

Comparison of Disturbed and Undisturbed Soil Core Methods to Estimate Nitrogen-Mineralization Rates in Manured Agricultural Soils

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Ion exchange resin / soil cores are a common in situ approach to estimating soil nitrogen (N) mineralization rates. However, no studies compare the two common methods of core preparation (disturbed and undisturbed). The objective of our study was to compare N mineralized and soil temperature in disturbed versus undisturbed cores of manured agricultural soils. Undisturbed cores were prepared by driving aluminum tubes (25 cm long with 10 cm inner diameter) into soil, removing the tubes, and then inserting an ion-exchange resin bag beneath the soil at the bottom of the tube. Disturbed cores were prepared with the same materials, but soil was excavated, mixed, and then filled into tubes fitted with ion-exchange resin bags at the bottom. Soil from six agricultural fields (five of which had more than 10 years of regular dairy manure application) was incubated over four time periods during summer and winter. A total of 13 soil / incubation-period combinations were tested. Disturbed cores tended to have more N mineralized than undisturbed cores ($P < 0.10$), especially in cores prepared with the lowest clay content soil. However, variability of N mineralized was lower in disturbed cores than undisturbed cores for 11 of the 13 soil / incubation periods. This lower variability was significant in two of the four incubation periods ($P < 0.10$). There was little difference in mean soil temperatures in disturbed versus undisturbed cores or within cores versus outside but adjacent to cores. However, in summer, the daily temperature range inside cores was significantly greater than the temperature range in soil outside cores ($P < 0.01$).

Keywords Ion-exchange resins, mineralization, nitrogen, soil core method, testing methodology

Introduction

Ion-exchange resin / soil cores (IER/SCs) are an in situ approach for estimating soil nitrogen (N) mineralization rates. Tubes for IER/SC typically are made of polyvinyl chloride, aluminum, or galvanized pipe that measure 5 cm in inner diameter by 15 to 50 cm long (DiStefano and Gholz 1986; Myrold, Baumeister, and Moore 1992; Kolberg et al. 1997; Sullivan et al. 1999; Eghball 2000). The tubes are open at the top and bottom to allow

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exchange of gases and water; however, the bottom of each tube is fitted with ion-exchange resins that capture nitrate nitrogen ($\text{NO}_3\text{-N}$) and ammonium nitrogen ($\text{NH}_4\text{-N}$) leaching from the soil core. Because the tubes exclude living plant roots, there is no plant uptake of mineral N, which allows a fairly direct estimate of net N mineralized.

Cores can be constructed with relatively undisturbed soil by pounding tubes into the ground or with excavated (disturbed) soil hand-packed into tubes. Using cores filled with hand-packed homogenized soil may reduce the high variability inherent in the spatial distribution of soil N. However, soil disturbance in hand-packed core preparation may increase N-mineralization rates (Raison 1987). Theoretically, this is because disturbing the soil destroys some of the protective capacity of soil aggregates, thereby allowing formerly protected organic N compounds to be mineralized and/or mineral N compounds to be released into the soil solution (Paul and Clark 1989). Thus, undisturbed cores more closely mimic natural soil conditions and may provide more accurate estimates of soil N mineralization. The extent to which this is true may depend in part on soil clay content.

Low-clay-content soils generally have less protected organic matter, and less organic matter overall, than high-clay-content soils (Paul and Clark 1989; Van Veen, Ladd, and Frissel 1984). The protection process involves both adhesion of organic molecules to clay particles and isolation of organic matter and soil microbial biomass inside aggregates (Hassink et al. 1993; Six et al. 2002). Disturbing soil by physical mixing reduces the protective capacity of aggregates, allowing a short-term flush of mineralization, but has little effect on organic matter adsorption to clay. Franzluebbers and Arshad (1997) found comparatively greater organic carbon (C) mineralization in lower-clay-content soils after crushing aggregates. They hypothesized that the low-clay soils may have had a greater percentage of organic C protected inside aggregates as compared to adsorbed onto clay minerals.

Similarly, if N-mineralization rates differ between disturbed and undisturbed cores, the extent to which this is true may depend in part on soil clay content. Although the effect of soil disturbance on N mineralization has been studied (Franzluebbers 1999), we are aware of no published studies comparing disturbed and undisturbed IER/SCs in different soils. The objective of this study was to make that comparison, using cores from manured agricultural soils. Mineralized N and core temperature in disturbed and undisturbed cores were compared during four incubation periods.

Materials and Methods

Mineralization experiments were conducted on soil samples from six agricultural fields in Oregon's Willamette Valley (Washington and Polk Counties) using IER/SCs. Soils, cropping systems, and manure histories are summarized in Table 1. All but field F had at least 10-year histories of dairy manure applications, but none of the fields had received manure, other organic amendments, or fertilizer within 2 months of core collection. This minimized labile organic N from previous manure applications. Soils were collected from four or five sites in each field, spaced approximately 25 m apart. Soil samples from recently harvested corn fields were collected at the midpoint between rows to avoid fertilizer bands.

Core Preparation and Incubation

To prepare undisturbed cores, aluminum pipes (10-cm inner diameter) were placed in a straight line, with each pipe spaced 10 cm from the adjacent pipe, and driven to a depth of 20 cm with a rubber mallet. Weeds and detritus larger than 1 cm in length were removed

Table 1
Characteristics of agricultural fields used in this study

Field	Soil series ^a	Clay (%)	C (%)	N (%)	Nmin index ^b (mg kg ⁻¹)	Previous crop
A	Newberg fsl	11.9	1.20 (0.07)	0.11 (0.01)	42.1 (4.8)	Silage corn ^c
B	McBee sicl	23.8	3.42 (0.25)	0.32 (0.02)	187.9 (15.2)	Grass hay ^c
D	Woodburn sil	13.1	3.94 (0.40)	0.32 (0.03)	113.9 (8.8)	Silage corn ^c
E	Wapato sicl	19.7	1.68 (0.07)	0.16 (0.01)	66.6 (10.7)	Silage corn ^c
F	McBee sicl	35.0	2.08 (0.14)	0.21 (0.02)	21.8 (2.0)	Silage corn ^d
G	Woodburn sil	16.6	3.02 (0.21)	0.24 (0.02)	106.2 (7.4)	Silage corn ^c

Notes. Values in this table are averaged over all incubation periods.

Values in parentheses are SE.

^aSoil series per NRCS soil surveys: fsl = fine sandy loam, sil = silt loam, sicl = silty clay loam.

^bNmin Index is 7-day anaerobic incubation N mineralization.

^cHistory of over 10 years of regular dairy manure application.

^dNo manure applications within past 10 years.

from the soil surface before driving the pipes. The pipes were removed by inserting a steel rod through two holes drilled in the upper 5 cm of the pipe and then lifting up on the steel rod with a cable attached to a wooden lever. Using this technique, excess soil remained attached to the core bottom and was trimmed off in the field. The cores were sealed with foil, placed in polyethylene bags and refrigerated at 4 °C within 5 h of collection. Before incubation, the lower 3 cm of soil was removed from inside each core and two ion-exchange resin (IER) bags were fitted into the bottom (Figure 1). The IER bags were nylon mesh and contained equal volumes of Sybron Ionac C-249 and ASB-1P. The upper IER bag contained 104 g moist IER and the lower IER bag contained 80 g moist IER. The bags were held in place with polyester fabric (Drain-Sleeve filter fabric sock) fastened to the outside of the tube with aluminum foil duct tape.

Soil for the disturbed cores was collected immediately after extracting the undisturbed cores from the field. Soil between the holes resulting from undisturbed core extraction was excavated to a depth of 17 cm with a metal 3-in. stiff chisel scraper. This loose soil was sealed in clean plastic pails and refrigerated at 4 °C within 5 h of collection. Just before incubation, the loose soil was mixed thoroughly and sieved through a 10-mm mesh. Detritus and gravel larger than 10 mm in diameter were removed and discarded. The IER bags were inserted into the bottom of 10-cm-diameter aluminum pipes in the same configuration used for the undisturbed cores and held in place with heavy nylon mesh. The sieved disturbed soil was added through the top of each tube and compacted with a wooden dowel to a dry bulk density of 1.00 g cm⁻³. By comparison, the undisturbed core mean dry bulk density varied from 0.88 to 1.50 and averaged 1.12 g cm⁻³ (SE = 0.01). All cores were refrigerated at 4 °C until incubation.

This soil core construction process is similar to that described in the literature (DiStefano and Gholz 1986; Kolberg et al. 1997; Sullivan et al. 1999; Raison 1987), with two modifications. First, we used 10-cm-diameter cores rather than the more commonly used 5-cm-diameter cores. Compared to the more traditional smaller diameter pipe, our larger diameter pipe reduced compaction when hammered into the ground to collect undisturbed samples. Large diameter cores also require significantly more IER (we used 104 g IER in the upper bag compared to the more commonly used 20–25 g IER). Our second

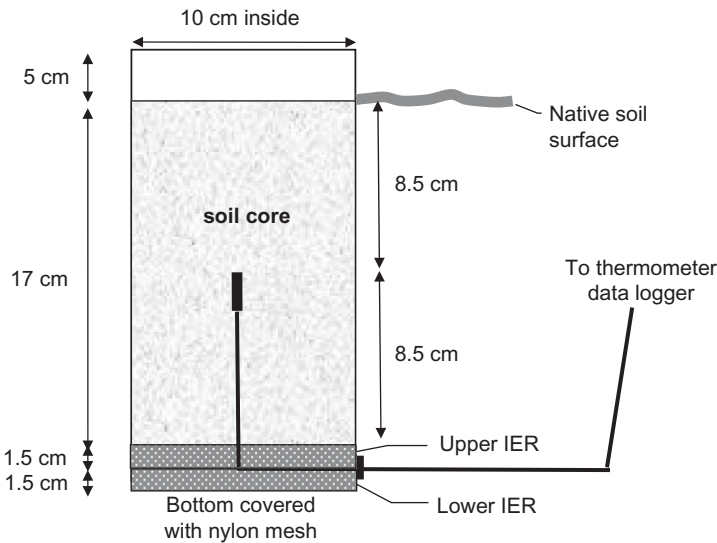


Figure 1. Diagram (not to scale) of IER/SC construction using 10-cm-inside-diameter aluminum pipe. Finished disturbed and undisturbed IER/SCs were identical, except disturbed cores were compacted to 1.00 g cm^{-3} whereas undisturbed core dry bulk densities varied from 0.88 to 1.50 and averaged 1.12 g cm^{-3} (SE = 0.01). One core from each field was fitted with a recording thermometer with a cable entering the lower side through a rubber septum as shown. The recording point of the cable was in the center of the core.

modification was to use two IER bags in each core rather than the more commonly used single IER bag. The upper IER captured $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ leaching out of the soil core. The lower IER prevented contamination from below in case a seasonally high water table saturated the soil surrounding the core.

One undisturbed and one disturbed core from each field were fitted with a Hobo recording thermometer (Onset Computer Corporation 2011). Cores were installed with a completely randomized design (Mendenhall and Sincich 1995) in a plot within a field seeded to soft white winter wheat (*Triticum aestivum* L.) for winter incubations and a field planted to silage corn (*Zea mays* L.) for the summer incubation.

A Hobo recording thermometer was installed in the field soil adjacent to the cores and at the same depth (8.5 cm) as the sensors inside the cores. Temperatures were automatically recorded every 1 to 2 h throughout the incubations. The fields, dates of soil collection, beginning incubation dates, harvest dates, and replication numbers are summarized in Table 2.

The incubation fields are near Forest Grove, Oregon (45.59° N , 123.14° W), approximately 43 kms west of Portland and within 100 km of the fields where the soil was collected. The incubation fields are in the Willamette Valley ecoregion (EPA 2008) and have 30-year (1971 to 2000) mean annual precipitation of 118 cm and mean annual air temperature of 11.7° C . The coldest and wettest month is December (4.1° C mean temperature and 20.1 cm mean precipitation). The warmest month is August (mean temperature 20.4° C), and the driest month is July (mean precipitation 1.5 cm) (Western Regional Climate Center 2011).

Table 2
Incubation trial summary

Parameter	Incubation period			
	1	2	3	4
Incubation start	28 Jun. 2003	1 Nov. 2003	27 Oct. 2004	27 Oct. 2004
Incubation end	22 Sep. 2003	18 May 2004	15 Dec. 2004	4 Feb. 2005
Incubation period (days)	86	199	49	100
Undisturbed cores (n)	10	36	20	20
Disturbed cores (n)	20	12	20	20
Fields	D,E,F	A,D,E	A,B,F,G	A,B,F,G

Note. Field F cores in summer 2003 were monitored for soil temperature but not for N mineralization.

Eghball (2000) reported that IER can be destroyed by freezing temperatures. However, Myrold, Baumeister, and Moore (1992) successfully used the IER/SC method during winter in western Oregon. (In the trials described here, the coldest temperature recorded within the IER/SC was only -0.6°C and inspection after incubation revealed no physical damage to the IER in these experiments.)

Soil and IER Analysis

Excess loose soil from the disturbed core preparation was analyzed for baseline (preincubation) soil moisture and chemistry. After incubation, tubes were removed from the ground, sealed with foil, and placed in plastic bags. Tubes were refrigerated at 4°C within 4 h of excavation until analyzed for moisture and chemistry.

Prior to all analyses, soil was mixed thoroughly, sieved through a 10-mm screen to remove stones and larger pieces of detritus, and then subsampled as follows. A 100-g subsample was used to determine moisture content (Gardner 1965). A separate air-dried subsample was analyzed by the Oregon State University Central Analytical Laboratory for 7-day anaerobic mineralizable N; percentages of clay (pipette method), total C, and total N (Leco CNS-2000 macro-analyzer; LECO Corp., St. Joseph, Mich.); and pH (1:2 soil to water) (Keeney 1982; Horneck et al. 1989). A separate 20-g moist soil subsample was extracted with 100 mL 2 M potassium chloride (KCl) (1 h on a reciprocating shaker) and then filtered with a Whatman 934-AH glass microfibre filter or a Whatman Puradisc 25AS $0.45\text{-}\mu\text{m}$ syringe filter (Whatman, Kent, Maine). The filtered extracts were colorimetrically analyzed for $\text{NO}_3\text{-N}$ by cadmium reduction and diazotization and for $\text{NH}_4\text{-N}$ by the alkaline phenol / hypochlorite method (Astoria-Pacific 1998).

The lower IER bags were not analyzed. The upper IER from each core was emptied from the nylon bag, weighed, and mixed thoroughly. A 10-g moist sample from each upper IER was placed in a fresh nylon bag and extracted with four sequential 50-ml aliquots of 2 M potassium chloride (KCl) with 15 min of shaking. Following each sequential extraction, the extract was decanted from the bag by pouring the contents through a funnel. After the extract drained from the IER bag with each extraction, the bag was rinsed with a separate 10-mL aliquot of 2 M KCl. Rinses and extracts were combined for each bag and analyzed for $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ with the same colorimetric analyses used for the soils.

This method of IER extraction is similar to that described by Eghball (2000) and yielded 95% of the $\text{NO}_3\text{-N}$ and 106% of the $\text{NH}_4\text{-N}$ in a trial with standard solutions adsorbed onto IER. The masses of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ extracted from the 10-g IER sample were multiplied by 10.4 to calculate the total mineral N mass that had been captured by the 104-g upper IER bag.

The IER $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ mass was normalized to the mass of dry soil in each core and added to the postincubation soil $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ analyses to determine the total postincubation mineral N. The baseline soil $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ concentrations were subtracted from the postincubation analyses to determine net mineralization. The Levene statistic was used to compare variance of N mineralization. Tukey box-and-whisker plots were used to illustrate the variability of the two IER/SC methods. All statistical analyses were done with SPSS (1999).

Results and Discussion

Nitrogen mineralized, averaged over all fields and trials, was greater in disturbed cores ($40.9 \text{ mg N kg}^{-1} \text{ soil}$) than undisturbed cores ($31.6 \text{ mg N kg}^{-1} \text{ soil}$) ($P < 0.10$). Ten of the 13 field / incubation-period combinations showed more N mineralized in the disturbed cores. Figure 2 illustrates N mineralized in the disturbed and undisturbed cores for the winter incubations (trials 2, 3, and 4). Table 3 provides mean N mineralized for all incubation periods (trials 1, 2, 3, and 4).

Notably, field A disturbed cores had consistently more N mineralized than undisturbed cores, significantly so during trials 2 and 4. Field A soil had the lowest clay content (11.9%) of all fields tested (Table 1). This result is consistent with that of Franzluebbers and Arshad

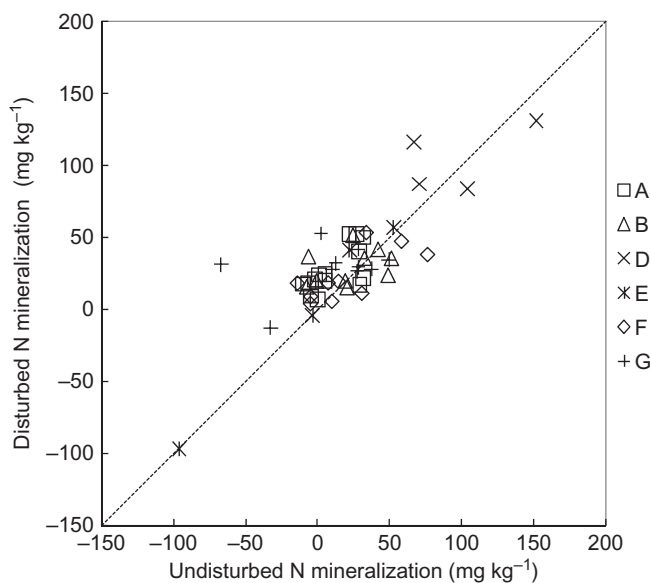


Figure 2. Comparison of net N mineralization in disturbed and undisturbed cores for winter incubations in soils from six agricultural fields (A–G). Summer 2003 data are not charted. Each point is the average N mineralized in the cores from one site in a field during one incubation period. Dashed line is $y = x$ for comparison.

Table 3
Comparison of mean N mineralization in undisturbed and disturbed IER/SCs

Incubation period	Field ^a	Net mineralization in cores (mg N kg ⁻¹)		t-test ^b
		Undisturbed	Disturbed	
1	D	91.9 (10.3)	88.1 (3.1)	n.s.
1	E	49.3 (7.0)	47.5 (1.7)	n.s.
2	A	27.2 (2.4)	48.4 (2.7)	**
2	D	98.2 (12.5)	104.2 (11.5)	n.s.
2	E	-6.1 (17.1)	-0.9 (34.5)	n.s.
3	A	5.1 (7.0)	15.8 (3.4)	n.s.
3	B	10.8 (10.8)	22.2 (3.9)	n.s.
3	F	4.4 (8.1)	12.2 (2.9)	n.s.
3	G	-2.9 (16.5)	33.7 (4.8)	*
4	A	4.6 (6.6)	19.7 (1.2)	*
4	B	33.8 (5.8)	36.7 (5.1)	n.s.
4	F	37.2 (13.4)	32.4 (9.0)	n.s.
4	G	22.1 (14.2)	24.0 (9.6)	n.s.
All	All	31.6 (5.1)	40.9 (4.0)	*

Notes. Values in parentheses are SE.

^aSoils from some fields were tested in multiple incubation periods.

^bOne-tailed Student's t-test of difference between means.

*, **Difference between means significant at 0.10 and 0.01 probability levels, respectively.

n.s. denotes difference between means not significant at $P < 0.10$.

(1997), who found comparatively greater organic C mineralization in lower-clay-content soils after crushing aggregates and hypothesized that the low-clay soils had a greater percentage of organic N protected inside aggregates as compared to adsorbed onto clay minerals. Another possible explanation for the increased mineralization in field A disturbed cores is the relative strength of aggregates. Field A soil was observed to have weak aggregates that thoroughly broke into pieces much smaller than the 10-mm screen during disturbed IER/SC preparation prior to incubation. For other fields, soil aggregates broke into pieces that were often only slightly smaller than the 10-mm screen.

Nitrogen-Mineralization Variability

The standard error (SE) of N mineralized was greater in undisturbed cores compared to disturbed cores for 11 of the 13 field/trial combinations (Table 3). The Levene statistic demonstrated a significant difference in variance between the disturbed and undisturbed cores for trials 3 and 4 ($P < 0.10$). Tukey box-and-whisker plots in Figure 3 graphically illustrate this difference in variability.

This greater variability in undisturbed core N mineralization is consistent with the differences in soil volumes used to prepare cores. Each disturbed core was constructed from a well-mixed sample of approximately 35,000 cm³ of soil, and each undisturbed core only represented a 1,300-cm³ soil sample. Because soil organic matter typically exhibits

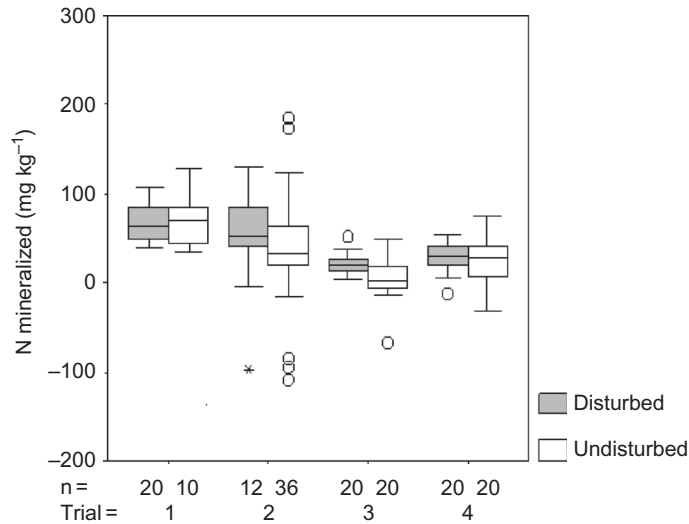


Figure 3. Tukey box-and-whisker plots of disturbed and undisturbed cores from all trials. The horizontal line in the middle of each box is the median, the bottom and top of each box are the 25th and 75th percentiles, respectively, and the whiskers show the range of data values that are within 1.5 box lengths of the bottom and top of each box. The circles are data outliers (1.5 to 3 box lengths from the bottom or top of the box) and the asterisk (*) is an extreme value (more than 3 box lengths from the bottom of the box).

high spatial variability in agricultural fields (Cahn, Hummel, and Brouer 1994), it is not surprising that the undisturbed cores exhibited greater variability in N mineralization.

Soil Temperature

Soil temperature is an important factor influencing N mineralization (Vigil and Kissel 1995). Table 4 summarizes mean core temperatures and daily temperature ranges for the summer 2003 and the winter 2004 to 2005 incubations. There was no significant difference in mean daily temperatures in disturbed versus undisturbed cores in the summer or winter incubations. There was a small but significant ($P < 0.05$) tendency for the undisturbed cores to have a greater daily temperature range (maximum minus minimum daily temperature) in both summer and winter. In the summer, the mean daily temperature range was 0.46 °C greater in the undisturbed cores. In the winter, the mean daily temperature range was 0.42 °C greater in the undisturbed cores.

Table 4 also provides “in-field” temperature data for the soil outside of, but adjacent to, the IER/SC. During summer, the mean daily soil temperature outside the cores was 20.39 °C, which was slightly but significantly ($P < 0.01$) less than either the disturbed (20.70 °C) or undisturbed (20.67 °C) mean temperatures inside the cores. The mean daily temperature range in the soil outside of the cores was 3.03 °C, which was significantly ($P < 0.01$) less than either the disturbed (5.57 °C) or undisturbed (6.03 °C) mean daily temperature ranges inside the cores. The greater daily temperature fluctuation inside the cores during the summer may have been due to the aluminum pipes extending 5 cm above the soil surface. We expect that this exposed pipe collected solar radiation during the day and radiated heat away from the cores at night.

Table 4
Temperatures in undisturbed and disturbed IER/SCs

Field	Core temperatures (°C) ^a			
	Daily mean ^b		Daily range ^c	
	Undisturbed	Disturbed	Undisturbed	Disturbed
1 Jul.-21 Sep. 2003				
D	20.52a	20.50a	5.34	5.09
E	20.79b	20.76b	6.56	6.47
F	20.70c	20.85c	6.21	5.13
Mean ^d	20.67d	20.70d	6.03	5.57
in field	20.39		3.03	
6 Nov. 2004-17 May 2005				
G	7.92e	7.89e	4.94	4.09
A	7.64f	7.75f	4.19	4.28
F	7.67g	7.56g	4.36	3.86
Mean ^d	7.74h	7.74h	4.50	4.08
in field	7.62		4.74	

^aSoil core temperatures at 8.5-cm depth.

^bMean daily core temperature.

^cMean of 24-h maximum minus minimum core temperature.

^dTemperature taken at 8.5-cm depth outside of but adjacent to cores.

Note. Results followed by the same lowercase letter are not significantly different at $P < 0.10$.

During winter, the mean daily soil temperature outside the cores was 7.62 °C, which was slightly but significantly ($P < 0.05$) less than either the disturbed or undisturbed (both were 7.74 °C) mean temperature inside the cores. The mean daily soil temperature range outside the cores was 4.74 °C. This was slightly but significantly ($P < 0.05$) greater than the disturbed (4.08 °C) and insignificantly greater than the undisturbed (4.50 °C) mean daily temperature ranges inside the cores. We expect that the exposed aluminum pipe collected and radiated less heat during winter when the weather was often cloudy than during summer when the sky was often clear.

Conclusions

This study compared two methods of IER/SC preparation: disturbed and undisturbed. The disturbed method used soil samples that were thoroughly mixed before core preparation, and the undisturbed method used soil samples that were collected by driving tubes into intact soil with little disturbance.

The N mineralized, averaged over all fields and incubation periods, was greater in disturbed cores (40.9 mg N kg⁻¹ soil) than in undisturbed cores (31.6 mg N kg⁻¹ soil) ($P < 0.10$). Of the 13 field / incubation-period combinations, 10 exhibited greater N mineralization in disturbed cores than in undisturbed cores. Difference in N mineralized in disturbed versus undisturbed cores was greatest in soil from field A, which had the lowest clay content (11.9%) of all soils tested.

Disturbed cores had less variability in N mineralization than undisturbed cores. Because reduced variability allows the use of fewer cores in mineralization studies and

because the construction and analysis of cores is expensive, decreased variability is advantageous. This study indicates that there is a trade-off in the use of disturbed versus undisturbed IER/SC incubations to study N mineralization: Disturbed cores in this trial had lower variability but tended to have increased N mineralization rates.

The mean core temperatures were not significantly different between disturbed and undisturbed cores, and there was little difference between the soil temperatures inside and outside of the cores. There was also little difference between disturbed and undisturbed core daily temperature ranges. During the winter, the daily temperature *range* inside cores was slightly (less than 0.25 °C) greater than the soil outside of the cores. However, during the summer, the daily temperature range was more than 2.0 °C greater inside the cores compared to the soil outside of the cores. These results indicate that both disturbed and undisturbed cores tended to match natural soil temperature regimes well during the cloudy and cool Pacific Northwest winter but not as well during the summer. It may be beneficial to thermally insulate the exposed surfaces of cores incubated during summer.

Overall, the disturbed core method tended to increase N-mineralization rates, especially in the low-clay-content soil, and reduce N-mineralization variability compared to the undisturbed core method. Soil core temperatures were similar for both methods. The disturbed method of IER/SC preparation is an acceptable option for estimating N mineralization in agricultural soils, especially when reduced variability of results is desirable.

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