheless, under the injected application, yield and estimated milk production means were always centered at or above the surface application (figures 1 and 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 preplant 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 C-min and SIR. The C-min values were greater in 2016 (mean = $0.36 \text{ mg C g dry wt. soil}^{-1} \text{ d}^{-1}$) than 2017 (mean = $0.11 \text{ mg C g dry wt. soil}^{-1} \text{ d}^{-1}$) and 2018 (mean = $0.10 \text{ mg C g dry wt. soil}^{-1} \text{ d}^{-1}$; 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 C substrates, possibly increasing decomposition of soil C through priming (Fierer et al. 2005; Strickland et al. 2015). In addition, providing a N source to drive decomposition of more recalcitrant C 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, $R^2 = 0.04$ and $R^2 = 0.03$, respectively. For SIR, all years were significantly different ($p < 0.0001$) with the following means: 2016 = $0.70 \text{ ug C g dry wt. soil}^{-1} \text{ h}^{-1}$, 2017 = $0.11 \text{ ug C g dry wt. soil}^{-1} \text{ h}^{-1}$, and 2018 = $0.33 \text{ ug C g dry wt. soil}^{-1} \text{ h}^{-1}$ (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 C compounds; however, the relationship to total C was unclear (Beyer 1995).

Figure 3

Carbon (C) mineralized during 30-day laboratory incubations by site and sampling time in (a) 2016, (b) 2017, and (c) 2018 (one month, pre-sidedress nitrate test [PSNT], four months, and postharvest). Carbon mineralized was estimated by integrating carbon dioxide (CO_2) production over days 1, 5, 10, 20, and 30 of the incubation. Where applicable, significant differences between manure application methods 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.

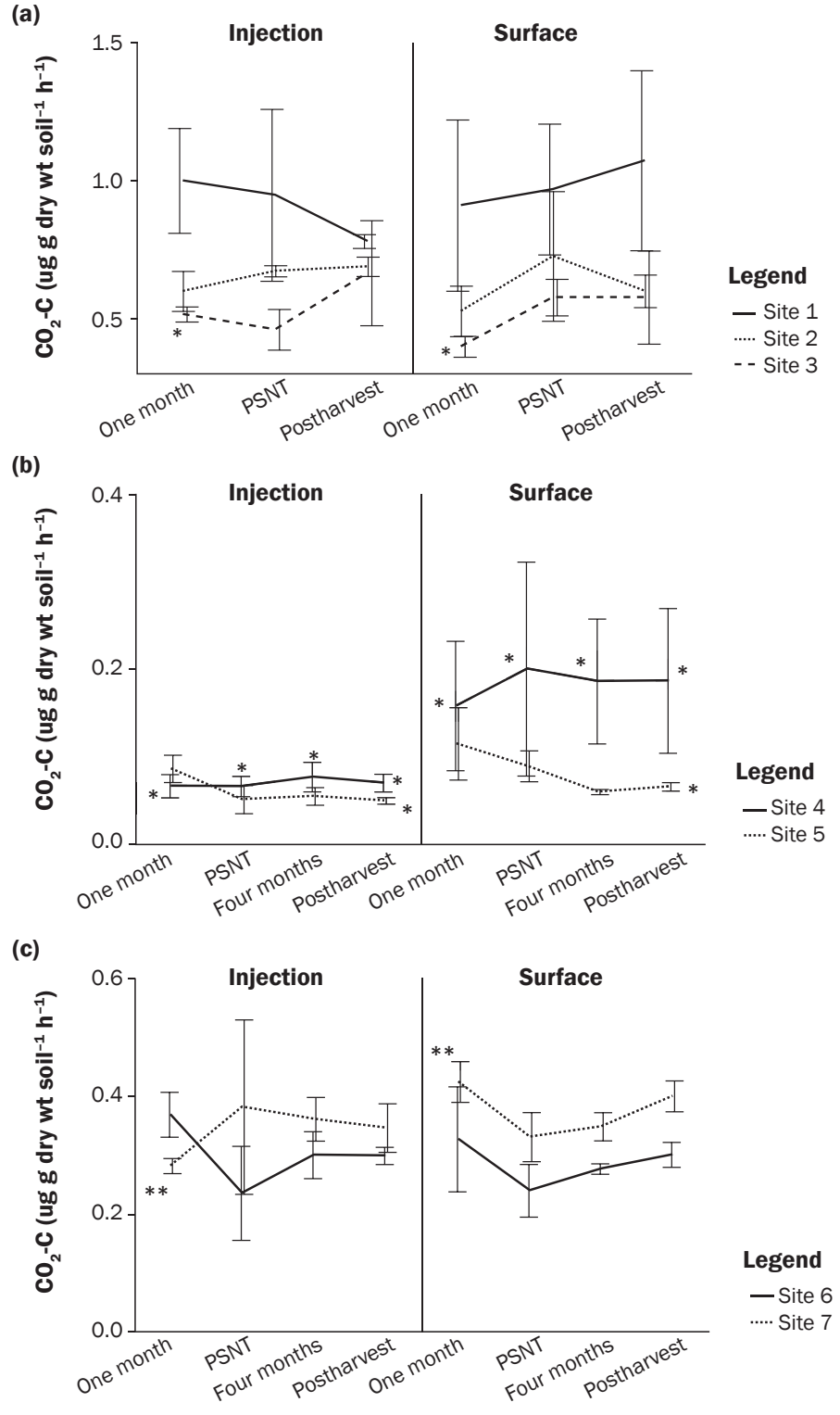


In the present study, sampling time had a significant effect on both C-min ($p = 0.0007$) and SIR ($p = 0.0159$), indicating the need to identify a sampling window or protocol for when biological testing should occur. Chang and Trofymow (1996), reported that SIR values differed by sampling date when 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 C/N additions. Several studies reported that 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 one month after manure was applied. In 2017, SIR of injected plots was 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 one 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 ($R^2 = 0.64$ and Pearson's correlation $r = 0.80$), 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

Figure 4

Substrate induced respiration during four-hour laboratory incubations after addition of an autolyzed yeast broth substrate. Incubations were performed by site and sampling time in (a) 2016, (b) 2017, and (c) 2018 (one month, pre-sidedress nitrate test [PSNT], four months, and postharvest). Where applicable, significant differences between manure application methods 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.



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 (18×18 m) and reported a moderately high CV (~26%) relative to the quantity of samples taken ($n \sim 150$). 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 CV of ~20% for arable soils, recommending six to eight pooled subsamples per sample for proper representation. For comparison, by site, this study observed a CV 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 that represent N 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. 2016; 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).

Summary and Conclusions

The present study recommends an ideal equispaced sampling technique for fields injected with manure when the direction of injector travel is known; however, a stan-

dard method proved adequate and both methods proved superior to a labor-intensive weighted method. In addition, 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 limited 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 C-min biological test proved to be logistically intensive and provided little useful information regarding short-term differences in management. The SIR test was less logistically demanding but was unable to consistently differentiate between manure application methods and should not be recommended to producers until practical interpretations of the test are clear.

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