Monolith Soil Core Sampling to Develop Nitrate Testing Protocol for Manure Injection

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Abstract

Injecting manure and commercial fertilizer beneath the soil surface is an important nutrient management practice that conserves ammonia-nitrogen (N) but creates distinct bands of N below the soil surface. To date, no widely accepted soil nitrate sampling protocol has been developed to account for the extreme heterogeneity created by injection. To develop sampling recommendations for Pre-Sidedress Nitrate Test (PSNT), we quantified patterns of NO3–N concentrations in soil from of corn (Zea mays L) plots injected with liquid dairy cattle (Bos taurus L) manure at 76 cm spacing over two years. Soil monoliths were collected to allow precise sampling of 30 cm deep by 2.5 cm soil cores from which a mid-season PSNT was determined. Monte Carlo simulation was conducted to simulate the effects of alternative soil sampling protocols on bias and error. Results from the simulation support the following equispaced sampling protocol: five, 30-cm deep soil cores are spaced 15 cm apart and oriented in a line perpendicular to the injected manure bands, collected at four locations in the field, to produce a single composite of 20 samples for NO3- analysis. It is not necessary to know manure band location. As spatially discrete manure application patterns become more prevalent with the expansion of manure injection, we believe this PSNT sampling protocol balances risk of error with practical concerns needed to promote adoption.

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Core Ideas

As part of the submission process, we ask authors to prepare highlights of their article. The highlights will consist of 3 to 5 bullet points that convey the core findings of the article and emphasize the novel aspects and impacts of the research on scientific progress and environmental problem solving.

The purpose of these highlights is to give a concise summary that will be helpful in assessing the suitability of the manuscript for publication in the journal and for selecting appropriate reviewers. If the article is accepted the highlights may also be used for promoting and publicizing the research.

Core Idea 1: â€¢ Monolith soil sampling provided unique ability to explore nitrate distribution after manure injection.

Core Idea 2: â€¢ Analysis of nitrate from monolith soil cores provided recommendation for soil collection protocol.

Core Idea 3: â€¢ Recommended soil nitrate testing procedure can be consistent with current PSNT protocols.

Core Idea 4: â€¢ Novel spatial analysis model can be used for future soil nutrient distribution work.

Core Idea 5: CUST_CORE_IDEA_5 :No data available.

TITLE

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CORE IDEAS

- Monolith soil sampling provided unique ability to explore nitrate distribution after manure injection.
- Analysis of nitrate from monolith soil cores provided recommendation for soil collection protocol.
- Recommended soil nitrate testing procedure can be consistent with current PSNT protocols.
- Novel spatial analysis model can be used for future soil nutrient distribution work.

ABSTRACT

Injecting manure and commercial fertilizer beneath the soil surface is an important nutrient management practice that conserves ammonia-nitrogen (N) but creates distinct bands of N below the soil surface. To date, no widely accepted soil nitrate sampling protocol has been developed to account for the extreme heterogeneity created by injection. To develop sampling recommendations for Pre-Sidedress Nitrate Test (PSNT), we quantified patterns of NO_3^--N concentrations in soil from of corn (*Zea mays L*) plots injected with liquid dairy cattle (*Bos taurus L*) manure at 76 cm spacing over two years. Soil monoliths were collected to allow precise sampling of 30 cm deep by 2.5 cm soil cores from which a mid-season PSNT was determined. Monte Carlo simulation was

conducted to simulate the effects of alternative soil sampling protocols on bias and error. Results from the simulation support the following equispaced sampling protocol: five, 30-cm deep soil cores are spaced 15 cm apart and oriented in a line perpendicular to the injected manure bands, collected at four locations in the field, to produce a single composite of 20 samples for NO_3^- analysis. It is not necessary to know manure band location. As spatially discrete manure application patterns become more prevalent with the expansion of manure injection, we believe this PSNT sampling protocol balances risk of error with practical concerns needed to promote adoption.

ABBREVIATIONS

PSNT Pre-Sidedress Nitrate Test

NO_3^--N Nitrate Nitrogen

KEY WORDS

Manure injection

Soil nitrate

Soil monolith

Pre-Sidedress Nitrate Test

Soil Test Model

INTRODUCTION

Compared to surface manure application, injection provides conservation of N due to lower volatilization (Dell et al., 2011), leading to increased N available for conversion to plant-available NO_3^--N (Bierer et al., 2017). Banding manure with commercially available tools provides heterogenic micro- and mesovariation in soil (Stecker et al, 2001), creating systematic zones with chemical, physical, and biological disparities (Van Vuuren et al., 2000). Soil testing for N focuses on NO_3^--N concentrations. The Pre-Sidedress Nitrate Test (PSNT) in corn is an early season indicator of N availability for the crop. PSNT sampling protocols were developed in humid environments of northeastern U.S. where soils were assumed to have received evenly distributed N by broadcast manure or fertilizer applications (Magdoff et al., 1984; Fox et al., 1989; Roth & Fox, 1990, Magdoff, 1991). PSNT sampling is especially useful on lands with manure application or recent legume crop history (Fox et al., 1989; Roth & Fox, 1990). PSNT protocols have been adapted for utilization in a number of states (e.g., Delaware - Sims et al., 2013; Indiana – Brouder & Mengel, 2003; Iowa – Sawyer & Mallarino, 2017; Maryland – Coale et al., 2010; Massachusetts – Spargo et al., 2013, New Jersey – Heckman, 2003; New York – Ketterings et al., 2012; Ohio – Watters & LaBarge, 2017; Pennsylvania – Beegle et al., 1999; Vermont – Jokela et al., 2017; Virginia – Maguire et al., 2019; Wisconsin – Laboski & Peters, 2012), recommending sampling soil when corn is at the 4 to 6 leaf growth stage (30-46 cm tall), with most states recommending random collection of 30 cm deep soil cores, with the number of composited soil sample cores ranging from 10 to 40 (7 of the 12 states listed recommended 20 cores at the top of their collection range). PSNT results become suspect on grounds receiving injected manure due to heterogeneity of N distribution. Random sampling near concentrated manure injection bands may give artificial confidence in N availability, while samples away from bands may indicate unnecessary need for N sidedressing (Assefa & Chen, 2008; Tewolde et al., 2013).

Intense soil sampling for NO_3^--N concentration across the two-dimensional areas perpendicular to a manure injection bands such as those recommended by Shapiro (1988), Kitchen et al. (1990), Mahler (1990), Ashworth et al. (1994), James and Hurst (1995), Tewolde et al. (2013), and Westerschulte et al. (2015) are not practical in field situations, especially if conducted at multiple sites within the field as recommended by Cline (1944) and PSNT protocols. Exploration of lateral movement of N after manure injection demonstrated that nitrification began at the periphery of manure injection band (Comfort et al., 1988). Higher concentrations were expected to decline rapidly with distance from the manure band (Westerschulte et al., 2015, Bierer et al., 2021) with expected lateral migration of 10 to 17 cm (Poffenbarger et al., 2015) to 20 cm (Assefa & Chen, 2008) through the growing season. Disparity between elevated NO_3^--N levels near the band compared to bulk soil away from the band decreases through the growing season in corn and is influenced by crop N uptake (Bierer et al., 2021). Poffenbarger et al. (2015) modeled randomly selected 30 cm deep soil cores, that were at least 10 cm apart, to find that a ratio of four to six samples away from injected pelletized poultry manure bands for every core within 5 cm of the band was the best predictor of mineral N at all corn growth stages. The current study recommends a protocol first suggested in conference proceedings (Meinen & Beegle, 2015) that emerged as a preferred practical sampling method and termed equispaced sampling by Bierer et al. (2020).

This research is the first study to approach a NO_3^--N soil sampling protocol development by analyzing adjoining soil cores across the entire two-dimensional space between, and oriented perpendicular to, injected manure bands extracted with a unique monolith soil sampling tool that allowed exploration of multiple sampling protocols for certainty and practicality of implementation. The objective of this research was to explore soil nitrate distribution in a two-dimensional orientation perpendicular to manure injection bands in corn and to recommend a nitrate soil sampling protocol for such scenarios.

MATERIALS AND METHODS

Plots were established at the Pennsylvania State University's Russel E. Larson Agricultural Research Center at Rock Springs, Pennsylvania in fields of Murrill channery silt loam, a well-drained colluvium ultisol derived from sandstone over residuum weathered limestone with less than 3% slope and hydrologic group B.In 2011, a single large field-scale 13.7 m wide and 82 m long plot was established for manure injection application. Sampling in 2011 was conducted at random locations within the large plot. In 2012, three 4.6 m wide and 16 m long plots were established within a larger randomized complete block design experiment that contained other manure application treatments that are not referred to in this manuscript. Soil was obtained from each individual plot in 2012. In both years all plots were previously harvested as corn for grain and free of manure application or legume crops for several years prior to research plot establishment. All manure was sourced from the same commercial dairy liquid Slurrystore (CST Industries, Kansas City, MO) storage. Manure analysis indicated total N and ammonium N contents of 3.60 and 1.62 kg $1{,}000^{-1}$ in 2011, and total N and ammonium N contents of 4.36 and 2.07 kg $1,000^{-1}$ in 2012 respectively. Approximately 45 and 47% of total manure N was in NH_4^+ form at the time of application in 2011 and 2012, respectively. Total P_2O_5 was 1.20 kg $1,000^{-1}$ in 2011 and 1.42 kg $1,000^{-1}$ in 2012. Total K_2O analysis was 2.69 kg $1,000^{-1}$ in 2011 and 3.29 kg $1,000^{-1}$ in 2012. Manure was applied on 12 May 2011 and 1 May 2012, prior to no-till corn planting on 13 May 2011 and 4 May 2012. Total manure N was applied at a rate of approximately 202 and 245 kg Total N ha^{-1} in 2011 and 2012, respectively. No other N sources were applied. In injected plots expected manure N availability to the corn crop was 50% (Penn State Extension, 2021). Corn (Growmark FS 5099VT3 (100 DRM) at 32,000 spa at 1.5" depth in 2011; Dekalb DKC 61-21 R1B (111 DRM) at 32,000 spa at 1.5" depth in 2012) was planted at 76 cm spacing. Travel of all planting and manure injection operations were in the same parallel direction. There was no attempt to standardize proximity of seed placement to injection manure band locations. Manure injection was conducted using the Penn State Research Manure Spreader with six Yetter Avenger (Colchester, IL) shallow-disc, toolbar-mounted injection units with a spacing of 76 cm between injection bands. The equipment placed manure in a slot that extended 10 to 15 cm below the soil surface and provided slot closure and soil coverage to minimize manure surface exposure to the free air stream.

Soil Sampling

A unique monolith sampler developed by the USDA-NRCS (Figure 1) was used to remove a large (76 cm X 15 cm X 50 cm deep) block of soil when corn leaf stages were V4 in 2011 and V6 in 2012. Soil sampling was conducted 72 days after manure application in 2011 and 57 days after manure application in 2012, with the unusually long period before corn growth approached recommended PSNT sampling height in 2011 attributed to drought conditions. The monolith sampler covered a rectangular soil surface area that measured 76 cm by 15 cm and was placed so the long edge of the sampler was perpendicularly to the direction of travel of all manure injection. The monolith sampler was pounded into the soil to a depth of 50 cm or more and then excavated and lifted from its sampling position with a backhoe tractor. The monolith sampler was then laid flat so one side of its long edge could be removed, exposing the unearthed monolith soil face (Figure 1D).

Figure 1. Photos of monolith sampling. A) Monolith sampler used hydraulics to drop a heavy weight onto the monolith sampling unit to drive the sampling unit into the soil. B) The monolith sampling unit after pounding into the soil and before excavation. C) Excavation of the monolith sampling unit. D) The monolith sampling unit was laid on its sideand a face of the sampling unit was removed to access the monolith soil sample, and special tools were utilized to precisely remove 30 side-by-side 2.54 cm square x 30 cm deep soil core samples. Drought stress to the corn is apparent in these 2011 photos.

When it was possible to locate manure injection bands, the monolith sampler was positioned with the manure band near the center. Thus, approximately 38 cm of soil was removed from each side of the band location, representing a two-dimensional, cross-sectional area of the soil face perpendicular to the direction of manure band application. From the exposed soil faces, thirty adjacent soil 'cores' were systematically removed, each being 2.5 cm square in shape and 30 cm in depth from the original soil surface (Figure 1D). In some cases, soil profile structure integrity issues at the outer edge of the removed monolith sample resulted in the inability to collect 30 samples, thus some monoliths have less than 30 data points. Each of these samples was analyzed individually for NO_3^--N concentration. In 2011, monolith sampling was conducted from 6 locations in the single large injection plot. In 2012, monolith sampling occurred once on in each of the three separate injection plots in the same field.

After leaving the field, soil samples were rapidly air-dried to minimize microbial transformations of N. Nitrate analysis was conducted by the Penn State Agricultural Analytical Services Laboratory. Briefly, 20 g of soil was extracted using 0.04 *M* $(NH_4)_2SO_4$ on a reciprocating shaker at 180 excursions per minute, filtered using Whatman 41 and analyzed for NO_3-N according to SM 4500 NO_3^- D (APHA, 2005) with modifications described by Griffin et al. (2011) for soil analysis.

Model Development and Statistical Analysis

Several parameters were specific to developing the PSNT protocol from this research. A 76 cm manure band spacing was used in this research which is a typical band spacing.

Soil cores were shaped in square cylinders 2.5 cm per side by 30 cm deep, and potential sampling protocols were limited to possible combinations of these thirty uniformly spaced contiguous samples extracted from the monolith sampling unit across the width of the manure injection spacing. This is a practical spacing unit (2.5 cm = 1 inch) in the US for development of a sampling protocol that will be acceptable and replicable in the field. For this reason, some data are presented with units of both cm and inches. To be consistent with practical implementation and existing PSNT protocols (e.g., Beegle et al., 1999, Maguire et al., 2019), modeling in this work was confined to collection of a maximum of 20 total cores. The desired protocol would not require identification of the manure band location because locating the band at PSNT time is often problematic. All protocols considered collected soil cores in a perpendicular orientation to the direction of the manure injection band.

Different proposed PSNT sampling protocols for injection plots were modeled with R (R Core Team, 2020) with differences reported as significant when $p < 0.05$. An in-depth explanation of model run input, processes, and data are presented in Supplement material. The model was run for only one year at a time. The inputs for the model were the specific sampling protocol for selecting soil cores from the available 30-core sets from individual monolith-sampled injection locations, which included the number of soil cores from each monolith, and the designated spacing between cores across the contiguous samples of that monolith. For the year of interest, a monolith sample was selected at random, and from that monolith the specified number of soil cores were then selected at random (from the available 30 cores in the monolith) while adhering to the soil core spacing indicated by the sampling protocol. This process was repeated until the desired total number of cores were selected. The average nitrate level was determined for each monolith set, and then the sample mean was determined from the resulting monolith averages.

Because some sampling protocols would require sampling from more monoliths than available, data replacement during model analysis was sometimes necessary. Data replacement means that once soil core data was randomly selected from a single monolith, all data from that monolith was placed back into the eligible data set for the next selection of monolith, making every monolith eligible for the subsequent random selection. This meant that the model run with data replacement could randomly choose data from the same monolith multiple times for any run of the model, and it was even possible that the same set of cores where randomly chosen from that monolith. In other cases, data replacement was not necessary. When the monoliths were selected randomly without data replacement, every monolith was eligible to be selected at most once in each run of the sampling protocol. In other highlighted cases data replacement was not conducted by choice.

RESULTS AND DISCUSSION

Summary data is shown in Table 1. The NO_3^--N concentration mean for all six 2011 injection plots combined was 16.77 mg kg^{-1}. The NO_3^--N concentration mean for all three 2012 injection plots combined was 17.05 mg kg^{-1}. Fitted plots for the individual 30-core injection monolith samples are presented in Figure 2. For the 2011, dry soil conditions prevented NO_3^--N production, which resulted in parity of NO_3^--N concentrations across the 30-core monolith samples. This was likely due to unfavorable N cycling in severely dry soil conditions. Duncan et al. (2017) also observed limited N cycling in manure plots, and reported rainfall data, at the same research farm in 2011. Poffenbarger et al. (2015) observed similar mineral N trends in dry soil conditions. While results were contrary to expectations, this does highlight the importance of moisture in soil nutrient cycling, mobility, and availability. Plots in 2012 were more responsive (Figure 2) although discrepancy between mean NO_3^--N concentrations is apparent between Plot 6 (25.48 mg kg^{-1}) and Plots 9 (13.21 mg kg^{-1}) and 10 (12.48 mg kg^{-1}).

Table 1. Nitrate concentration means from 2011 and 2012 manure injection monolith sample cores. Summary for individual monoliths and all monoliths combined by year are presented.

Year	Monolith Identifier	N	Mean Nitrate Concentration mg kg^{-1}	Standard Deviation mg kg-1	Minimum Nitrate Value mg kg^{-1}	Maximum Nitrate Value mg kg^{-1}
2011	Monolith 1	30	10.65	4.29	4.2	19.4
	Monolith 2	30	10.56	5.65	3.5	25.6
	Monolith 3	30	13.80	8.54	4.5	36.3
	Monolith 4	26	19.87	10.31	7.0	53.1
	Monolith 5	26	25.59	20.06	2.6	71.4
	Monolith 6	30	21.74	24.28	3.0	102.0
	2011 monoliths combined	172	16.77	15.09	2.6	102.0
2012	Plot 6	29	25.48	15.69	7.2	59.7
	Plot 9	28	13.21	11.30	2.9	37.6
	Plot 10	30	12.48	11.51	2.3	45.8
	2012 monliths combined	87	17.05	14.16	2.3	59.7

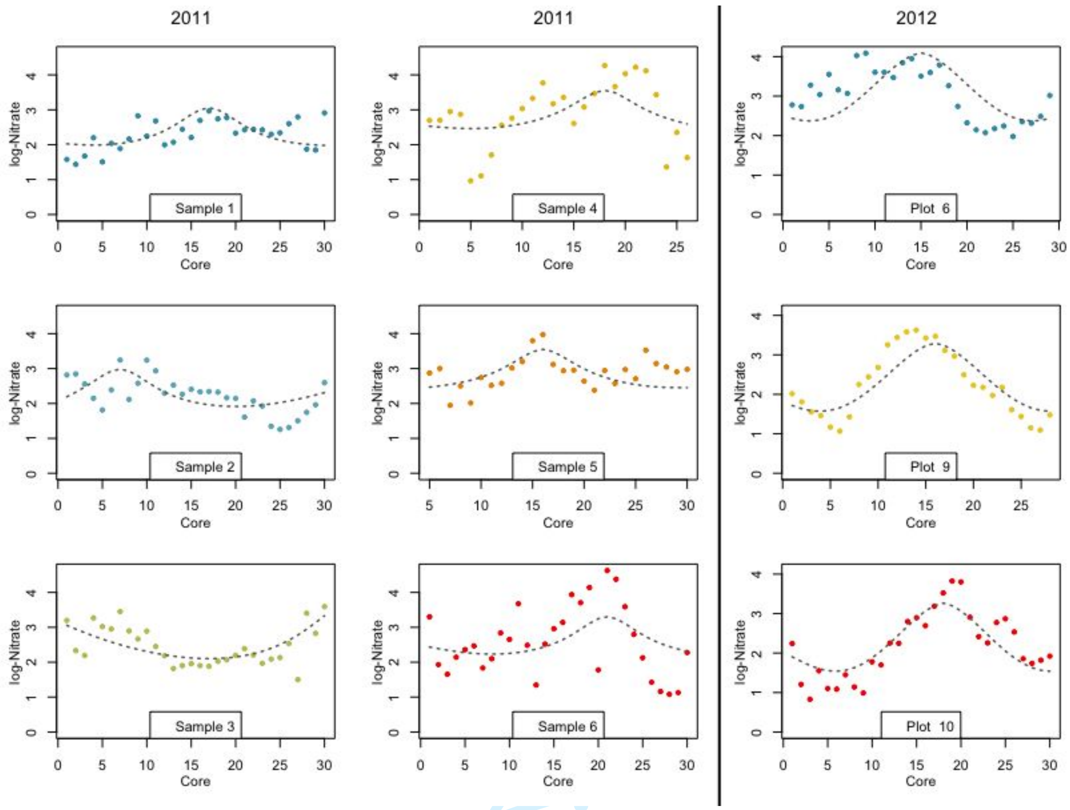

Figure 2. Scatterplot for log-Nitrate concentration levels vs. the core location along with the fitted lines from 30-core monolith sampling in soils receiving injected liquid dairy manure in bands spaced at 76 cm for 2011 (Left) and 2012 (Right).

Development of PSNT Soil Sampling Protocol for Injection Plots

Dry soil conditions and limited N conversions presented difficulty when applying the 2011 data to PSNT soil sampling protocol development. For this reason, the more responsive 30-core injection data from 2012 was utilized to investigate soil sampling protocols. Subsequently, favored models from the 2012 data were then employed to verify that the proposed model would produce satisfactory results under the conditions of low nitrate production and treatment response of 2011.

Selected nitrate soil sampling protocol combinations are outlined in Table 2 for 2012 injection monoliths. These models were developed as described earlier and in the

Supplemental material, but with the following stipulations. In these models no data replacement was utilized and sets of core data were selected randomly exactly one time from each of the three 2012 injection monoliths. Therefore, total cores in each model were determined by the number of selected cores per monolith times three plots. MSE values consider both bias and variance, with lower MSE values considered indicative of favorable soil sampling protocol options. Spacing comparisons provided for sampling protocols with two, five, and six cores collected per monolith indicate that sampling options with optimal uniform spacing across the manure injection band spacing, termed equispaced sampling (Bierer et al., 2020) were always best compared to other spacing options with the same number of cores, as indicated by lower MSE (Table 2, highlighted with ***bold italicized*** text). The four-core protocol also performed well.

Table 2. Select soil NO_3^--N sampling protocols. In these models, each protocol was conducted once in each of the 2012 manure injection monolith plots with NO_3^--Nfrom the three plots averaged. Data replacement was not used. Each model scenario was run 1,000 times.

Cores per Monolith	Spacing (cm)	Spacing (inches)	Total Cores	Data Replacement	Nitrate-N Concentration Mean mg kg^{-1}	Sampling Bias	Sampling SD mg kg^{-1}	Sampling MSE
2	2.5	1	6	No	17.63	0.58	7.22	52.46
2	25.4	10	6	No	20.57	3.52	3.29	23.24
2*	***38.1***	***15***	***6***	***No***	***17.82***	***0.77***	***4.19***	***18.17***
3	15.2	6	9	No	20.98	3.93	2.43	21.32
4	17.8	7	12	No	17.32	0.28	1.55	2.49
5	2.5	1	15	No	18.90	1.85	6.56	46.45
5	7.6	3	15	No	21.45	4.40	2.81	27.24
5	12.7	5	15	No	18.40	1.35	1.78	4.98
5*	***15.2***	***6***	***15***	***No***	***17.11***	***0.06***	***1.62***	***2.63***
6	10.2	4	18	No	18.85	1.80	1.36	5.11
6*	***12.7***	***5***	***18***	***No***	***16.97***	***-0.08***	***1.47***	***2.18***
10	7.6	3	30	No	17.10	0.05	0.94	0.88

*For protocols selecting 2, 5, and 6 cores per monolith those with lowest MSE were protocols that had equispaced soil core selection, compared to other sampling spacing models with the same number of cores.

Because desired in-field sampling protocols will collect up to 20 total soil cores data replacement was necessary with our data set. Table 3 uses the same sampling protocol as Table 2 but with data replacement. In Table 3 sampling bias is comparable to those before data replacement (Table 2), however sampling standard deviation noticeably increased because the three 2012 plots (notably Plot 6) have very different statistical qualities (Figure 2) leading to replacement selection of poorly representative samples in a significant fraction of total samples. Similar to output without data replacement, the equispaced sampling protocols demonstrate lower MSE (Table 3, highlighted with ***bold italicized*** text). Bierer et al. (2020) found that equispaced and random sampling of injection plots for NO_3^--N had similar CV values, but here equispaced sampling showed clear advantages for estimation accuracy compared to other spacings or random sampling (data not shown).

Table 3. Select soil nitrate sampling protocols with data replacement. Sampling protocols from Table 2 are presented with similar model input parameters except monolith data replacement was utilized in statistical modeling. Each model scenario was run 1,000 times.

Cores per Monolith	Spacing (cm)	Spacing (inches)	Total Cores	Data Replacement	Nitrate-N Concentration Mean mg kg^{-1}	Sampling Bias	Sampling SD mg kg^{-1}	Sampling MSE
2	2.5	1	6	Yes	17.50	0.45	7.54	57.06
2	25.4	10	6	Yes	20.52	3.47	5.76	45.15
2*	***38.1***	***15***	***6***	***Yes***	***17.51***	***0.46***	***5.58***	***31.31***
3	15.2	6	9	Yes	20.88	3.83	5.51	45.06
4	17.8	7	12	Yes	17.16	0.11	3.65	13.34
5	2.5	1	15	Yes	18.89	1.84	7.43	58.63
5	7.6	3	15	Yes	21.59	4.54	5.05	46.10
5	12.7	5	15	Yes	18.56	1.51	3.97	18.05
5*	***15.2***	***6***	***15***	***Yes***	***17.14***	***0.09***	***3.83***	***14.71***
6	10.2	4	18	Yes	19.10	2.05	4.12	21.16
6*	***12.7***	***5***	***18***	***Yes***	***17.02***	***-0.03***	***3.81***	***14.55***
10	7.6	3	30	Yes	17.08	0.04	3.53	12.47

*For protocols selecting 2, 5, and 6 cores per monolith those with lowest MSE were protocols that had equispaced soil core selection, compared to other sampling spacing models with the same number of cores.

Since it was desired to develop a repeatable, practical sampling protocol that required collection of no more than 20 total soil cores as recommended in many current state PSNT guidance documents, further model exploration was narrowed to the five protocols that could meet these requirements in the field. The five chosen protocols demonstrated desirable statistical characteristics over other protocols that collect the same number of cores from a monolith and have the practical characteristic of equispacing (Table 3). These protocols were 2, 4, 5, 6, and 10 cores collected from 10, 5, 4, 3, and 2 different locations in a field, respectively (Table 4). The 6-core option produced a total of 18 total cores, while other options produced 20 total cores. The 4-core protocol was considered with spacing of 17.8 cm (7 inches) since this matched the dimensions of collected core units in this research (each 2.5 cm or 1 inch across), although uniform mathematical spacing with 4 cores would be 19 cm (7.5 inches); a spacing not available in our data.

Table 4. Practical sampling protocols selected for evaluation.

Cores per Sampling Location	Spacing between Cores (cm)	Spacing between Cores (inches)	Number of Monoliths	Total Cores
2	38.1	15	10	20
4	17.8	7	5	20
5	15.2	6	4	20
6	12.7	5	3	18
10	7.6	3	2	20

Data replacement was used for all runs of the model in 2012 (Table 5), which was necessary to obtain the total cores needed in the favored scenarios presented in Table 4. Sampling data from 2011 was then run with two different data management scenarios. The first run of 2011 data used data replacement (Table 6). The second run of 2011 data did not use replacement for the 4, 5, 6, and 10 core runs because it was not necessary since data was collected from six monolith locations in that year (Table 7). Each sampling run in Tables 5, 6, and 7 were conducted as outlined in Table 4, and each model was run 500,000 times.

Table 5. Comparison of 2012 soil nitrate sampling protocols from 30-core sampling data from injection monoliths using data replacement. The times each protocol was repeated, as well as the number of total cores, are provided in Table 4.

Cores per Monolith	Spacing (cm)	Spacing (inches)	Data Replacement	Nitrate Sampling Mean mg kg^{-1}	Sampling Bias	Sampling SD mg kg^{-1}	Sampling MSE
2	38.1	15	Yes	17.0940	0.0450	2.7660	7.6530
4	17.8	7	Yes	17.2580	0.2090	2.9140	8.5380
5	15.2	6	Yes	17.0510	0.0020	3.3240	11.0520
6	12.7	5	Yes	17.0530	0.0030	3.7670	14.1900
10	7.6	3	Yes	17.0540	0.0050	4.3890	19.2640

Table 6. Comparison of 2011 soil nitrate sampling protocols from 30-core sampling data from injection monoliths using data replacement. The times each protocol was repeated, as well as the number of total cores, are provided in Table 4.

Cores per Monolith	Spacing (cm)	Spacing (inches)	Data Replacement	Nitrate Sampling Mean mg kg^{-1}	Sampling Bias	Sampling SD mg kg^{-1}	Sampling MSE
2	38.1	15	Yes	16.8010	0.0334	3.1175	9.7201
4	17.8	7	Yes	16.9318	0.1641	3.3287	11.1075
5	15.2	6	Yes	16.8452	0.0775	3.4924	12.2031
6	12.7	5	Yes	16.8467	0.0790	4.0825	16.6734
10	7.6	3	Yes	16.9160	0.1483	4.2777	18.3204

Table 7. Comparison of 2011 soil nitrate sampling protocols from 30-core sampling data from injection monoliths. Because samples were obtained from six monoliths in 2011 data replacement was not necessary for models that selected 4, 5, 6, and 10 cores from single monoliths. For the protocol selecting 2 cores from 10 monoliths, data replacement was necessary. The 2-core results are a separate run of the model than that presented in Table 6. The times each protocol was repeated, as well as the number of total cores, are provided in Table 4.

Cores per Monolith	Spacing (cm)	Spacing (inches)	Data Replacement	Nitrate Sampling Mean mg kg^{-1}	Sampling Bias	Sampling SD mg kg^{-1}	Sampling MSE

2	38.1	15	Yes	16.8007	0.0330	3.1134	9.6941
4	17.8	7	No	16.9148	0.1471	2.3004	5.3134
5	15.2	6	No	16.8035	0.0359	2.6951	7.2646
6	12.7	5	No	16.8174	0.0497	3.5084	12.3114
10	7.6	3	No	16.8876	0.1200	3.8572	14.8925

Information from Tables 5, 6, and 7 were used to determine a PSNT soil sampling protocol in fields with banded manure. Selection of the preferred protocol was based on both data analysis as well as practicality of implementation. We recommend equispaced sampling with five, 30 cm deep soil cores be collected at 15 cm spacing in an orientation perpendicular to the 76 cm space between the manure bands and repeated four times.

The 5-core, 15 cm-equispaced sampling protocol provided an average 2012 soil NO_3-N value of 17.05 mg kg^{-1} (Table 5), which matches the true average of the soil cores collected from the three plots (Table 1). Model runs for this protocol with 2011 data as described in Tables 6 and 7 provided sampling means closest to the true 2011 average of 16.77 mg NO_3^-- N kg^{-1} (Table 1). With a goal of finding an unbiased estimator of nitrate concentration, the 5-core, 15 cm-equispaced protocol yielded the lowest sampling bias in each of Tables 5, 6, and 7. The chosen protocol provided standard deviations and MSE values that were neither highest nor lowest compared to others within the same table scenarios, yet not extremely removed from the lowest values. The 5-core sampling protocol is repeated at four locations in the field to produce a total of twenty cores. The twenty cores are composited for removal of a single subsample of the composited material for NO_3^--N analysis. It is not necessary to know the location of the manure band, but crucial to know the direction of travel during manure band placement.

Similar to the over estimation of standard deviation noted when comparing 2012 data with data replacement (Tables 2 and 3), our 2011 data demonstrates that models without data replacement (Table 7) performed better than models with replacement (Table 6), suggesting that confidence in 2012 protocol development may have been greatly improved with monolith sampling from a fourth injection plot so that data replacement was not necessitated. Similarly, field collection would be conducted in four locations, thus removing data replacement influence from field results.

The pragmatic 5-core equispaced protocol provides opportunity to account for micro, macro, and meso variations when cores are taken at four locations within a field. The soil sampling protocol developed here for NO_3^--N testing has practical and repeatable advantages of using a reasonable number of soil cores that can be collected with tools that are standard in the industry and with no need to mark the injection band during application or locate the band when sampling soil. Our experience is that the exact band location is often not apparent after application and can be very hard to locate, especially after additional field operations have occurred, some time has passed, or the soil surface has been subjected to precipitation. When samples are collected that contain the manure band, it is expected that an overestimate of available soil nutrient level will occur (Kitchen et al., 1990, Rehm et al., 1995, Stecker et al., 2001, Tewolde et al., 2013, Westerschulte et al., 2015). Cores that contain visible manure content should be discarded because they may provide artificial inflation of N availability. Collection of soil with the protocol from more than four locations is recommended and should provide greater certainty in soil sampling results and subsequent N fertilizer sidedress recommendations.

The soil nitrate sampling protocol developed through this research obtains samples from soils with manure injection bands when corn is around the 4 to 6 leaf stage of growth, before corn uptake demand is at its highest. The number of total cores collected here is practical and comparable to the widely implemented NO_3^--N sampling protocols for lands receiving broadcast manure that recommended 10 to 20 random cores (e.g., Beegle et al., 1999; Maguire et al., 2019). This protocol does not require development of an unwieldy tool that would acquire slice samples in a variety of soils with 10 to 12 (James & Hurst, 1995) to 15 (Ashworth et al., 1994) samples and damage growing crops. In Figure 3 we present the possible soil core sample collection positions of our square 2.5 cm, side-by-side research protocol, with the centerline of the injection band zone represented as a finite point. With the protocol developed here the furthest distance a core could be from the centerline of the manure band is 5.0 to 7.6 cm, yielding a 4:1 ratio of cores outside of 7.6 cm to every one inside that distance.

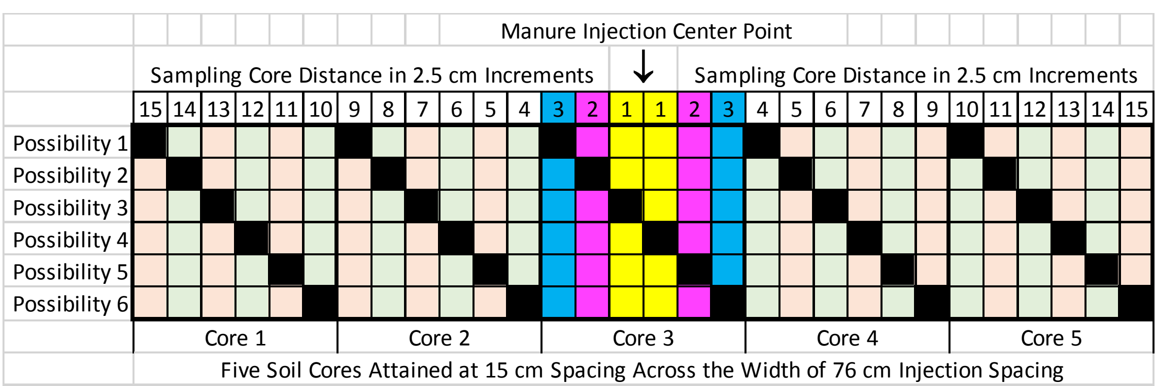

Figure 3. This schematic shows the six possible positionings of core selection from the 2.5 cm wide soil cores collected across the monolith samples of injection manure plots in

this research when the proposed 5-core, 15 cm-equispacing sampling protocol is utilized. Soil collection locations are represented with solid black cells across each possibility. The yellow, pink, and blue sampling core distances indicate samples within 2.5, 5, and 7.6 cm of the band centerline respectively.

In the field this sampling protocol would be repeated four times for a total of twenty soil cores. Although a zonal soil volume surrounding the manure injection band will contain elevated nitrate levels, sampling positions are presented here as a distance measure from the centerline of the manure injection band zone in the center of this diagram. The ratio of soil cores collected outside of 7.6 cm (outside of yellow, pink, and blue sampling core distances 1, 2, and 3 in Figure 3) to those inside of that distance is 24:6, or 4:1, which matches the ratio suggested by Mahler (1990) for mobile nutrients. Here, the ratio of cores collected outside 5 cm (outside of yellow and pink sampling core distances 1 and 2) to those inside that distance is 26:4, or 6.5:1. This ratio is slightly higher than that recommended by Poffenbarger et al. (2015), who suggested the ratio range between 4:1 to 6:1 (24:6 to 25.7:4.3) within 5 cm of the injection point with 30 cm deep cores, and much lower than that of Kitchen et al. (1990) and Tewolde et al. (2013), who suggested ratios that specifically included the band center (injection point) of 20:1 and 18:1, respectively, with 15 cm deep cores.

Our methods did not test injection band spacing less than 76 cm and applying the protocol here to narrower spacing should be approached cautiously. Our protocol could be associated with dividing band spacing by five to obtain sample collection spacing, but when spacing is narrower the ratio of samples outside of the area of band influence to the area inside band influence will drop below 4:1. It is problematic to compare our protocol to formulas presented in earlier work that purposefully sought to collect a sample that included the manure band and were based on field work that considered only soluble P fertilizer (Kitchen et al., 1990), or considered a suite of nutrients including N and P (Tewolde et al., 2013) in sampling protocol recommendation development.

CONCLUSIONS

Manure injection conserves N in comparison to broadcast application but banded placement presents soil sampling challenges. In our study, monolith soil sampling was used to conduct analysis of NO_3^--N levels in a perpendicular direction to travel of manure injection equipment demonstrated that five 30 cm deep samples equispaced 15 cm apart in positions perpendicular to the manure band provided a reliable and repeatable sampling method to estimate the mean NO_3^--N concentration in the soil. Four sets of samples taken in this manner (20 soil cores in total), when composited, provided confidence of soil nitrate prediction. Marking of manure bands was not necessary with this protocol as at least one of five cores will be taken within 7.6 cm of the centerline of the manure band. Testing can be performed at random locations in the field. Sampling more than four locations is recommended to increase confidence in results and if soil characteristics vary within the field. Adoption of this practical equispaced PSNT soil sampling protocol provides an excellent tool to support agronomic, economic, and environmental optimization of manure nitrogen. Some manure injection implements can be used with minimal soil surface disturbance that is acceptable within no-till guidelines. In the mid-Atlantic region manure injection is expected to become more common as economics and regulations drive increased nutrient conservation, and as producers utilize injection to decrease potential odor conflict associated with manure application. Further work is needed to determine accuracy of this soil sampling protocol for prediction of sidedress nitrogen needs in corn and response to those fertilizer predictions, and when manure is injected in narrower bands. Additionally, research for utilization of this protocol for testing of phosphorus levels in soils with banded manure or fertilizers is needed.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interests.

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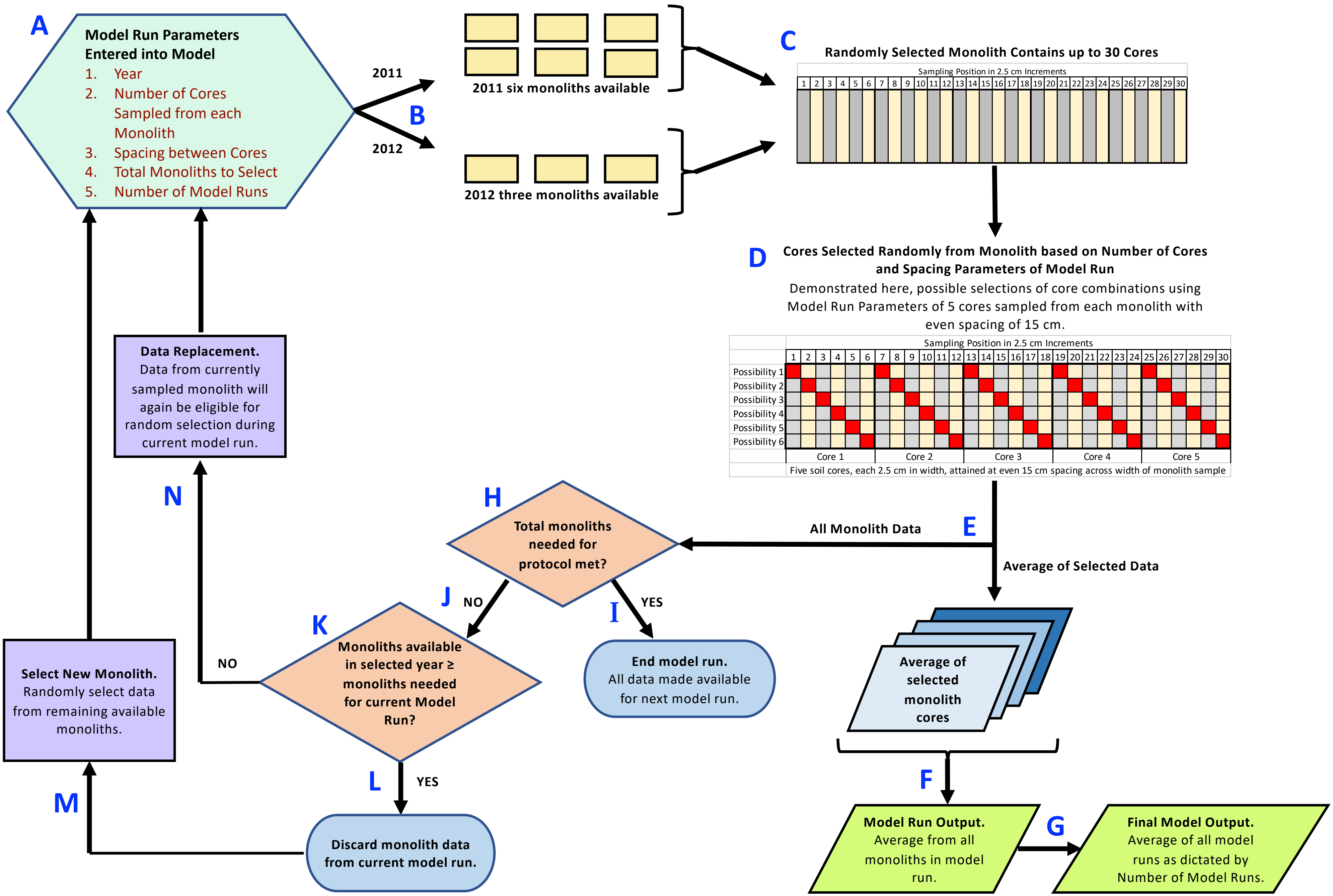
A
Model Run Parameters Entered into Model
1. Year
2. Number of Cores Sampled from each Monolith
3. Spacing between Cores
4. Total Monoliths to Select
5. Number of Model Runs
B
2011
2012
2011 six monoliths available
2012 three monoliths available
C
Randomly Selected Monolith Contains up to 30 Cores
Sampling Position in 2.5 cm Increments
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
D
Cores Selected Randomly from Monolith based on Number of Cores and Spacing Parameters of Model Run
Demonstrated here, possible selections of core combinations using Model Run Parameters of 5 cores sampled from each monolith with even spacing of 15 cm.
Sampling Position in 2.5 cm Increments
Possibility 1
Possibility 2
Possibility 3
Possibility 4
Possibility 5
Possibility 6
Core 1
Core 2
Core 3
Core 4
Core 5
Five soil cores, each 2.5 cm in width, attained at even 15 cm spacing across width of monolith sample
E
All Monolith Data
Average of Selected Data
Average of selected monolith cores
F
Model Run Output. Average from all monoliths in model run.
G
Final Model Output. Average of all model runs as dictated by Number of Model Runs.
H
Total monoliths needed for protocol met?
I
YES
End model run. All data made available for next model run.
J
NO
K
Monoliths available in selected year ≥ monoliths needed for current Model Run?
L
YES
Discard monolith data from current model run.
M
Select New Monolith. Randomly select data from remaining available monoliths.
N
NO
Data Replacement. Data from currently sampled monolith will again be eligible for random selection during current model run.

FIGURE CAPTION:
Flow diagram of model process.

Model input (**A**): The following parameters were assigned to model runs.

1. *Year.* The model was run for only one year at a time because of discrepancy between years in data values and responsiveness. Six monoliths were collected in 2011 and three in 2012 (**B**), which becomes important when determining if data replacement is necessary in the model run.
2. *Number of Cores Sampled from each Monolith.* Each monolith contained up to 30 soil cores (**C**), each 2.5 cm square and 30 cm deep. Cores were assigned sequential spatial numbering 1 through 30 as depicted in the diagram (**C**). This parameter assigned the number of cores out of the 30 to sample. From the assigned year, the model randomly selected a single monolith data set of 30 cores.
3. *Spacing between Cores.* The number of cores sampled from each monolith were selected at evenly assigned spacing. An example is shown (**D**) to demonstrate the possible selection scenarios when the *Number of Cores Sampled from each Monolith* was set at 5 with *Spacing between Cores* set at 15 cm (equivalent of 6 core positions). In this example (**D**), there were six possible randomly selected scenarios of core monolith combinations that satisfied designated input criteria. One of these six possible combinations was randomly selected to provide an average nitrate value for that monolith group. In some exploratory steps the model was told to select the number of cores from random locations in the monolith, in which case even spacing was not dictated.
4. *Total Monoliths to Select.* Because a practical soil sampling protocol recommendation was desired for field work, the maximum number of soil cores was held to 20 or less. This influenced the total number of monolith data sets that needed to be selected to complete a model run. In the example (**D**) the *Number of Cores Sampled from each Monolith* was 5, which dictated that data from 4 monoliths could be used to attain 20 total cores to represent those that a field worker might collect.
5. *Number of Model Runs.* The model was run multiple times with the average of each run collected until the total *Number of Model Runs* was complete, then the output of all runs was averaged to provide a final nitrate value.

The model first randomly selected a monolith from the assigned *Year*, secondly the model randomly selected the correct number of soil cores at the specific spacing, and then averaged the nitrate values from the selected cores from that monolith (**E**). The average from that monolith was held while the model selected data from additional monoliths until the *Total of Monoliths to Select* parameter was satisfied, at which time the averages from individual monolith data sets (**E**) were averaged together to provide the final average nitrate value for the model run (**F**). Individual model run output was held until the total *Number of Model Runs* was completed, and the nitrate values for all model runs were averaged together to provide Final Model Output (**G**) nitrate concentration. Model runs were repeated up to 500,000 times.

After the average for each individual monolith was calculated (**E**) the model had to determine if the *Total Monoliths to Select* criteria were satisfied (**H**). If the *Total Monoliths to Select* criteria was satisfied, then the model run was ended (**I**) and all data was made eligible for the next model run. If the *Total Monoliths to Select* criteria were not satisfied (**J**), then the model needed to determine if there were enough monoliths available in the selected *Year* to satisfy the *Total Monoliths to Select* criteria (**K**). If the total monoliths available in the specified *Year* was greater or equal to the *Total Monoliths to Select*, then all data from the monolith in the model run was discarded (**L**) and the model randomly chose the next monolith from those that remained in the data pool (**M**). If the total monoliths available in the selected *Year* was less than the *Total Monoliths to Select*, then it was necessary to move all data from the current monolith back into the data pool for random selection, a process termed Data Replacement (**N**). This was sometimes necessary to satisfy the desired total number of cores to be selected as dictated by Model Run Parameters. In the example inserted into the diagram (**D**) a total of 4 monolith sets were needed to satisfy the Model Run Parameters. In this example scenario (**D**) Data Replacement was not necessary for 2011 because the 6 available monoliths were greater than the 4 monoliths needed to complete the model run(**B**), however Data Replacement was necessary in 2012 since the 3 available monoliths were less than the 4 monoliths needed to complete the model run (**B**).