Assessment of Denitrification Gaseous End-Products in the Soil Profile under Two Water Table Management Practices Using Repeated Measures Analysis

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ABSTRACT

The denitrification process and nitrous oxide (N_2O) production in the soil profile are poorly documented because most research into denitrification has concentrated on the upper soil layer (0–0.15 m). This study, undertaken during the 1999 and 2000 growing seasons, was designed to examine the effects of water table management (WTM), nitrogen (N) application rate, and depth (0.15, 0.30, and 0.45 m) on soil denitrification end-products (N_2O and N_2) from a corn (*Zea mays* L.) field. Water table management treatments were free drainage (FD) with open drains and subirrigation (SI) with a target water table depth of 0.6 m. Fertility treatments (ammonium nitrate) were 120 kg N ha^{-1} (N120) and 200 kg N ha^{-1} (N200). During both growing seasons greater denitrification rates were measured in SI than in FD, particularly in the surface soil (0–0.15 m) and at the intermediate (0.15–0.30 m) soil depths under N200 treatment. Greater denitrification rates under the SI treatment, however, were not accompanied with greater N_2O production. The decrease in N_2O production under SI was probably caused by a more complete reduction of N_2O to N_2, which resulted in lower N_2O to ($N_2O + N_2$) ratios. Denitrification rate, N_2O production and N_2O to ($N_2O + N_2$) ratios were only minimally affected by N treatments, irrespective of sampling date and soil depth. Overall, half of the denitrification occurred at the 0.15- to 0.30- and 0.30- to 0.45-m soil layers, and under SI, regardless of fertility treatment level. Consequently, sampling of the 0- to 0.15-m soil layer alone may not give an accurate estimation of denitrification losses under SI practice.

W ATER TABLE MANAGEMENT has been proposed as a beneficial management practice for bioremediation of nitrate N (NO_3^-–N) contaminated soils by enhancing denitrification (Elmi et al., 2002a; Jacinthe et al., 2000; Kliewer and Gilliam, 1995). Denitrification is the major biological process by which NO_3^-–N is reduced to N_2, with N_2O being an intermediary product. Nitrous oxide emissions have been a topic of increasing concern because N_2O contributes to the atmospheric greenhouse gas concentration and has a well-documented role in stratospheric ozone depletion (Duxbury et al., 1982).

Although most previous studies have concentrated on the top 0.15 m of the soil surface, some researchers have reported increased denitrification in subsoils if water-soluble carbon (WSC) is not limited (Ryan et al., 1998; Jarvis and Hatch, 1994). McCarty and Bremner (1992) measured denitrification in subsurface soils (up to a 2-m depth) and reported negligible denitrification in response to added NO_3^-–N alone, but the addition

of both NO_3^-–N and glucose stimulated denitrification activity. They concluded that denitrification processes in subsurface soils are primarily limited by the availability of WSC. Water-soluble C provides microorganisms with an energy source and thus may play a dominant role in defining the capacity of agroecosystems to act as NO_3^-–N sinks.

Denitrification processes occurring in the subsoil environment can be an important mechanism to help mitigate NO_3^-–N contamination of ground and surface waters. A series of studies conducted previously (Elmi et al., 2002a) and concurrent with the study reported here (Elmi et al., 2004) at the same field site reported that SI reduced NO_3^-–N accumulation in the soil profile and, consequently, improved drainage water quality. Whether NO_3^-–N removal by denitrification is beneficial to the wider environment, however, depends on whether the dominant end-product of NO_3^-–N reduction is N_2O or N_2. We hypothesize that N_2O produced at deeper soil depths will take longer to diffuse, providing an opportunity for further reduction of N_2O to N_2 before escaping to the atmosphere. Despite this potential for denitrification in subsurface soil to help mitigate NO_3^-–N pollution without a concomitant increase in N_2O emissions, the interactive effects of WTM and N fertilization practices on subsurface N_2O production have not been investigated under field conditions. Examination of subsoil denitrification will improve our knowledge gap in N transformations and future environmental risk assessment associated with the emissions of N_2O from agroecosystems. The objectives of this study were, therefore, to examine denitrification rates, N_2O production, and N_2O to ($N_2O + N_2$) ratio in the soil profile as influenced by WTM, N fertilization rate, and soil depth from a corn field during two cropping seasons.

MATERIALS AND METHODS

Field Management and Experimental Design

Experimental layout, treatment arrangements, and field operations are described in Elmi et al. (2002b). Briefly, we conducted this study on a corn field (4.2 ha) located at St-Emmanuel, Quebec, Canada. The soil is classified as a Soulanges fine sandy loam (fine silty; mixed, nonacid, frigid Humaquept, Gleysol, according to the FAO classification system). The fine sandy loam soil (0–0.25 m) was underlain by layers of sandy clay loam (0.25–0.55 m) and clay (0.55–1.0 m), and the clay layer impeded the natural drainage. The soil contained 50 g C kg^{-1} soil (fresh wt.) in the 0- to 0.25-m layer, 15 g C kg^{-1} soil (fresh wt.) in the 0.25- to 0.55-m layer, and a negligible amount of C below 0.55 m. The pH (0.45-m depth) was near

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Abbreviations: FD, free drainage; N120, 120 kg N ha^{-1}; N200, 200 kg N ha^{-1}; SI, subirrigation; WSC, water-soluble carbon; WTM, water table management.

neutral (6.8). Primary tillage after harvest consisted of mold-board plowing to a depth of 0.15 to 0.20 m. Secondary tillage consisted of disking before planting.

There were two WTM treatments: FD with open drains and SI with a target water table depth of 0.6 m, factorially combined with two N fertility rates: 120 kg N ha^{-1} (N120) and 200 kg N ha^{-1} (N200). Diammonium phosphate (18–46–0) was banded at planting to provide approximately 24 kg N ha^{-1} and 130 kg P$_2$O$_5$ ha^{-1}. In each year, one month after seeding, 97 and 178 kg N ha^{-1} were broadcast as ammonium nitrate (34–0–0) for the N120 and N200 treatments, respectively, to reach desired N levels. These second applications occurred on 10 June 1999 and 20 June 2000. The four treatment combinations of water table and fertility rate were randomized in 15- × 75-m plots. There were three blocks (120 m wide and 75 m long, including four buffer plots) separated by a 30-m-wide strip of undrained land. In the middle of each plot, 75-mm-diameter subsurface drain pipes were laid (1.0-m depth and 30-m spacing) on a 0.3% slope. The SI treatment was imposed two weeks after planting and maintained until crop maturity in late September. Subirrigation was switched to FD on 17 Sept. 1999 and 15 Sept. 2000.

Three observation wells (pipes wrapped with geotextile [Zodiac, London, ON, Canada]) were installed diagonally across each of the treatment and buffer plots to a depth of approximately 1.5 m. A graduated rod with a sonic water sensor was used to monitor water table levels. Following heavy rainfall events, pumping was stopped manually in SI plots and excess water drained until a 0.6-m water table depth was achieved. Water table levels under both WTM treatments fluctuated throughout growing seasons, responding to the amount of rainfall and intensity (Elmi et al., 2004).

Rainfall and air temperature data were obtained from an Environment Canada weather station 500 m from the site. Based on a 30-yr average, the climate at the experimental site has a monthly mean temperature of 15°C and a mean precipitation of 522.2 mm during the growing season (May–October). Total seasonal rainfall in 1999 was 13% higher than normal with almost half (47%) of the rainfall occurring in September and October. In 2000 it was about 12% higher than normal, with May being the wettest month (twofold the norm) and October the driest month (less than half the norm) of this growing season. Mean monthly temperatures at the site during the growing season (May–October) were 1.6°C higher in 1999 than the 30 yr average, while 2000 followed the long-term average.

Denitrification and Nitrous Oxide Measurements

Denitrification was measured using the intact core incubation method with acetylene (C$_2$H$_2$) inhibition (Ryden et al., 1987). Three incremental depths (0–0.15, 0.15–0.30, 0.30–0.45 m) were sampled simultaneously in pairs. Due to the heavy labor commitment, time constraints related to sampling and incubating cores, and analytical costs, it was only feasible to collect one pair of samples from each treatment plot for each sampling date. There were six (9 June, 7 July, 28 July, 26 August, 18 September, and 20 October) and eight (28 May, 22 June, 6 July, 19 July, 3 August, 17 August, 19 September, 20 October) sampling dates for 1999 and 2000, respectively.

On each sampling date, aluminum cylinders (50 mm in diameter × 150 mm long) were used to collect soil cores of three depth increments (0–0.15, 0.15–0.30, 0.30–0.45 m). Samples were collected from randomly selected locations in the non-wheel-tracked middle rows of each plot and they were never taken from the same location (hole) more than once within the growing season. Samples were placed in 2-L plastic jars fitted with rubber stoppers for gas sampling. One hundred milliliters of the headspace in the jars was removed from one sample of each pair and replaced with 100 mL of C$_2$H$_2$ to give a 5% (v/v) concentration. The second sample was not treated with C$_2$H$_2$. All samples were buried at the 0- to 0.15-m depth in an attempt to mimic field conditions for a 24-h incubation.

Headspace gas was thoroughly mixed by inserting a syringe and pumping several times before gas sampling. About 4 mL of headspace gas was removed from the jars and injected into a gas chromatograph (GC) (Model 5870 Series II; Hewlett-Packard, Palo Alto, CA) equipped with a ^{63}Ni electron capture detector (ECD) using Ar and CH$_4$ (95:5) as a carrier gas, with oven and detector temperatures adjusted to 70 and 400°C, respectively.

The consequence of C$_2$H$_2$ treatment is the inhibition of the N$_2$O-reductase enzyme, allowing N$_2$O to accumulate as a measure of total denitrification (N$_2$O + N$_2$) (Ryden et al., 1987). The production of N$_2$O by denitrification was estimated by the amount of N$_2$O evolved from soil cores without C$_2$H$_2$, whereas total denitrification (N$_2$O + N$_2$) was estimated from the amounts of N$_2$O produced from C$_2$H$_2$–amended soil cores. In the remainder of this paper, the quantities of N$_2$O produced with and without C$_2$H$_2$ addition are referred to as N$_2$O and N$_2$O + N$_2$, respectively. The mole fraction of N$_2$O, defined as N$_2$O to (N$_2$O + N$_2$) ratio, was estimated as the ratio of N$_2$O measured without C$_2$H$_2$ to that produced with C$_2$H$_2$ (Aulakh et al., 1984).

Water-Soluble Carbon

Three soil samples were collected from each plot using an auger before planting and after harvest. Samples were combined to make composite samples. To extract WSC, 10 g of field moist subsample was shaken in 100 mL of deionized distilled water for 1 h, centrifuged at 10 000 rpm for 10 min, and then filtered through #5 paper (Whatman, Maidstone, UK). Samples were analyzed using a Model TOC-5000A analyzer (Shimadzu, Kyoto, Japan).

Statistical Methods: Repeated Measures Analysis

Separate repeated measures analysis was completed for each of 1999 and 2000 because the levels of one of the factors of interest, sampling date, were different in the two years. For each year, the location in the field was considered as a random blocking factor, and the other four factors: fertilizer (N), WTM, depth, and sampling date (date) were considered fixed factors. Although the layout of the experiment does not allow randomization of the three depths, the levels of N and WTM were completely randomized. The levels of date cannot be randomized, as the response measurements [denitrification end products and their ratios; N$_2$O, N$_2$O + N$_2$, and N$_2$O to (N$_2$O + N$_2$) ratio] were collected repeatedly over six and eight sampling dates in 1999 and 2000, respectively. Because the values of the response variables follow a certain pattern (i.e., it is not random), the independence assumption on the error terms required for the analysis of variance (ANOVA) of a factorial model (Montgomery, 2001) was unlikely to be met.

Generally, with repeated measurements such as this one, two measurements taken at adjacent times are typically more highly correlated than two measurements taken several time points apart (Littell et al., 1996). Moreover, the variability in the response measurements from different dates tended to change with the magnitude of the measurements, which may lead to the violation of the constant variance (uniform sources of experimental error) assumption. Therefore, the appropriate assumption on the error terms for this experiment was normal

Table 1. The *p* values of all main and interaction fixed effects estimated using the restricted maximum likelihood (REML) method with compound symmetry (CS) covariance structure on the log-transformed values.

	$N_2O + N_2$		N_2O		N_2O to $(N_2O + N_2)$ ratio	
Source of variation	1999	2000	1999	2000	1999	2000
N	0.079	0.233	0.939	0.035	0.311	0.254
WTM†	0.001	0.001	0.001	0.649	0.001	0.001
N × WTM	0.004	0.700	0.231	0.612	0.724	0.406
Depth	0.001	0.001	0.040	0.013	0.001	0.001
N × depth	0.159	0.964	*0.043*‡	0.232	0.349	0.166
WTM × depth	0.593	0.001	0.843	0.075	0.239	0.286
N × WTM × depth	*0.028*	0.799	0.698	0.253	0.100	0.188
Date	0.001	0.001	0.001	0.001	0.002	0.001
N × date	0.297	0.013	0.210	0.043	0.051	0.001
WTM × date	0.001	0.001	*0.001*	*0.007*	0.004	0.163
N × WTM × date	*0.001*	0.064	0.230	0.221	0.425	0.037
Depth × date	0.002	0.001	*0.015*	*0.005*	0.001	0.161
N × depth × date	0.899	0.783	0.578	0.709	0.636	0.249
WTM × depth × date	*0.003*	0.725	0.203	0.782	*0.001*	0.602
N × WTM × depth × date	0.878	*0.028*	0.943	0.231	0.984	*0.045*

† Water table management.

‡ Means comparisons of interaction effects whose *p* values are shown in italic type are discussed in subsequent tables.

distribution with heterogeneous (nonconstant) variance by date, and a covariance structure of Σ. The appropriate covariance structure for each response variable was determined using Akaike's Information Criterion (AIC) and Schwarz's Bayesian Criterion (BIC), which are essentially log likelihood values penalized for the number of parameters estimated (Littell et al., 1996).

We used the mixed procedure in SAS (SAS Institute, 1999), specifically written for analyzing mixed effects and repeated measures with nonconstant variance and any covariance structure models, to analyze our data. In a preliminary analysis, the normality of the error terms for all three response variables was found to be violated on the original scale, but was met with logarithmic values. Also, according to the AIC and the BIC, the best covariance structure was determined to be compound symmetry (CS), with heterogeneous variance–covariance parameters for different sampling dates. Water-soluble C and pH were considered as covariates, but because they were not significant in any of the responses they were excluded from the model. The statistical test results are based on the log values, and with different variances for the different sampling dates, and with the CS covariance structure. As shown in Table 1, up to four factor interaction effects were significant. Therefore, means comparison results, starting with the highest order significant interaction, were completed by generating letter groupings of the least squares means. Because these significant interactions gave a large number of treatment combinations, the letter groupings were based on the Tukey–Kramer adjusted *p* values to control Type I error rates (SAS Institute, 1999).

To facilitate easy reading of the results presented in Tables 2 to 7, the least squares means were back-transformed to the original scale, and the groupings done within and across sampling dates.

RESULTS AND DISCUSSION

Denitrification Rates in the Soil Profile

A summary of repeated measures analysis results of the main and interaction effects for both years is presented in Table 1. In 1999, both WTM and N treatments interacted significantly with depth and date of sampling (Table 1), with highest denitrification rates being pro-

duced on 7 July under both WTM treatments (Table 2). Within the SI treatment, plots receiving higher N rate (200 kg N ha^{-1}) produced greater denitrification rates than the lower N rate (120 kg N ha^{-1}) only on 7 July at the 0- to 0.15-m depth (Table 2). In contrast, denitrification rates were not affected by N treatment under FD at any sampling date. Generally, our results indicate that the addition of 200 kg N ha^{-1} relative to 120 kg N ha^{-1} does not play a significant role in soil denitrification.

The lack of N rate effect appears contradictory to the widely reported (Ellis et al., 1998; MacKenzie et al., 1997) findings that the application rate of N fertilizers has a significant influence on denitrification. One plausible explanation may be that there was sufficient NO_3^-–N in the soil even at the lowest rate of N application for denitrification to occur. If this is correct, an important management implication would be that manipulating N fertilizer rate alone may not be a sufficient strategy to control N_2O emissions under certain situations. The NO_3^-–N concentrations in the soil solution as well as in drainage effluent have been reported in Elmi et al. (2004). Briefly, NO_3^-–N average levels were greater than 5 mg kg^{-1} at a depth of 0 to 0.75 m. The NO_3^-–N levels were greater during the growing season (May–October) under all treatments. With soil NO_3^-–N concentrations at that level, it is apparent that ammonium was high enough to support nitrification.

The lack of differences between the two N treatments may also be related to the sampling scheme. Both denitrification and N_2O are strongly episodic in nature and peak values that can contribute significantly to overall production might have been missed with the kind of sampling intensity used in this study. As noted previously, the work reported is labor-intensive and more frequent sampling was not feasible. From a practical point of view, more frequent sampling would also have caused extensive damage (depressional areas) to the field, creating technical difficulties for water table management and future field operations.

With respect to sampling depth, the effect was not consistent under either WTM treatment (Table 2). Within FD, for example, 7 July was the only sampling date

Table 2. Mean denitrification rate (back-transformed to the original scale) of all treatment combinations of two significant three-factor (N × water table management [WTM] × date; WTM × depth × date) interaction effects in 1999.†

WTM‡	N	Depth	Date					
			9 June	7 July	28 July	26 August	18 September	20 October
	kg N ha^{-1}	m			g ha^{-1} d^{-1}			
SI	120		B 4.94	A 14.40 b	B 6.36 a	B 6.64 a	C 1.50 a	C 2.05 a
	200		B 6.53	A 70.97 a	B 7.05 a	B 6.65 a	C 1.19 a	C 0.94 b
FD	120		B 5.27	A 15.59 b	C 0.82 b	C 0.23 b	C 0.34 ab	C 0.65 b
	200		A 3.52	A 5.14 b	B 0.54 b	C 0.13 b	C 0.08 b	B 0.70 b
SI		0.15	B 10.99	A 50.50 a	B 9.05 a	B 8.96 a	BC 2.83 a	C 1.43 a
		0.30	BC 7.53	A 19.83 b	AB 13.10 a	AB 14.26 a	C 2.32 a	C 2.01 a
		0.45	B 2.22	A 32.62 ab	B 2.53 ab	B 2.30 b	B 0.36 ab	B 0.93 ab
FD		0.15	B 5.02	A 33.50 ab	C 0.82 bc	C 0.24 c	C 0.16 b	C 0.74 b
		0.30	A 10.03	A 9.34 bc	C 0.97 bc	C 0.18 c	C 0.18 b	C 0.66 b
		0.45	BC 1.59	AB 2.29 c	C 0.36 c	C 0.11 c	C 0.17 b	C 0.63 b

† Means preceded by the same uppercase letter (within rows) and means followed by the same lowercase letters (within columns) are not significantly different (Tukey–Kramer adjusted $p > 0.05$).
‡ SI, subirrigation; FD, free drainage.

when the denitrification rate in the two upper layers (0–0.15 and 0.15–0.30 m) was significantly ($p < 0.05$) greater than the deepest (0.45 m) soil depth. Furthermore, depth effects diminished as sampling period progressed, with only a trace amount of denitrification measured under FD regardless of sampling depth. Under SI, however, 26 August was the only sampling date when denitrification in the two upper layers (0–0.15 and 0.15–0.30 m) was significantly ($p < 0.05$) greater than in the deepest (0.45 m) soil layer (Table 2). When comparing between SI and FD, it is evident that SI produced greater denitrification than FD in most sampling dates at the corresponding depths, but the effect was more pronounced at the surface (0–0.15 m) and intermediate (0.15–0.30 m) depths. Enhanced denitrification in the 0.15- to 0.30-m soil layer under SI indicates the existence of favorable conditions for denitrification to occur at a depth below 0.15 m.

In 2000, there was a significant ($p < 0.05$) four-way (N × WTM × depth × date) interaction (Table 1), making the interpretation of the data somewhat difficult. We therefore presented the data graphically (Fig. 1) to make it easier to understand the results, with detailed statistical findings reported in Table 3. Denitrification rates under FD did not change consistently with time, regardless of N level and depth (Fig. 1, Table 3). For example, denitrification rates from the upper soil layers (0.15- and 0.30-m depths) under FD with 200 kg N ha^{-1} applied were not affected by the sample date (Table 3). Under SI denitrification rates remained high throughout the season, reaching maximum on 3 August in all sampling depths (Fig. 1), six weeks after N was applied. These observations are consistent with Koops et al. (1996), who estimated that denitrification rates from the 0- to 0.40-m soil layer increased twofold a few weeks after N fertilizer application on a grassland ecosystem. Similarly, Velthof et al. (1996) reported that N$_2$O losses from deeper layers were most significant after application of N fertilizer. With respect to depth effect, greater denitrification rates were measured at the uppermost (0–0.15 m) and intermediate (0.15–0.30 m) depths than at the lowest depth (Table 3). The effect, however, was more pronounced under SI combined with the higher (200 kg N ha^{-1}) N rate (SI + N200) than FD receiving similar N rate (200

kg N ha^{-1}). This observation suggests that N effects on denitrification may be more pronounced under SI management than under FD management.

Soil moisture exerts an important control on denitrification and the proportion of gaseous end-products. Consistent with our previous report (Elmi et al., 2002b), soil water contents (water-filled pore space) were higher under SI than FD at nearly each depth and ranged between 40 to 60% under FD and 60 to 80% under SI. Filling more soil pores with water under SI would have increased volume of anaerobic zones within soil profile, creating conditions conducive to denitrification processes. We calculated that under the conditions of the denitrification assay, dissolved N$_2$O would represent approximately 5% of headspace N$_2$O and thus estimates of denitrification reported represent 95% of actual denitrification rate.

Nitrous Oxide Production in the Soil Profile

In 1999, N$_2$O production at the 0.45-m depth was significantly lower than at the other two soil depths under N120, but did not change with soil depth under N200, which resulted in a significant ($p = 0.043$) N × depth interaction (Table 1). With respect to WTM, N$_2$O production was significantly greater under SI than FD in three sampling occasions following N application (Table 4). Also, N$_2$O production did not differ between the 0- to 0.15- and the 0.15- to 0.30-m soil layers, but it was significantly lower in the 0.45-m soil layer than in the two other layers on the 28 July and 26 August sampling dates (Table 4). For N$_2$O production in 2000, there were three significant two-factor (N × date, WTM × date and depth × date) interactions (Tables 1 and 5). The effects of N application were not significant, except on the 19 September sampling date. Similarly, SI produced greater N$_2$O than FD only on the 3 August sampling date. Differences among depths were significant only on 6 July where the 0.15- to 0.30-m depth produced the largest amount of N$_2$O (Table 5).

Generally speaking, our findings are within the lower range of the values reported by Williams et al. (1992), who compiled and evaluated available data of N$_2$O emissions from different agroecosystems and found N$_2$O
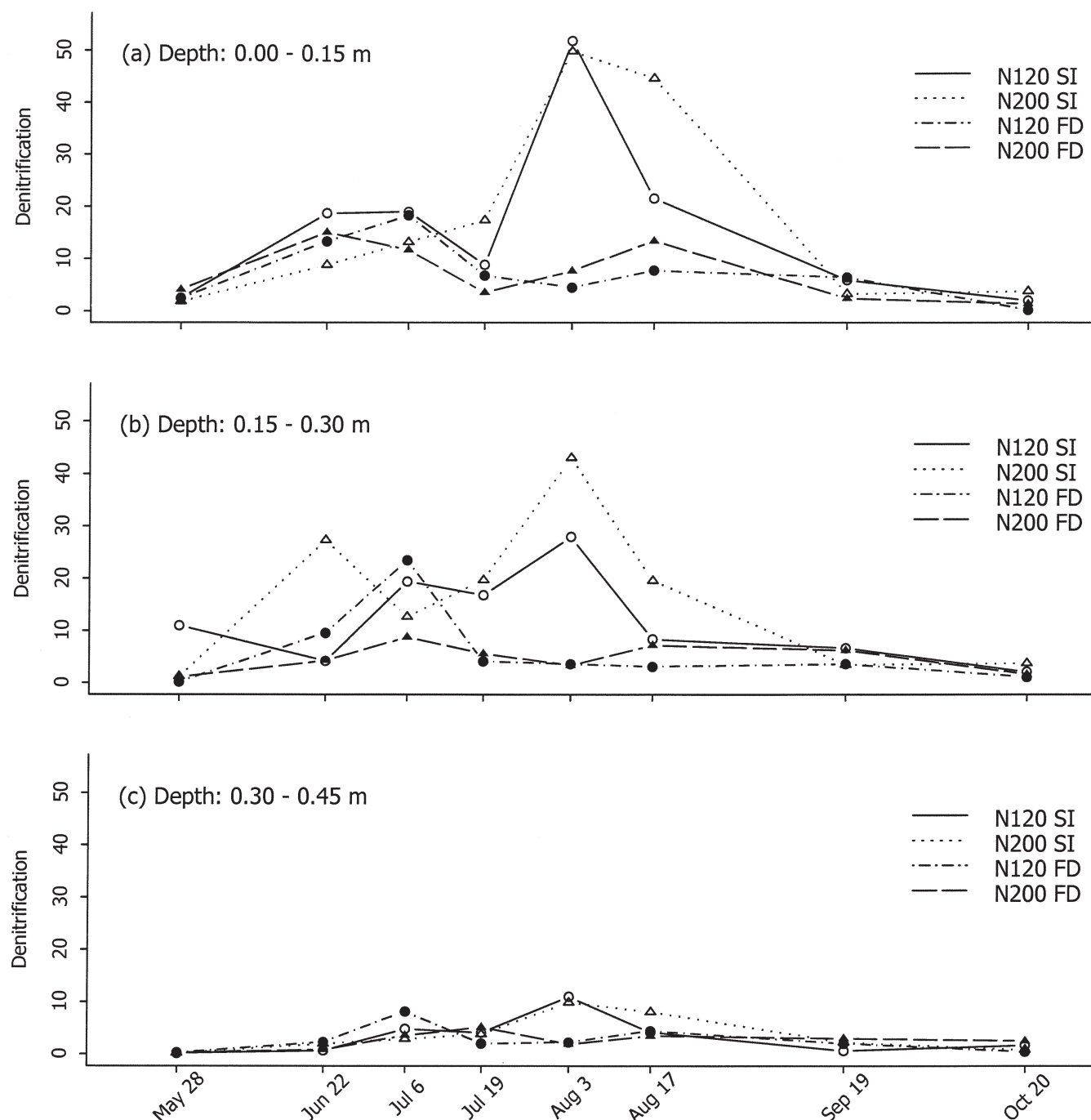
Fig. 1. Interaction effect of fertilizer, water table management (WTM), and date at (a) 0- to 0.15-, (b) 0.15- to 0.30-, and (c) 0.30- to 0.45-m depths on denitrification (g d^{-1} ha^{-1}) measured in 2000.

emissions ranging from 2.4 to 136 g d^{-1} ha^{-1}. The amount of N losses through denitrification obtained in this experiment may be small enough to have little economic or agronomic importance, but significant in terms of their effects on atmospheric chemistry.

Nitrous Oxide to Denitrification Ratio

The N_2O to $(N_2O + N_2)$ ratios at various sampling dates and depths are presented in Tables 6 and 7. The N_2O to $(N_2O + N_2)$ ratio varied between the two growing seasons and provided an interesting contrast. In 1999, N rate had no consistent effect on N_2O to $(N_2O + N_2)$ ratio (Table 6). The N_2O to $(N_2O + N_2)$ ratio was greater from N120 treatment than from the N200 treatment in 28 July, and the opposite was observed in 20 October (Table 6). As shown in Table 6, differences among depths within SI were not significant, except for the 18 September sampling, when the largest N_2O to $(N_2O + N_2)$ ratio for the season was recorded at the 0.30- to 0.45-m depth. Under FD, the largest fraction of N_2O was observed under the 0.30- to 0.45-m depth.

Table 3. Mean denitrification rate (back-transformed to the original scale) of all treatment combinations of the significant four-factor (N × water table management [WTM] × depth × date) interaction effect in 2000.†

N	WTM‡	Depth	28 May	22 June	6 July	19 July	3 August	17 August	19 September	20 October
kg N ha^{-1}		m					g ha^{-1} d^{-1}			
120	SI	0.15	B 2.53 ab	AB 18.72 a	AB 18.97 ab	AB 8.87 ab	A 52.07 a	AB 21.53	AB 5.97	B 2.02
		0.30	10.95 a	4.17 ab	19.40 ab	16.80 a	27.95 ab	8.36	6.68	2.13
		0.45	B 0.20 b	B 0.70 b	A 4.81 ab	A 4.08 ab	A 11.01 ab	A 4.20	B 0.66	AB 1.69
120	FD	0.15	A 2.45 ab	A 13.34 a	A 18.31 ab	A 6.81 ab	A 4.55 b	A 7.77	A 6.48	B 0.23
		0.30	C 0.20 b	AB 9.54 ab	A 23.45 a	AB 4.15 ab	AB 3.61 b	AB 3.11	AB 3.61	BC 1.11
		0.45	B 0.30 b	AB 2.32 ab	A 8.13 ab	AB 2.01 b	AB 2.27 b	AB 4.44	AB 2.03	B 0.46
200	SI	0.15	B 1.82 ab	AB 8.85 ab	AB 13.20 ab	AB 17.35 a	A 50.00 a	A 44.80	B 3.29	B 3.77
		0.30	C 1.22 ab	AB 27.29 a	AB 12.64 ab	AB 19.67 a	A 42.97 a	AB 19.57	BC 3.46	BC 3.73
		0.45	B 0.19 b	AB 1.95 b	AB 2.87 b	A 3.78 b	A 9.89 ab	A 8.03	AB 2.23	AB 0.82
200	FD	0.15	4.08 ab	15.06 a	11.69 ab	3.57 b	7.70 ab	13.42	2.41	1.40
		0.30	1.10 ab	4.23 ab	8.71 ab	5.58 ab	3.34 b	7.17	6.20	1.64
		0.45	B 0.24 b	AB 0.85 b	AB 3.55 ab	A 5.13 ab	AB 1.92 b	AB 3.47	AB 2.99	AB 2.51

† Means preceded by the same uppercase letter (within rows) and means followed by the same lowercase letters (within columns) are not significantly different (Tukey–Kramer adjusted $p > 0.05$).
‡ SI, subirrigation; FD, free drainage.

Table 4. Mean nitrous oxide production rate (back-transformed to the original scale) of all treatment combinations of two significant two-factor (irrigation × date and depth × date) interaction effects in 1999.†

WTM‡	Depth	9 June	7 July	28 July	26 August	18 September	20 October
	m				g ha^{-1} d^{-1}		
SI		B 1.66	A 7.26 a	B 1.43 a	B 0.64 a	C 0.13	C 0.12
FD		B 1.55	A 3.59 b	C 0.38 b	D 0.02 b	D 0.04	D 0.13
	0.15	B 1.47	A 7.27	B 1.26 a	B 0.41 a	C 0.05	C 0.13
	0.30	AB 2.56	A 4.85	BC 0.98 a	CD 0.33 a	D 0.08	D 0.12
	0.45	B 1.10	A 3.78	C 0.33 b	D 0.01 b	CD 0.10	CD 0.12

† Means preceded by the same uppercase letter (within rows) and means followed by the same lowercase letters (within columns) are not significantly different (Tukey–Kramer adjusted $p > 0.05$).
‡ SI, subirrigation; FD, free drainage.

Table 5. Mean nitrous oxide production rate (back-transformed to the original scale) of all treatment combinations of the three significant two-factor (N × date, water table management [WTM] × date, and depth × date) interaction effects in 2000.†

N	WTM‡	Depth	28 May	22 June	6 July	19 July	3 August	17 August	19 September	20 October
kg N ha^{-1}		m					g ha^{-1} d^{-1}			
120			B 0.29	B 0.46	A 3.98	A 2.68	A 4.13	A 1.69	B 0.44 b	B 0.39
200			D 0.24	BC 0.76	A 4.10	A 2.21	A 3.35	A 2.54	AB 1.72 a	CD 0.58
	SI		C 0.26	C 0.59	AB 3.51	A 1.88	A 7.15 a	B 2.11	C 0.60	C 0.44
	FD		C 0.27	C 0.59	A 4.65	AB 3.15	AB 1.93 b	AB 2.03	BC 1.27	C 0.52
		0.15	BC 0.37	BC 0.53	A 2.70 b	A 2.73	A 2.93	AB 1.12	BC 0.70	C 0.26
		0.30	D 0.44	CD 0.98	A 7.45 a	BC 1.91	AB 3.86	ABC 2.29	CD 0.82	D 0.65
		0.45	C 0.11	BC 0.39	A 3.28 ab	A 2.78	A 4.54	A 3.47	AB 1.15	B 0.65

† Means preceded by the same uppercase letter (within rows) and means followed by the same lowercase letters (within columns) are not significantly different (Tukey–Kramer adjusted $p > 0.05$).
‡ SI, subirrigation; FD, free drainage.

In 2000, there were two noticeable trends. First, sampling date (time) was significant only under FD and SI receiving 120 kg N ha^{-1} at the 0.15- to 0.30-m sampling depth (Table 7). Second, there were instances when the N_2O to ($N_2O + N_2$) ratio was greater than 1.0 under FD at the 0.30- to 0.45-m sampling depth (Table 7). These results are in general agreement with those reported by Jacinthe et al. (2000) who observed that concentrations of N_2O remained high at depths lower than 0.40 m of soil columns. They attributed this to a low activity of N_2O-reductase at that depth and, consequently, N_2O was not being reduced at a significant rate.

The average values of N_2O to ($N_2O + N_2$) ratios were generally higher under FD than under SI during both growing seasons. This was particularly noticeable at the 0.30- to 0.45-m depth, where the N_2O to ($N_2O + N_2$) ratios were close or exceeded 1.0 in several sampling dates (Tables 6 and 7). Under some conditions, N_2O can be produced simultaneously by nitrification and denitrification, so the production of N_2O from nitrification could affect calculated N_2O to ($N_2O + N_2$) ratios. If nitrification is a source of N_2O then the denitrification rate could be underestimated by the C_2H_2 inhibition technique (Malone et al., 1998), resulting in N_2O to ($N_2O + N_2$) ratios of >1.0. As indicated earlier, water-filled pore space was greater under SI than FD. Consequently, denitrification presumably produced the major share of N_2O and that the major proportion of the gas-

Table 6. Mean percentage ratio of N_2O to $(N_2O + N_2)$ (back-transformed to the original scale) of all treatment combinations of the significant two-factor (N × date) and three-factor (water table management [WTM] × depth × date) interaction effects in 1999.†

WTM‡	Depth	N	9 June	7 July	28 July	26 August	18 September	20 October
	m	kg N ha^{-1}						
SI	0.15		A 0.20	A 0.21 b	A 0.30 ab	A 0.13	B 0.01 b	A 0.15
SI	0.30		0.35	0.29 ab	0.15 b	0.14	0.10 b	0.04
SI	0.45		0.35	0.20 b	0.23 ab	0.05	0.67 a	0.12
FD	0.15		AB 0.20	0.15 b	0.72 a	0.5	AB 0.54 a	B 0.10
FD	0.30		0.25	0.44 ab	0.52 ab	0.35	0.04 b	0.3
FD	0.45		0.98	0.98 a	0.52 ab	0.54	0.72 a	0.22
		120	A 0.33	A 0.34	A 0.42 a	B 0.09	B 0.05	B 0.07 b
		200	0.32	0.27	0.29 b	0.16	0.1	0.24 a

† Means preceded by the same uppercase letter (within rows) and means followed by the same lowercase letters (within columns) are not significantly different (Tukey–Kramer adjusted $p > 0.05$).
‡ SI, subirrigation; FD, free drainage.

eous N production would have been emitted as N_2. The relative contribution of nitrification to N_2O production may be addressed by differentially inhibiting nitrification and denitrification by varying C_2H_2 concentration. An C_2H_2 concentration of 5 to 10% (v/v) is needed to block N_2O-reductase (Tiedje et al., 1989), but a concentration of 0.01% (v/v) is sufficient to block nitrification (Davidson et al., 1986). Field studies of this kind, however, are generally lacking because this approach is prohibitively expensive and can damage the field by removing large-quantity soil core samples. In a study conducted on a different site, Elmi et al. (2003) concluded that the main source of N_2O is denitrification at water-filled pore space > 60%. We interpret that wet conditions under SI may increase the residence time of N_2O in the soil by restricting diffusion and, consequently, enhance the reduction of N_2O to N_2. Supporting this argument is the fact that all occasions in which N_2O to $(N_2O + N_2)$ ratio was greater than 1.0 occurred only under FD, possibly because of better aeration encouraging the nitrification process. Under forest soil, Mogge et al. (1998) reported denitrification ratios of >1.0 for 50% of all measurements, suggesting nitrification was much greater importance for N_2O production than denitrification. Jarvis and Pain (1994) proposed a value of 0.25 (1:4) mole fraction to be used in budget studies to estimate denitrification losses from N_2O measurements. While this value is favorably comparable with the mole fraction of N_2O

under SI in our study, it will probably underestimate the contribution of N_2O in agricultural systems where FD is practiced.

These results may have implications when devising strategies to minimize N pollution from various agricultural practices. According to our findings, a value of 0.40 to 0.45 mole fraction ratio appears to be appropriate for freely drained soils. The values of N_2O to $(N_2O + N_2)$ ratios obtained in our study are greater than those reported by Kliewer and Gilliam (1995) who found the N_2O fraction represented only a small fraction (2%) of total denitrification. In their study, water tables were set at a much shallower depth than ours (up to 0.15 m), which apparently saturated soil columns and promoted a more complete reduction of N_2O to N_2. For a practical purpose, however, such shallow water tables can only be recommended during the nongrowing period, in order not to interfere with crop growth, tillage, and other field operations.

It is important to point out, however, that the diffusion of the N_2O generated at a certain depth in the soil profile to the surface soil is a critical factor that determines the final emission at the soil surface. Nitrous oxide produced near the soil surface would probably have readily diffused out of the soil into the atmosphere. In contrast, N_2O produced at deeper depths may have taken longer to diffuse from the soil, providing more

Table 7. Mean percentage ratio of N_2O to $(N_2O + N_2)$ (back-transformed to the original scale) of all treatment combinations of the significant four-factor (N × water table management [WTM] × depth × date) interaction effect in 2000.†

N	WTM‡	Depth	28 May	22 June	6 July	19 July	3 August	17 August	19 September	20 October
kg N ha^{-1}		m								
120	SI	0.15	0.46	0.04	0.11 b	0.29 ab	0.21 ab	0.12	0.06 ab	0.09
120	SI	0.30	AB 0.05	AB 0.27	A 0.77 ab	AB 0.14 ab	AB 0.24 ab	AB 0.22	B 0.02 b	AB 0.25
120	SI	0.45	0.36	0.14	0.67 ab	0.47 ab	0.61 ab	0.44	0.37 ab	0.46
120	FD	0.15	0.69	0.04	0.13 b	0.88 a	0.29 ab	0.19	0.05 ab	1.07
120	FD	0.30	AB 0.30	B 0.03	AB 0.22 ab	AB 0.56 ab	A 0.82 ab	AB 0.18	AB 0.23 ab	AB 0.69
120	FD	0.45	0.53	0.36	0.39 ab	1.15 a	1.13 ab	0.80	0.85 a	0.7
200	SI	0.15	0.10	0.09	0.28 b	0.07 b	0.12 b	0.05	0.27 ab	0.15
200	SI	0.30	0.24	0.05	0.21 ab	0.05 b	0.13 b	0.19	0.60 ab	0.11
200	SI	0.45	0.59	0.16	0.65 ab	0.86 a	0.85 ab	0.56	0.75 a	0.52
200	FD	0.15	0.07	0.09	0.24 ab	0.86 a	0.11 b	0.05	0.84 a	0.15
200	FD	0.30	0.93	0.51	1.76 a	0.43 ab	0.60 ab	0.27	0.27 ab	0.66
200	FD	0.45	0.42	0.90	1.73 a	0.81 a	1.52 a	1.4	0.83 a	0.66

† Means preceded by the same uppercase letter (within rows) and means followed by the same lowercase letters (within columns) are not significantly different (Tukey–Kramer adjusted $p > 0.05$).
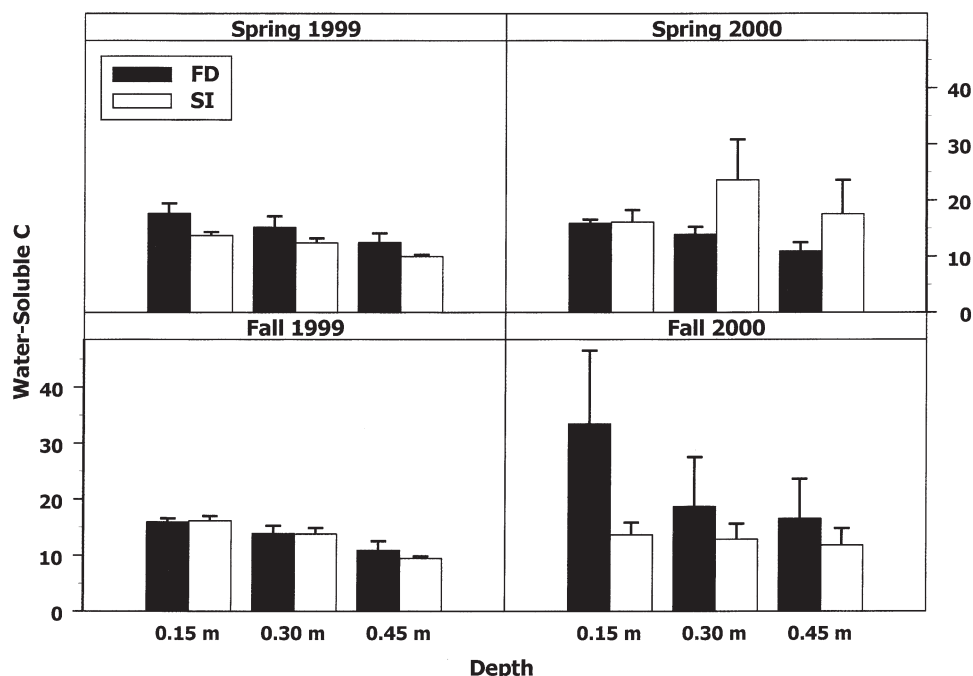‡ SI, subirrigation; FD, free drainage.

Fig. 2. Water-soluble carbon (WSC) concentration (mg kg^{-1}) in the soil profile in spring and fall of 1999 and 2000. Vertical bars represent standard error of the mean ($n = 6$).

opportunity for N_2O to be reduced to N_2 rather than being allowed to diffuse into the atmosphere.

Role of Water-Soluble Carbon in Denitrification Activity

Although both denitrification and N_2O production decreased with depth in both years, measurable denitrification occurred at depths lower than 0.15 m (Tables 2–5). McCarty and Bremner (1992) reported very low rates of subsoil (0.25- to 2-m depth) denitrification in response to added NO_3^-–N, but addition of both NO_3^-–N and glucose stimulated denitrification activity. They concluded that the low rate of denitrification in subsoils was due to a lack of available organic C.

In our study, however, there were no clear trends with WSC concentrations in the soil profile (Fig. 2). The WSC concentrations remained relatively uniform, ranging from 5 to 30 mg WSC L^{-1}. Despite the relationship between soil organic C and denitrification that was established decades ago (Burford and Bremner, 1975; Bremner and Shaw, 1958), the critical level of WSC needed for denitrification to proceed is not yet well-defined, particularly in soils with different drainage systems. For example, Beauchamp et al. (1980) observed that regressions of denitrification rate on WSC were nonsignificant with poorly drained soils whereas they were highly significant with well or moderately drained soils. They also noted that a WSC content greater than 40 mg kg^{-1} soil was required to support denitrifiers. In the Burford and Bremner (1975) study, however, data presented suggest that denitrification can occur at lower WSC levels.

In the present study, concentrations of WSC (Fig. 2) may suggest that WSC in this soil was sufficiently high

for denitrification to proceed. Mean WSC concentrations were generally similar or higher in FD than SI (Fig. 2), but the differences were not significant ($p > 0.05$) in all sampling periods. Trends toward lower WSC under SI than FD in spring 1999 and fall 2000 may suggest that WSC in SI plots might have been metabolized at a greater rate than in FD plots.

Water-soluble C is dynamic and can be influenced by the growth of crops. Although changes in WSC differed little due to sampling period (Fig. 2), we acknowledge that WSC values measured during spring and fall may not reflect accurately the influence of WSC on denitrification dynamics during the growing season. On the basis of the data available, we cannot be definitive how WSC concentrations would have changed within the crop root zone if sampling was continued throughout the growing season. Further study on the dynamic changes of WSC during active plant growth is required to determine whether WSC can explain a significant portion of denitrification process.

CONCLUSIONS

Denitrification capacity at soil depth has received surprisingly little attention. Information on N_2O to (N_2O + N_2) ratio during denitrification is important in assessing the amount of N_2O production from denitrification and its potential environmental impact. This work reports on a two-year field study during which denitrification, N_2O production, and their ratio in the subsurface environment (0–0.45 m) as influenced by WTM and N application rate were quantified. Denitrification rates in the soil profile were generally greater under SI than FD in most sampling dates, irrespective of soil depth and N rate. We found that ratios of N_2O to (N_2O + N_2) were

lower in SI plots than FD, suggesting that SI treatment created conditions that enhance further reduction of N_2O. This is an indication that higher denitrification rates under SI than FD do not necessarily add to concerns over global atmospheric N_2O loadings. Significant amounts of denitrification were measured at deeper soil layers, especially the 0.15- to 0.30-m layer. We therefore conclude that the assumption that denitrification in the top soil layer (0–0.15 m) is representative of the overall rate of denitrification from the soil may not always be true. We recognize that WSC measured before planting and after harvest may not reflect the influence of WSC on denitrification during the growing season. Our results showed no consistent differences between 120 and 200 kg N ha^{-1} rates at any soil depth. Therefore, we conclude that emphasis on N rate management alone may not be a sufficient strategy to overcome environmental concerns associated with N applications.

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REFERENCES

Aulakh, M.S., D.A. Rennie, and E.A. Paul. 1984. Gaseous N losses from soil under zero-till compared with conventional-till management systems. J. Environ. Qual. 13:130–136.

Beauchamp, E.G., C. Gale, and J.C. Yeomans. 1980. Organic matter availability for denitrification in soils of different textures and drainage classes. Commun. Soil Sci. Plant Anal. 11:1221–1233.

Bremner, J.M., and K. Shaw. 1958. Denitrification in soil II: Factors affecting denitrification. J. Agric. Sci. 51:40–52.

Burford, J.R., and J.M. Bremner. 1975. Relationships between the denitrification capacities of soils and total, water soluble and readily decomposable soil organic matter. Soil Biol. Biochem. 7:389–394.

Davidson, E.A., W.T. Swank, and T.O. Perry. 1986. Distinguishing between nitrification and denitrification as sources of gaseous nitrogen production in soil. Appl. Environ. Microbiol. 52:1280–1286.

Duxbury, J.M., D.R. Bouldin, R.E. Terry, and R.L. Tate. 1982. Emissions of nitrous oxide from soils. Nature (London) 298:462–464.

Ellis, S., E. Yamulk, R. Dixon, R. Harrison, and S.C. Jarvis. 1998. Denitrification and N_2O emissions from a UK pasture soil following early spring application of cattle slurry and mineral fertiliser. Plant Soil 202:15–25.

Elmi, A.A., C. Madramootoo, M. Egeh, G. Dodds, and C. Hamel. 2002a. Water table management as a natural bio-remediation technique of nitrate pollution. Water Qual. Res. J. Can. 37:563–576.

Elmi, A.A., C. Madramootoo, M. Egeh, A. Liu, and C. Hamel. 2002b. Environmental and agronomic implications of water table and nitrogen fertilization management. J. Environ. Qual. 31:1858–1867.

Elmi, A.A., C. Madramootoo, C. Hamel, and A. Liu. 2003. Denitrification and nitrous oxide to nitrous oxide plus dinitrogen ratios in the soil profile as influenced by tillage systems and crop residue in a sandy loam soil. Biol. Fertil. Soils 38:340–348.

Elmi, A.A., C. Madramootoo, M. Egeh, and C. Hamel. 2004. Water and fertilizer nitrogen management to minimize nitrate pollution from a cropped soil in southwestern Quebec, Canada. Water Air Soil Pollut. 151:117–134.

Jacinthe, P.-A., W.A. Dick, and L.C. Brown. 2000. Bioremediation of nitrate-contaminated shallow soils using water table management techniques: Production and evolution of nitrous oxide. Soil Biol. Biochem. 32:371–382.

Jarvis, S.C., and D.J. Hatch. 1994. Potential for denitrification at depth below long-term grass swards. Soil Biol. Biochem. 26:1629–1636.

Jarvis, S.C., and B.F. Pain. 1994. Greenhouse gas emissions from intensive livestock systems: Their estimation and technologies for reduction. Clim. Change 27:27–38.

Kliewer, B.A., and J.W. Gilliam. 1995. Water management effects on denitrification and nitrous oxide evolution. Soil Sci. Soc. Am. J. 59:1694–1701.

Koops, J.G., O. Oenema, and M.L. Van Beusichem. 1996. Denitrification in the top and sub soil grassland on peat soils. Plant Soil 184:1–10.

Littell, R.C., G.A. Milliken, W.W. Stroup, and R.D. Wolfinger. 1996. SAS system for mixed models. SAS Inst., Cary, NC.

MacKenzie, A.F., M.F. Fan, and F. Cardin. 1997. Nitrous oxide emissions as affected by tillage, corn-soybean-alfalfa rotations and nitrogen fertilization. Can. J. Soil Sci. 77:145–152.

Malone, J.P., R.J. Stevens, and R.J. Laughlin. 1998. Combining the 15N and acetylene inhibition techniques to examine the effect of acetylene on denitrification. Soil Biol. Biochem. 30:31–37.

McCarty, G.W., and J.M. Bremner. 1992. Availability of organic carbon for denitrification of nitrate in subsoils. Biol. Fertil. Soils 14:219–222.

Mogge, B., E.-A. Kaiser, and J.-C. Much. 1998. Nitrous oxide emissions and denitrification N-losses from forest soils in the Bonhöved lake region (northern Germany). Soil Biol. Biochem. 30:703–710.

Montgomery, D.C. 2001. Design and analysis of experiments. 5th ed. John Wiley & Sons, New York.

Ryan, M., D. Noonan, and A. Fanning. 1998. Relative denitrification rates in surface and subsurface layers of mineral soil. Ir. J. Agric. Food Res. 37:141–157.

Ryden, J.C., J.H. Skinner, and D.J. Nixon. 1987. Soil core incubation system for the field measurement of denitrification using acetylene inhibition method. Soil Biol. Biochem. 19:753–757.

SAS Institute. 1999. SAS online doc. Version 8. SAS Inst., Cary, NC.

Tiedje, J.M., S. Simkins, and P.M. Groffman. 1989. Perspectives on measurement of denitrification in the field including recommended protocols for acetylene based methods. Plant Soil 115:261–284.

Velthof, G.C., A.B. Brader, and O. Oenema. 1996. Seasonal variations in nitrous oxide losses from managed grasslands in the Netherlands. Plant Soil 181:263–274.

Williams, E.J., G.L. Hutchinson, and F.C. Fehsenfeld. 1992. NO_x and N_2O emissions from soil. Global Biogeochem. Cycles 6:351–388.