DIVISION S-3—SOIL MICROBIOLOGY AND BIOCHEMISTRY

Influence of Sample Size on Measurement of Soil Denitrification[1]

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ABSTRACT

The influence of sample size on the magnitude and variability of soil denitrification was studied by collecting soil cores, ranging in size from 1.7 to 5.4 cm in diameter, from no-till and conventional-till corn plots. Estimates of natural denitrification rates were obtained by incubating intact soil cores with C_2H_2 and monitoring gaseous N_2O production. In addition, maximum denitrification potential was determined by monitoring N_2O production in anaerobic slurries amended with glucose, NO_3^- and C_2H_2. Natural rate estimates were highly skewed and approximated lognormal distributions. The spatial variability of denitrification was characterized by large variation at small distances of <10 cm and only weak spatial dependence at distances of 10 to 100 cm. Studies of the effect of sample size on denitrification suggest that soil cores >4.2 cm in diameter yielded the most reliable estimates of natural denitrification rates. Using a computerized random resampling technique, we estimated that approximately 10 to 15 kg of soil was necessary to obtain a representative soil mass for estimating natural denitrification rates. The results of this study are consistent with the hypothesis that the source of variability associated with the natural denitrification rates is the patchy distribution of denitrifying "hot spots" in soil. Some implications associated with the application of classical statistical methods to lognormal data are also discussed.

Additional Index Words: **lognormal, spatial variability, geostatistics, nitrous oxide.**

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GASEOUS N LOSS from denitrification is one of the least well quantified of the soil N cycle processes. A better understanding of soil and environmental controls as well as better quantitative estimates of denitrification are required before

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management practices can be developed to moderate the effect of this process on agricultural systems. Difficulties associated with quantification of denitrification can be attributed to the lack of methodology to both measure the process and to deal with the spatial and temporal variability.

Recently, a variety of techniques have been developed in an attempt to estimate natural rates of soil denitrification. These include measurements of: (i) ^{15}N-gas flux rates from ^{15}N-fertilized soils (Rolston et al., 1982; Siegel et al. 1982) (ii) N_2O flux rates from the soil surface in C_2H_2-amended soil chambers (Ryden and Dawson, 1982; Burton and Beauchamp, 1984; McConnaughey and Duxbury, 1986), and (iii) N_2O production rates from C_2H_2-amended intact soil cores (Rice and Smith, 1982; Aulakh et al., 1984; Parkin et al., 1985). Regardless of the relative merits and limitations of these approaches, a problem inherent to all attempts to quantify natural rates of denitrification has been the high spatial variability associated with this process. This high variability has been acknowledged, yet few studies have focused on this problem (Folorunso and Rolston, 1984; Folorunso and Rolston, 1985).

Recent research on the variability of other soil properties and the application of novel statistical methods has aided in the characterization of this variability. The purpose of this study was to investigate the influence of sample size on estimates of natural denitrification rates and on the variability associated with these rate estimates. The statistical nature of these analyses also requires a discussion and critical evaluation of the commonly used statistical methods for hypothesis testing.

MATERIALS AND METHODS

Field Site and Sampling

Samples were collected from a field site located on the University of Maryland Plant Research Farm, Beltsville, MD. The site had long-term (12 yr) plots of no-till and conventional-till continuous corn. The soil at this site was a Beltsville silt loam (Typic Fragiudult) having a pH of 6.5 and total organic N (Kjeldahl) and C (persulfate digestion) contents of 0.8 g/kg and 5.1 g/kg, respectively. All plots received 168 Kg N/ha of NH_4NO_3 fertilizer in April, 1984. The conventional-till plots were plowed at the end of May 1984 and all plots were planted to corn in early June 1984.

An experiment consisted of collecting soil samples from 36 blocks (20 by 30 cm experimental units utilizing two block spacing patterns; Table 1). In the two spring experiments (prior to planting) the blocks were spaced adjacent to one another in a 6 block by 6 block grid which covered a 1.2 m by 1.8 m area. In the two summer sampling experiments the blocks were spaced between corn rows at 76-cm intervals.

Five different sized soil cores were collected at random from within each block for each experiment (Table 2). Cores were obtained by pounding steel tubes, fit with hardened cutting bits, 16 cm into the ground. The intact soil cores contained within the steel tubes were then transferred into plastic tubes which were then stoppered. The intact soil cores fit loosely in the plastic tubes to facilitate gas diffusion into and out of the soil. After all five soil cores were removed from the block, the remaining soil was removed using rectangular steel templates (20 by 30 cm), which were also driven 16 cm into the soil. The soil from the large blocks was not maintained in an intact state but sieved (0.5 cm mesh) and stored at 4°C overnight. This sampling protocol produced a total of 216 soil samples (36 of each of the 5 different size soil cores plus the 36 blocks of soil) for each experiment.

Table 1. Description of sampling dates, treatments, and experimental unit spacing (block spacing) for the sample size experiment.

Experiment no.	Date	Tillage treatment	Block spacing, cm
1	1 May 1984	Conventional-fallow	0
2	14 May 1984	No till-fallow	0
3	19 June 1984	Conventional-corn	76
4	24 July 1984	No till-corn	76

Natural Denitrification Rate Measurements

Natural denitrification rate estimates of the intact soil cores were begun immediately upon returning to the laboratory using an C_2H_2 block technique. The gas pressure in each core was first brought to atmospheric levels by venting with a needle. Then an appropriate volume of C_2H_2 was added to each sized core to achieve a final C_2H_2 partial pressure of approximately 10 kPa. All C_2H_2 was generated by reacting CaC_2 with distilled water immediately prior to use. The pressure increase from injecting the known volumes of C_2H_2 was recorded for each core using a pressure transducer. The pressure values were used to calculate the total gas-filled volume in each of the samples as described by Parkin et al. (1984).

Gas in the soil cores was then mixed to distribute C_2H_2 throughout the core by alternately drawing and releasing a vacuum on the samples using a 60-mL syringe. This mixing procedure, in addition to the loose fit of the intact soil cores in the tubes, facilitated C_2H_2 distribution into, and N_2O distribution out of, the soil pores. Following the gas mixing procedure, the gas overpressure in the soil cores resulting from the initial C_2H_2 injection was vented.

Cores were incubated at 24 to 26°C and gas samples withdrawn 3, 6, and 18 h following the C_2H_2 additions. Gas samples were obtained by adding 5 mL of air to each core, mixing the gas in the cores using the above procedure, then removing a 5-mL gas sample. The 5-mL gas samples were stored in 3-mL evacuated vials for later N_2O analyses.

Contents of the vials were analyzed for N_2O using an electron capture detector–gas chromatograph equipped with an automatic gas sampler (Parkin, 1985). Denitrification rates were then calculated from the rates of N_2O production after correcting for the dilution resulting from the 5-mL air additions during sampling and correcting for the N_2O dissolved in the soil water (Tiedje, 1982). The water content of the samples was determined gravimetrically by drying overnight at 105°C. Rates are expressed per g oven dry soil.

Denitrification Enzyme Activity

Denitrification enzyme activity (Smith and Tiedje, 1979; Tiedje, 1982) was determined immediately following the last gas sampling of the intact core incubations. The soil samples

Table 2. Description of sample sizes and approximate mass of fresh soil collected from each experimental unit (block).

Sample size†	Diameter	Volume	Soil mass‡
	cm	cm^3	kg
1	1.73	38	2.0
2	2.15	58	3.3
3	3.45	149	8.9
4	4.15	216	12.3
5	5.40	366	20.7
6	20 by 30	8770	570.0

† Sample size 1–5 were soil cores and size 6 was a rectangular block.
‡ Approximate soil mass (wet weight) of 36 of each of the different sample sizes collected in each experiment.

were sieved, mixed, and a 25-g subsample placed in a 125-mL Erlenmeyer flask containing 25 mL of a glucose–nitrate–chloramphenicol solution (1 m*M* glucose, 1 m*M* KNO_3, 1 g/L chloramphenicol). In addition to the soil from the intact cores, soil from the large blocks was also incubated. The soil slurries were made anaerobic by alternately flushing the Ar and evacuating the flasks four times. Five-milliliter gas samples were withdrawn 0.5, 1, 1.5, and 2 h following the addition of 20 mL of C_2H_2 to the flasks. Gas samples were stored in evacuated vials and analyzed for N_2O as described above. Replicate soil samples from some of the large blocks of soil (size 6) were analyzed to determine the analytical variability associated with these anaerobic soil slurry incubations.

Surface Area to Volume effects

A study was also made of the influences of surface area to volume ratio (sa/v) of soil cores on measured denitrification rates. Denitrification rates were measured on a 4.15 cm diam soil core over a 24-h incubation in a manner similar to that described above. The surface area which was exposed to the incubation atmosphere was 222 cm^2 (area of side + area of the top) and the average volume was 216 cm^3 (sa/v = 1.03). After the initial denitrification rate determinations these intact cores were subcored down the center using a small sampler having a diameter of 2.0 cm. This subcoring procedure resulted in a small soil core and an annular cylinder of soil. Each of these fractions had an exposed sa/v of 2.06. The denitrification rates of the annular soil cylinders and the subcores were then determined in a subsequent 24-h incubation. A composite denitrification rate was calculated by summing the N_2O production rates of the annular cylinder and the small core fractions and dividing by the total mass of soil present in the two fractions. This composite rate was then compared to the original rate of the large intact soil core. This procedure allowed the comparison of denitrification rates of the same soil mass but incubated under differing sa/v ratios (1.03 vs. 2.06).

Statistical Considerations

Natural denitrification rate estimates measured with the intact soil cores as well as the denitrification enzyme activity rates were skewed and approximated lognormal distributions. Non-normal data are typically analyzed by transforming the data to obtain normality and then performing the appropriate classical statistical tests. Unfortunately, this approach is often performed mechanically with little thought given to the implications and consequences of analyzing lognormal data on a transformed scale. The following discussion is given to examine and clarify these implications.

Testing Location

For a lognormally distributed variable the mean does not equal the median; therefore, one must choose which location parameter to test. Medians can be tested by performing a classical parametric test on the means of the log-transformed data. Nonparametric methods such as the Wilcoxen test have been used to test differences in location for nonnormal data. However, nonparametric tests of location are based on ranking of the untransformed data, hence, these methods also test for differences in medians and not means.

A test of means on the untransformed scale requires the joint testing of both the mean and variance of the log-transformed data, a situation for which theory does not exist (Aitchinson and Brown, 1957). Overlap of confidence intervals calculated about the means of two samples may offer a method of comparing means on the untransformed scale. The limitation of this technique is that the exact significance level is unknown.

The appropriateness of testing on the transformed or untransformed scale depends on the objective of the study. The transformed scale would be adequate if one is interested in the relative comparisons among treatments or if the median is the target location parameter of choice. If one is interested in a quantitative comparison of the variable on the untransformed scale, however, then the mean should be the location parameter of choice.

Testing Scale

A primary objective of this study was to determine the influence of sample size on variability. This requires that the spread or scale of the data for each size sample be evaluated and compared. Unfortunately, there are no suitable methods for determining differences in scale for a lognormally distributed variable. Due to the relationship between the mean and variance of a lognormally distributed variable, a test of variances of log-transformed data indicates little about the variances on the untransformed scale. Classical tests of variance are not sensitive to small differences in the variance, however, small differences in variances, undetected on the log-transformed scale, may be amplified to yield large differences in variances on the untransformed scale.

Due to the problems and limitations which currently exist in the analysis of lognormally distributed variables, several different statistical techniques were applied to the data (both transformed and log-transformed) of this study.

Data Analyses

Parameter estimation

The mean, variance, and coefficient of variation (CV) were calculated by the minimum variance estimation techniques of Finney (1941), and confidence intervals about the mean were calculated by the procedure of Sichel (1966).

Comparisons of denitrification rates and denitrification enzyme activities of the different core sizes were carried out using log-transformed data and untransformed data. Analyses of log-transformed data utilized standard analysis of variance (ANOVA) for a randomized complete block with 36 replicates (Steel and Torrie, 1980) and Tukey's LSD procedure to compare means. Bartlett's test and individual *F* tests were performed to compare variances on the log-transformed and untransformed scale. Untransformed data was evaluated using nonparametric procedures (Daniel, 1978). Differences in location (medians) were evaluated using the Wilcoxen matched pairs signed rank test. Results of this test were identical to those obtained from Tukey's test on log-transformed data. The Kolmogorov-Smirnov goodness-of-fit fest was used to compare distributions of the untransformed data. An additional test of location for the untransformed means was performed by comparing overlap of upper and lower 95% confidence limits.

Analyses of spatial variability were done using geostatistical methods (Journel and Huijbregts, 1978). Average two-dimensional semivariograms for the log-transformed denitrification data were constructed using the computer programs provided by Vieira et al. (1983). For these analyses, data from core sizes 2 and 3 were pooled and data from sizes 4 and 5 were pooled to provide better spatial information at the smaller sampling distances.

Representative soil masses required to provide reliable estimates of denitrification were obtained using a computerized random resampling technique. This technique was developed on the basis of the computer intensive statistical techniques of Efron (1979, 1982). Computer intensive statistics are nonparametric, in that, no assumptions are made concerning the underlying population distribution (Diaconis and Efron, 1983). The only assumption inherent in this approach is that the sample must be representative of the pop-

ulation, an assumption which is inherent in any statistical method. For this analysis, denitrification rate data from all the cores (sizes 1–5) of a given sampling experiment were pooled. Random subsets from these pooled samples were drawn and weighted average denitrification rates were calculated for these subsets of data by dividing the total amount of N denitrified in the subset by the total amount of soil represented in the subset. This procedure was repeated 10 000 times, and estimates of the representative soil mass for denitrification rates were determined from plots of the weighted average denitrification rates vs. mass of soil. These values were compared to the total weighted average denitrification rate of each experiment [total N denitrified/total amount of soil in the 180 soil cores (ca. 50 kg)], which was taken to be the population mean.

RESULTS AND DISCUSSION

Denitrification Enzyme Activity

Sample histograms of denitrifing enzyme activity were slightly skewed to the right (Fig. 1). Generally, histograms of the small core sizes (sizes 1 and 2) exhibited a larger range, with a higher percentage of samples occurring in the upper tail region (Fig. 1A-H). With increasing sample size the frequency of samples in the upper tail decreased and the range decreased such that histograms of the largest size (size 6) exhibited little spread and were approximately symmetric (Fig. 1U-X).

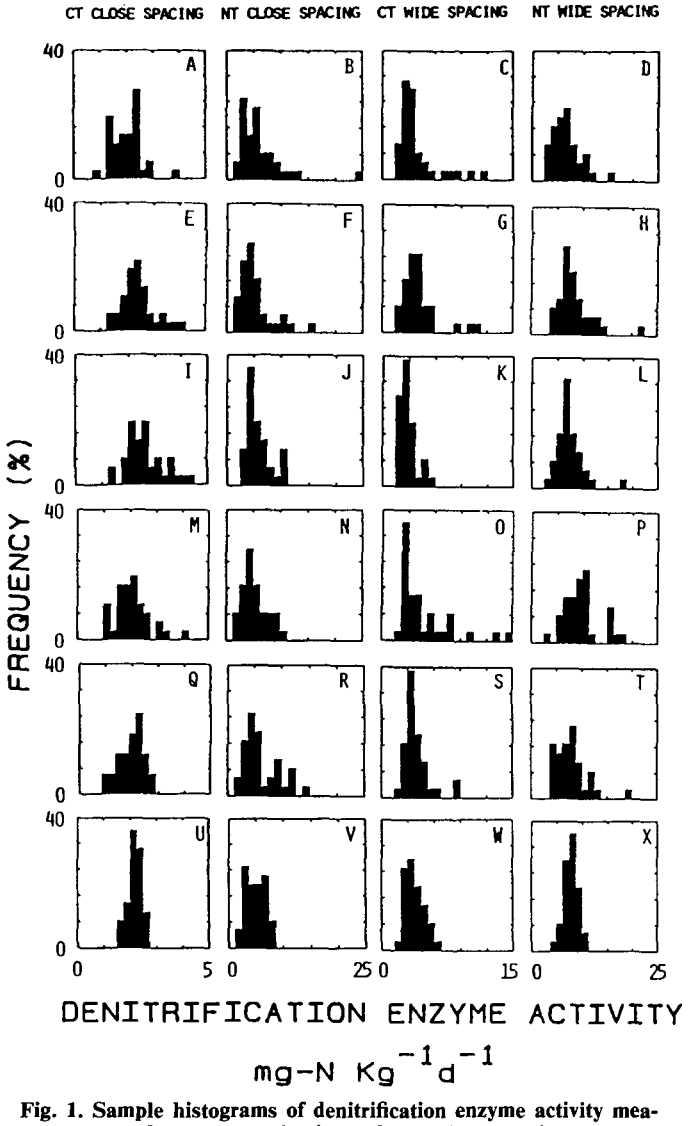

Fig. 1. Sample histograms of denitrification enzyme activity measurements for each sample size and sampling experiment. Rates are in units of μg N kg^{-1} d^{-1}.

Comparisons of denitrification enzyme activity as a function of sample size are presented in Table 3. The parameters estimated for the large soil blocks (size 6) were taken to be the best estimates of the population parameters because the mass of soil collected in the large soil blocks represented approximately 92% of the total mass of soil collected in each experiment. Comparisons of location parameters (either means or medians) of the soil cores (sizes 1–5) with the values obtained for size 6 yielded similar trends for each experiment, namely: there was a general lack of significance between rates from the core samples and the rate obtained for the size 6 samples although occasionally differences were observed among core samples. Also, the largest cores (size 5) were never significantly different from size 6.

Variability of the denitrification enzyme activity measurements, as indicated by the standard deviation estimates (Table 3), showed patterns consistent with sample histograms, i.e., small core sizes tended to have higher standard deviations than the large sample sizes. The variability of the denitrification enzyme activity assay was estimated in a separate experiment and was found to be low, with CVs in the range of 4 to 15%. This low assay variability can be attributed to the variability associated with sieving, mixing and subsam-

Table 3. Summary statistics for denitrification enzyme activity measurements of four sampling experiments. Rates are in units of μg N kg^{-1} d^{-1}.

Experiment	Sample size	Median rate*	Mean rate**	Standard deviation†	% CV	Lower 5% CL	Upper 5% CL
Conventional till close spacing	1	1 980a	2 060a	620a	30.1	1 900	2 300
	2	2 360ab	2 440ab	654a	26.8	2 270	2 690
	3	2 540b	2 640b	758a	28.7	2 440	2 930
	4	1 980a	2 070a	637a	30.8	1 910	2 320
	5	1 960ab	1 990a	373b	18.7	1 840	2 240
	6	2 140ab	2 160a	300b	13.8	2 080	2 150
No till close spacing	1	5 530a	6 390a	3 700a	57.9	5 410	8 100
	2	4 820a	5 580a	3 260ab	58.4	4 720	7 090
	3	5 602a	6 010a	2 340bc	38.9	5 370	7 030
	4	4 790a	5 260a	2 410bc	45.8	4 610	6 340
	5	4 890a	5 340a	2 380bc	44.6	4 700	6 400
	6	4 760a	5 040a	1 780c	35.3	4 550	5 810
Conventional till wide spacing	1	3 520a	3 930a	1 950a	49.6	3 440	4 710
	2	3 730a	4 050a	1 740ab	43.0	3 610	4 740
	3	2 650b	2 810b	990c	35.2	2 550	3 190
	4	4 050a	4 620a	2 530a	54.7	4 000	5 650
	5	3 730a	3 910a	1 220bc	31.2	3 590	4 370
	6	3 610a	3 730a	970c	26.0	3 480	4 090
No till wide spacing	1	6 340a	6 790a	2 612a	38.5	6 110	7 820
	2	8 090bc	8 550abc	2 940a	34.4	7 790	9 700
	3	7 180ac	7 550a	2 490a	33.0	6 910	8 530
	4	9 240b	9 850c	3 640a	37.0	8 910	11 300
	5	7 440ac	7 880abc	2 800a	35.5	7 162	8 990
	6	7 700ac	7 860ab	1 600b	20.4	7 440	8 460

* Medians followed by the same letter for individual experiments are not significantly different ($P < 0.05$) as determined by Tukey's LSD test on log transformed values.

** Means followed by the same letter for individual experiments are not significantly different as determined by overlap of upper and lower 5% confidence limits.

† F test on log transformed values, values followed by the same letters are not significantly different ($P < 0.05$).

pling, as well as variability associated with the incubation procedure (e.g., injection error). Thus, variability of the assay procedure accounts for approximately one-half of the variability observed in the field using the largest sample size.

Spatial structure of the denitrification enzyme activity was negligible (Fig. 2). For the close spacing experiments the spatial variability of the core samples exhibited a weak spatial component as evidenced by the slight increase in semivariance with increasing sampling distances (Fig. 2A, B). Variability of the large block size showed no spatial dependence (flat response of semivariance with distance). This general lack of spatial dependence was observed for the wide spacing experiments for all sample sizes (Fig. 2C, 2D). These results indicate that there is a random "patchy" dispersion of active denitrifying enzymes which accounts for most of the observed variability within the soil cores. However, dispersion of active enzymes between blocks is more homogeneous.

Natural Denitrification Rate Estimates

Natural denitrification rate estimates (intact soil cores), exhibited highly skewed distributions (Fig. 3), and a distinct relationship between histogram shape and sample size was observed. The small cores (Fig. 3A-H) generally contained a greater frequency of very low denitrification rates along with an occasional very high rate. Rates of the larger cores were more evenly distributed with a lower frequency of samples yielding low rates (Fig. 3M-T).

Tests of location parameters, as well as the goodness-of-fit test of distributions, suggest that sample size had a significant influence on the magnitude of the measured denitrification rate estimates (Table 4). There was a weak but consistent pattern of increasing mean and median denitrification rate with increasing sample size. In all experiments the mean denitrification rates of the smallest cores (size 1) were significantly lower than rates of the largest cores (size 5). Also, mean denitrification rates of the two largest core sizes (sizes 4 & 5) were never significantly different from one another.

Two possible explanations for the differences in mean rates of the small and large cores are: (i) the differences could be an incubation artifact due to sa/v relationships resulting in an increased aeration state in the small core sizes, or (ii) the differences could be a real sampling effect and actually reflect the efficacy of the small core sizes in obtaining a representative sample.

Surface Area/Volume Effects

To determine the influence of increased sa/v on the magnitude of measured denitrification rate, measurements were done on the same soil mass but at two different sa/v (Table 5). In nearly all of the 12 individual samples the final composite rate (soil incubated with a sa/v of 2.06) was the same as the initial rate of the sample (sa/v = 1.03). This result indicates that increased sa/v had no significant influence on the denitrification rates. These results also illustrate the high

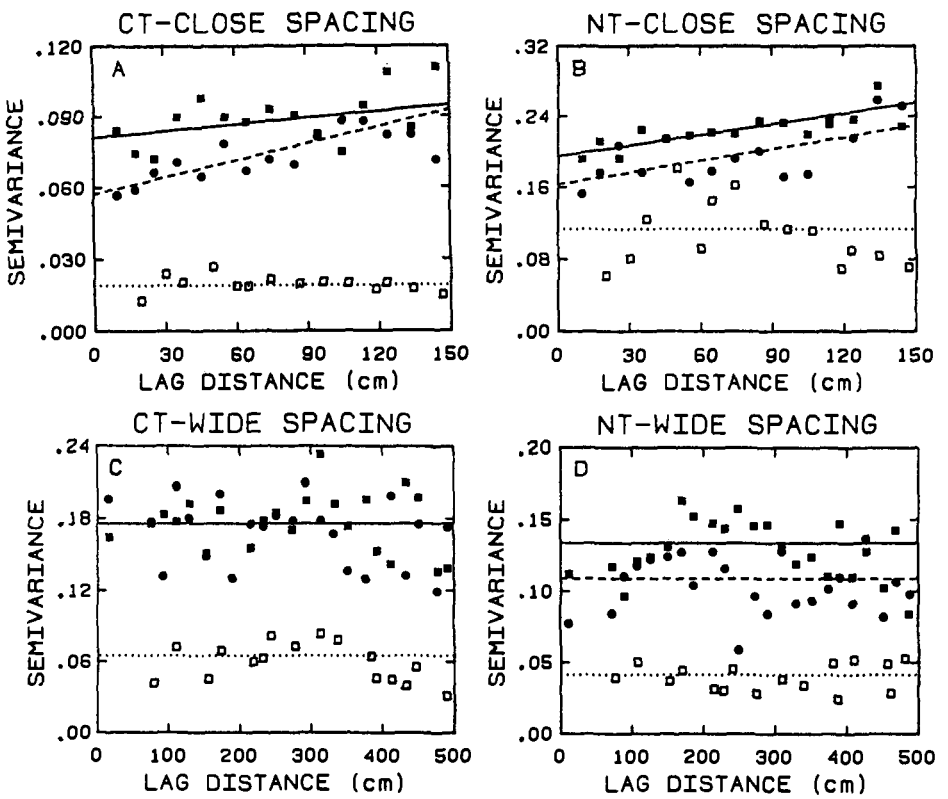

Fig. 2. Semivariograms of denitrification enzyme activity. Symbols indicate calculated semivariances (sizes 2-3, ●; sizes 4-5, ■; size 6, □) and lines indicate models fit to each semivariogram (size 2-3 dashed, size 4-5 solid, size 6 dotted). The CT-wide spacing experiment semivariograms of sizes 2-3 and 4-5 were fit with same linear model (solid line).

degree of spatial heterogeneity which exists within a single soil sample. For example, sample no. 4 (Table 5), had an initial rate of 9.6 μg N kg^{-1} d^{-1}, but when subsampled the specific denitrification rate associated with the small core was 10 times higher than the annular cylinder rate. Yet, the resulting composite rate (small core + annular cylinder) was the same as the initial rate of the sample. This spatial heterogeneity was observed in other samples and suggests that the denitrification rate of a given sample is a composite of the denitrification rates occurring in discrete microsites within the sample.

Sampling Efficacy

Estimates of the soil mass required for reliable estimation of the mean denitrification rate were calculated using a random resampling technique (Fig. 4). Each point on the graph represents a denitrification rate calculated by randomly combining actual rates from the core samples. Thus, for the conventional till, close spacing experiment (Fig. 4A) only 29.4% of the denitrification rates calculated from soil samples in the 0 to 2 kg range fall within ±50% of the population mean. Similar patterns were observed for all the sampling experiments (Fig. 4A, B, C, D) despite the fact that mean denitrification rates varied over a 100-fold range for the different experiments. Estimates of the representative soil mass from Fig. 4 indicate that the small core sizes did not provide a representative sample because 36 of the small cores (ca. 2 kg total soil) yield a mean denitrification rate estimate within ±50% of the population mean only 15 to 37% of the time. However, Fig. 4 predicts that 36 of the large size cores (ca. 20 kg of soil) should yield a mean denitrification rate within ±50% of the population mean, 79 to 98% of the time.

The sa/v experiments and the minimum soil mass estimates indicate that the low mean denitrification

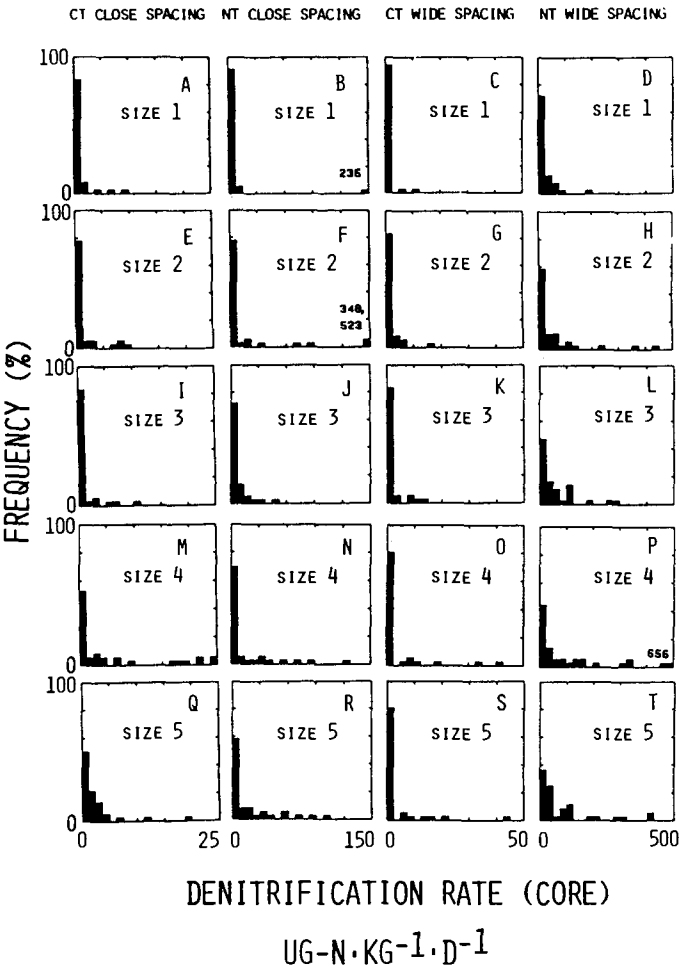

Fig. 3. Sample histograms of natural denitrification rate estimates as measured with intact soil cores. Rates are in units of μ N kg^{-1} d^{-1}. Histograms for each of the core sizes of each sampling experiment are presented.

Table 4. Summary statistics of natural denitrification rate estimates (soil core) of four sampling experiments. Denitrification rates in units of μg N kg^{-1} d^{-1}.

Experiment	Sample size	Median rate*	Mean rate**	Std. Dev. (untrans)	Std. Dev. (ln-trans)	% CV	Lower 5% CL	Upper 5% CL
Conventional till close spacing	1a	0.23a	0.61a	1.27	1.44	212	0.38	1.38
	2a	0.34a	1.22ab	3.14	1.64	257	0.69	3.41
	3a	0.26a	1.03ab	2.79	1.70	270	0.57	3.08
	4b	1.59b	6.59c	18.5	1.74	281	3.57	20.8
	5b	0.95b	2.44bc	4.95	1.41	203	1.53	5.38
No till close spacing	1a	0.82a	3.14a	8.41	1.69	267	2.13	5.49
	2ab	1.57ab	24.80b	95.1	2.47	383	16.7	66.8
	3ab	1.57ab	12.80b	45.9	2.14	359	8.44	28.0
	4b	3.10ab	16.60b	52.7	1.92	317	11.1	31.8
	5b	4.05b	26.40b	89.20	2.01	337	17.4	53.9
Conventional till wide spacing	1b	0.57ab	0.96a	1.23	1.04	128	0.70	1.57
	2a	0.31b	0.91a	2.10	1.52	231	0.55	2.26
	3b	0.69a	1.35a	2.09	1.18	155	0.93	2.46
	4bc	0.65a	2.30ab	5.83	1.63	253	1.31	6.33
	5bc	0.42ab	3.90b	14.3	2.21	366	2.57	8.87
No till wide spacing	1a	4.4a	22.3a	69.1	1.87	310	14.9	42.5
	2b	16.5b	84.8b	263.	1.87	310	56.5	161.
	3b	19.1b	75.5b	206.	1.71	273	51.1	133.
	4b	33.8b	131.0b	355.	1.70	271	89.0	230.
	5b	36.5b	91.9b	182.	1.40	198	65.4	142.

* Medians followed by the same letter for individual experiments are not significantly different ($P < 0.05$) as determined by Tukey's LSD test on log transformed values.

** Means followed by the same letter for individual experiments are not significantly different as determined by overlap of upper and lower 5% confidence limits.

† Sizes followed by the same letter in each experiment indicate that the population distributions are not significantly different ($P < 0.05$) as determined by the Kolmogorov-Smirnov goodness-of-fit test.

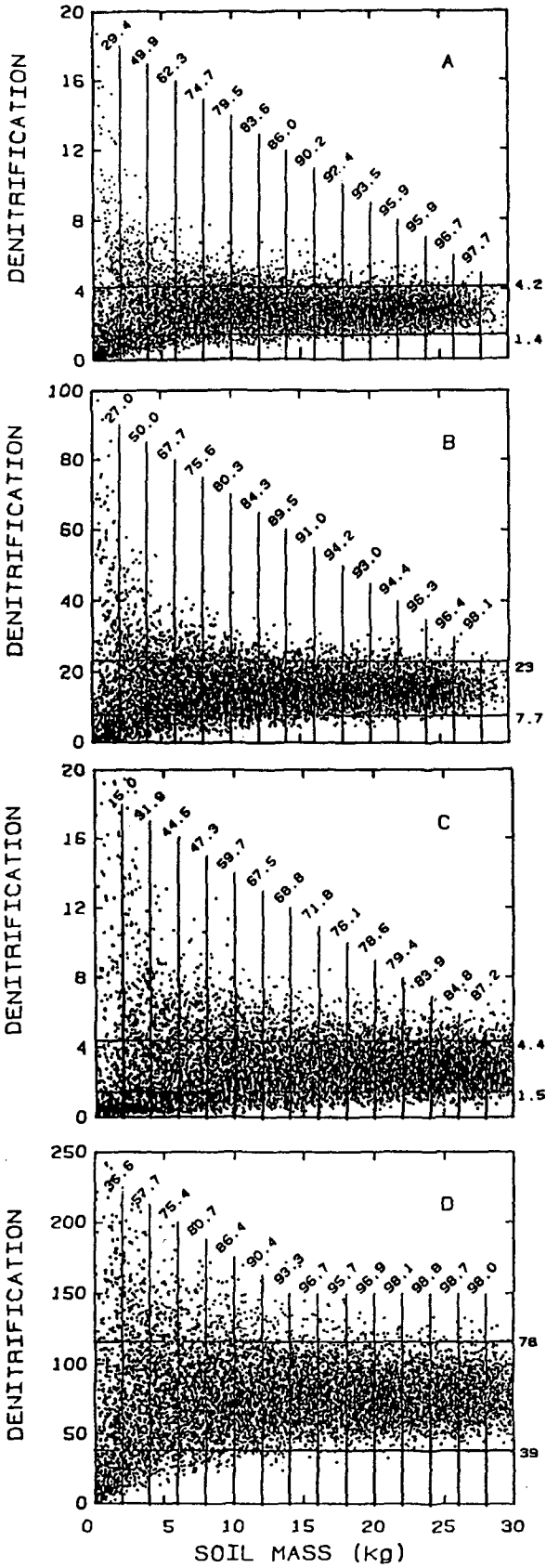

Fig. 4. Representative soil mass estimates for denitrification rates for all sampling experiments. Rates are in units of $\mu g\ N\ kg^{-1}\ d^{-1}$. The numbers at the top of each 2 kg soil mass segment indicate the percentage of samples which yielded a rate within ±50% of the population mean. The ±50% population mean range is indicated at the right of each panel.

rates obtained with the small cores are not due to a laboratory incubation artifact but are due to an undersampling of the heterogenous dispersion of denitrifying microsites in the field. We observed that the sa/v did not have a significant influence on measured denitrification rate. It might also be expected that if a laboratory artifact caused the core size differences then such differences would be systematic. However, rates of individual samples were not systematically lower and denitrification rates of individual samples of the small core sizes were occasionally as high or higher (Fig. 3B,F,H) than maximum rates observed in the larger core sizes. Therefore, the observed differences in rates as a function of sample size are the result of undersampling the patchy distribution of denitrifying microsites in the field which yield low estimates.

Influence of Sample Size on Variability of Natural Denitrification Rates

Due to the highly skewed nature of the denitrification rate estimates it is difficult to determine the precise influences of sample size on variability. Analysis of variance conducted on the log-transformed denitrification rates homogenized the variance in all the sampling experiments except the CT-Wide spacing experiment, suggesting that sample size has little influence on variability (Table 4, col. 5). As discussed previously, a test of variances on the log-transformed scale does not necessarily indicate that variances of the untransformed data are different. Standard deviations of the untransformed data (Table 4, col. 4) indicate that the variability associated with the small soil cores is 3 to 10 times lower than the large cores. This low variability is related to the undersampling problem associated with the small cores discussed above. The mean and variance of a lognormally distributed variable are positively correlated. Thus, if an underestimate of the mean is obtained, the corresponding standard deviation estimate will also be low. The direct relationship between the mean and standard deviation

Table 5. Influence of surface area to volume ration (sa/v) of soil samples on measured denitrification rate.

Sample no.	Denitrification rate			
	Large core	Annular cylinder	Small core	Composite rate†
	$\mu g\ N\ kg^{-1}\ d^{-1}$			
1	0.9	0.3	2.5	0.7
2	32.7	40.8	11.1	34.5
3	6.6	6.6	6.02	6.5
4	9.6	3.3	32.8	9.7
5	3.1	5.6	1.7	4.8
6	2.2	0.4	3.4	1.0
7	0.6	5.3	0.7	4.4
8	12.8	15.6	3.7	13.4
9	1.5	4.1	4.4	4.2
10	2.4	17.7	1.9	14.8
11	2.7	3.6	1.5	3.2
12	1.1	0.0	6.9	1.0
sa/v	1.03	2.06	2.06	2.06
Mean	5.87	10.1	5.89	8.50
Standard dev.	7.85	18.2	6.56	11.5
Lower 95% CL	3.18	4.86	3.69	4.79
Upper 95% CL	17.8	51.7	14.3	26.1

† The composite rate of each sample was calculated from the weighted average of the rates from the annular cylinder and small core corresponding to that sample.

is evident in Table 4, with the smaller core sizes generally having the lowest means as well as the lowest standard deviations.

A spatial component of the denitrification variability was observed for three of the four experiments (Fig. 5A, B. D), and except for the CT wide spacing experiment (Fig. 5C), there was little influence of sample size on the structure of the spatial variability. For the close spacing experiments, spatial dependence was linear up to distances of 150 cm (Fig. 5A, B) and the variability was dominated by a high degree of small scale variability, as indicated by the relatively large y-intercepts. In the wide spacing CT experiment (Fig. 5C), variability was spatially independent, with the variance being lower for the small sample sizes. For the no-till wide spacing experiment (Fig. 5D), the sample semivariograms were characterized by a spherical model. In this experiment, variability increased with increasing distance up to 100 cm. At distances >100 cm variability was spatially independent. The relatively high degree of microscale variability exhibited in all the experiments indicate that a large portion of the variability was occurring at distances of <10 cm.

Source of Denitrification Variability

Our results indicate that the relative variability associated with the denitrification enzyme activity was considerably less than the natural denitrification rate estimates. In the enzyme activity measurements, the variability associated with the environmental and soil factors which control denitrification in the field (i.e., carbon, nitrate, and anaerobiosis) have been removed. Thus, if variability associated with these factors is controlling the variability of the natural denitrification rates, then it is reasonable that the anaerobic incubations (amended with C and N) should exhibit a lower variability.

Semivariograms of denitrification indicate that the majority of the total variability observed occurs at distances <10 cm. Similar results were reported for denitrification by Folorunso and Rolston (1984). The highly skewed frequency distributions of denitrification along with the high microscale variability is similar to the pattern of variability exhibited by gold deposits, which also typically exhibit a lognormal distribution as well as a high degree of microscale variability (Matheron, 1963, Krige, 1981). In gold deposits, two adjoining samples may exhibit very different ore values if one of the samples happens to contain a large gold nugget. An analogous spatial phenomenon might also be occurring with soil denitrification.

Denitrification is widely recognized to be an anaerobic process and that O_2 inhibits both the synthesis and activity of denitrifying enzymes (Firestone, 1982), yet denitrification has been measured in well-drained soils having pore space O_2 contents which approach atmospheric levels (Rolston et al., 1982; Parkin and Tiedje, 1984). The existence of anaerobic microsites in soils (Currie, 1961; Greenwood, 1961) has been proposed as an explanation for the occurrence of denitrification in well-aerated soils. Models incorporating this microsite theory have been developed to describe

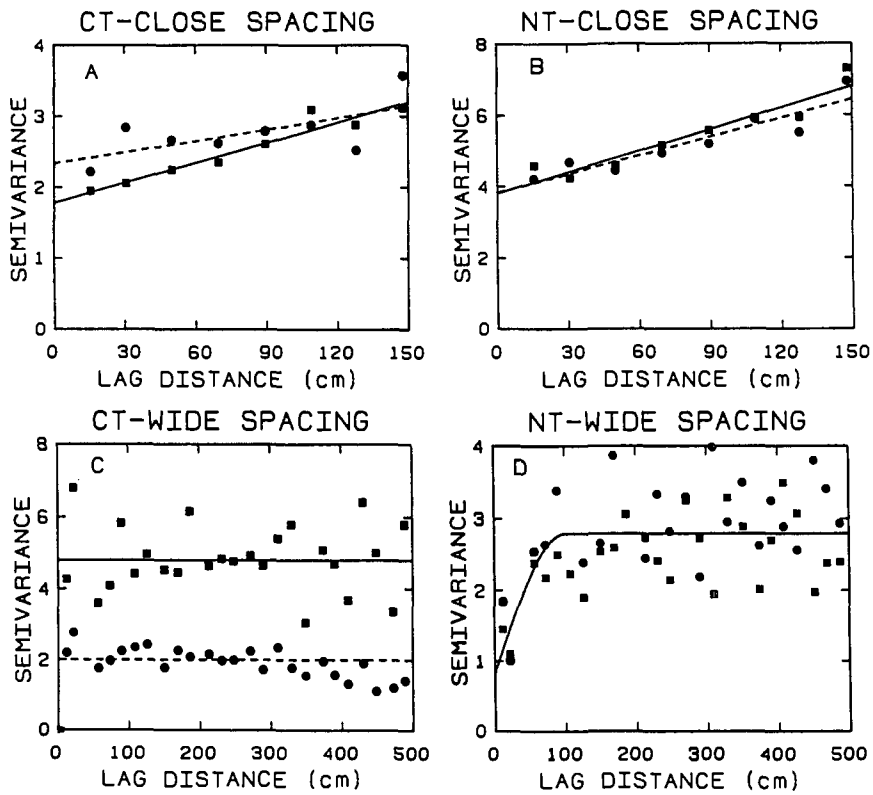

Fig. 5. Semivariograms of natural denitrification rate estimates. Symbols indicate calculated semivariances (sizes 2–3, ●; sizes 4–5, ■) and lines indicate the models fit to each semivariogram (size 2–3, dashed; size 4–5, solid). The NT-wide spacing experiment semivariograms were fit with the same spherical model (solid).

soil denitrification (Smith, 1980; Leffelaar, 1979), and recently, O_2 concentrations and denitrification rates have been directly measured in soil aggregates (Sexstone et al., 1985). From their statistical study of soil aeration status, Fluhler et al. (1976) found that variability of microsites is a significant component of the variability exhibited by soil aeration. The assumption that denitrification occurs in anaerobic microsites in soil is consistent with results observed in a recent study where isolated "hot-spots" of high denitrification activity were identified in soil (Parkin, 1987).

This view of microsite activity of denitrification relates to the observations of our study. If isolated hot-spots of denitrification exist in soil, then with the small core sizes there is a lower probability of including one of these hot spots in the sample and, thus, a greater likelihood that the resulting frequency distribution will contain many low values with an occasional very high value. By using the larger sized cores there is a greater probability of sampling a hot-spot, but the effect of the high activity hot spot on the measured rate of the sample is diluted because of the large mass of low activity soil. Thus, the frequency distributions of the larger sizes contain fewer extreme values and, therefore, result in a more accurate estimate of the true population mean and variance.

Statistical Considerations

The primary objective of this work was to determine the influence of sample size on both the magnitude and the variability of soil denitrification rates. Thus, consideration was given to the selection of the statistical methods used for data analysis. Natural denitrification rates, whether estimated by N-gas flux measurements from soil chamber techniques or by N-gas production measurements from intact soil cores, have been observed to be highly skewed and approximate lognormal distributions (this study; Folorunso and Rolston, 1984; Parkin et al. 1985). Therefore, if a parametric approach is to be taken in the analysis of such data, lognormality is a better assumption than normality when calculating parameter estimates and testing of hypotheses.

In evaluating the influence of sample size on the magnitude of the denitrification rate estimates, two location parameters were tested (the mean and median). Generally, tests of these parameters gave similar results; however, differences were occasionally observed. For the no-till close spacing experiment, the median test indicated that core sizes 1 and 2 were not significantly different (Table 4). The means differed by a factor of 8, however, a significant difference as indicated by the 95% confidence limits. The disparity between the results of the two tests can be explained by the fact that the median is a robust estimator, in that, the median is not markedly influenced by the occasional high values encountered when sampling skewed distributions. The consistency with which occasional extreme values occur suggest that these samples are not outliers or artifacts but are real and make a significant contribution to the magnitude of the denitrification-N loss estimates. Thus, since the mean places equal emphasis on all the samples, it is the relevant location parameter of this study.

In devising sampling strategies to accurately measure denitrification, consideration must be given to the underlying distribution of the population as well as to the size and number of samples to be collected. Our results indicate that a sufficient volume of soil must be collected in order to include a representative number of denitrifying hot-spots. At this experimental site, it was determined that from 10 to 15 kg of soil was needed to provide a reasonable estimate of denitrification.

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