Spatial Heterogeneity in the Distribution of Denitrifying Bacteria Associated with Denitrification Activity Zones

ROBERT E. MURRAY,^{1*} YVETTE S. FEIG,¹ AND JAMES M. TIEDJE²

*Department of Biology, Appalachian State University, Boone, North Carolina 28608,¹ and
Center for Microbial Ecology, Michigan State University, East Lansing, Michigan 48824²*

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Denitrifying enzyme activity (DEA) in artificially established denitrification activity zones was, on average, 3.5-fold greater than in bulk soil. Denitrifier biomass was between 1 and 4 orders of magnitude greater in activity zones. Denitrifier specific activity was lower in activity zones and more similar to specific activity values reported for pure cultures of denitrifying bacteria added to soil. Measurements of DEA and biomass carried out on a homogeneous subsample derived from a soil core containing a denitrification activity zone will overestimate the DEA and biomass of the bulk soil and underestimate the DEA and biomass of the activity zone.

Measurements of nitrogen gas loss from soil often exhibit large coefficients of variation (3, 4, 6–10), and nitrogen gas loss is poorly correlated with both the synthesis of denitrifying enzymes (denitrifying enzyme activity [DEA]) and denitrifier biomass (most-probable-number [MPN] counts [9]). Field measurements of denitrifier specific activity (the maximum potential rate of nitrogen utilization per cell) are often several orders of magnitude greater than that expected on the basis of specific activity values reported for pure cultures of denitrifying bacteria added to soil (5). This discrepancy has led to speculation that physiologically active cells are isolated from soil with much higher efficiency in MPN counts than older or inactive cells and that MPN-based isolation techniques thus underestimate denitrifier numbers in soil between 1 and 2 orders of magnitude (5).

In addition to the problem of recovering inactive organisms, another part of the explanation for the lack of correlation between nitrogen gas loss, DEA, and MPN counts may be related to the way in which soil microsites structure the population dynamics of denitrifying bacteria. The activity of denitrifying bacteria is not uniformly distributed in soil, and high rates of nitrogen gas loss are often associated with denitrification activity centers, or hot spots (1, 2, 7). Nitrogen gas loss from soil is usually measured from intact soil cores which maintain within-core spatial heterogeneity. DEA and population counts are usually measured on core subsamples which have been mixed and from which large pieces of decaying organic material have been removed. The disruption of soil heterogeneity prior to measurements of denitrifying enzyme activity and population density may have a serious and unintended effect on our perception of the relationship between nitrogen gas loss from soil and denitrifier population dynamics.

In this study we investigated how spatial heterogeneity in the distribution of denitrifying bacteria can cause poor estimates of whole-core rates of DEA, denitrifier biomass, and specific activity. We compared rates of DEA, denitrifier biomass, and specific activity in small sections of soil cores enriched with glucose and nitrate and larger core sections containing bulk soil.

Soil cores (2.2-cm diameter, 15 cm long) from conventional tillage (CT) and no-tillage soils planted with corn were col-

lected at the Michigan State University agricultural ecosystem Long-Term Ecological Research site in July 1993. Soil cores were stored at 4°C until used. All cores were used within a period of approximately 3 weeks. Denitrification activity zones were established in the cores by placing a section of dried (105°C for >48 h) corn leaf (approximately 2.5 by 4.5 cm), which had been saturated with a solution of 10 mM glucose and 10 mM nitrate and folded into a cylindrical shape, in the lower 3 cm of the core. A short vertical slice was made in the central region of the bottom of the cores, the leaf material was inserted into the opening formed by the slice, and the soil was closed around the leaf material. Denitrification rate was determined from measurements of nitrous oxide production from intact soil cores or soil core sections amended with acetylene, using the general procedure of Parsons et al. (9) and Tiedje et al. (13).

Denitrification activity zones were successfully established with dried leaf material soaked with glucose and nitrate. Denitrification rates in a CT soil core containing an established activity zone and a CT core flooded with a solution containing 10 mM glucose and 10 mM nitrate were similar (1.140 and 1.302 μg of $\text{N}_2\text{O-N}$ core h^{-1} , respectively, 19 h after amendment) and approximately 30-fold greater than the denitrification rate in an unamended CT control core (0.040 μg of $\text{N}_2\text{O-N}$ core h^{-1}). The denitrification rate in the control core

TABLE 1. Denitrification rates of core sections 40 h after establishment of denitrification activity zones

Core and section ^a	% of core dry wt	Denitrification rate (ng of $\text{N}_2\text{O-N}$ min ⁻¹)		% of total rate ^b
		Dry wt (g)	Section	
CT-1				
1	70	0.039	2.466	19
2	26	0.438	10.116	79
3	4	0.073	0.254	2
CT-2				
1	72	0.123	8.096	18
2	23	1.468	31.356	69
3	5	1.396	6.155	14

^a See text for compositions of sections.

^b Denitrification rate of core section divided by the sum of denitrification rates of three sections $\times 100$.

* Corresponding author. Phone: (704) 262-2666. Fax: (704) 262-2127.

TABLE 2. Distribution of DEA and denitrifier biomass in bulk soil sections and established activity zones

Core section ^a	% of core dry wt	% of total DEA	% of total biomass
CT-1	74	67	16.5
AZ	26	33	83.5
NT-1	89	75	0.2
AZ	11	25	99.8
NT-2	90	64	1.6
AZ	10	36	98.4
CT-3	80	28	0.2
AZ	20	72	99.8
NT-3	82	80	0.7
AZ	18	20	99.3
NT-4	90	95	41.6
AZ	10	5	58.4

^a AZ, activity zones; NT, no-tillage soil.

increased 68-fold ($2.736 \mu\text{g}$ of $\text{N}_2\text{O-N}$ core h^{-1}) 40 h after an activity zone was established in this core. Treatment of soil cores with glucose-and-nitrate-amended leaf material increased whole-core denitrification rates between 30- and 68-fold and successfully established denitrification activity zones.

Two soil cores containing established activity zones were divided into three sections. The three sections included the bulk soil (section 1), the area adjacent to the leaf material (section 2), and the area containing the leaf material and a small amount of attached soil (section 3). Sections 2 and 3 were collected in order to distinguish denitrifier activity in the soil adjacent to the leaf material from denitrifier activity associated with the leaf surface. The core sections were placed in 125-ml Erlenmeyer flasks and assayed for denitrification rate. Core sections containing established activity zones and representing between 23 and 26% of the total core dry weight were responsible for between 69 and 79% of the total core denitrification rate (Table 1). In both cores (Table 1), the highest rates of denitrification occurred in soil (section 2) adjacent to the

added leaf material (section 3), suggesting that activity zones were composed of indigenous organisms which were stimulated by the release of glucose and nitrate solution from the leaf material.

DEA was measured by the technique of Smith and Tiedje (11). Soil samples were thoroughly mixed but not sieved prior to subsampling for DEA and MPN determinations. The DEA assay media contained chloramphenicol, which prevents the synthesis of new denitrifying enzymes during the assay period. Activity zones established in six separate cores (AZ, Tables 2 and 3) contained between 5 and 72% of the total DEA. In all but one case, over 83.5%, and usually over 98%, of total denitrifier biomass was located in activity zones. In all cases, the majority of denitrifier biomass (enumerated by the MPN technique [9, 12]) occurred in established activity zones.

In core NT-1 (Tables 2 and 3), for example, a 7.9-g dry soil activity zone contained a total of 3.2×10^7 denitrifying bacteria. The bulk soil section (62.6 g of dry soil) contained a total of 6.6×10^4 denitrifying bacteria. If all of the denitrifying bacteria in core NT-1 were evenly distributed and the core was assayed for denitrifier biomass, a mean value of 4.5×10^5 denitrifier cells per g of dry soil would be determined. A homogeneous subsample from core NT-1 would overestimate the denitrifier biomass of the bulk soil by 1 order of magnitude and underestimate the denitrifier biomass of the activity zone by 2 orders of magnitude. Denitrifier population estimates determined from a homogeneous subsample derived from a soil core containing a denitrification activity zone do not provide an accurate measurement of the biomass of denitrifying bacteria within the bulk soil or the activity zone.

In five of six cores (Table 3), DEA in established activity zones was greater than DEA in bulk soil. DEA was 10-fold greater in the activity zone established in core CT-3 than in the bulk soil. Denitrifier biomass was greater in all established activity zones and was 3 orders of magnitude greater than bulk soil in four of six cores. Denitrifier specific activity (calculated by dividing DEA by denitrifier biomass) in bulk soil was similar to values reported for agricultural soils (5). In all cases, denitrifier specific activity was between 1 and 3 orders of magnitude lower in activity zones and more similar to specific activity values (0.7×10^{-16} to 4.4×10^{-16} g of N min^{-1} cell $^{-1}$ [5]) reported for pure cultures of denitrifying bacteria added to

TABLE 3. DEA, biomass (MPN), and specific activity in bulk soil sections and denitrification activity zones

Time elapsed from initiation of activity zones (h)	Soil ^a	DEA (SD) (ng of $\text{N}_2\text{O-N}$ g of dry soil $^{-1}$ min $^{-1}$)	n	MPN (SD) (log cells g of dry soil $^{-1}$)	n	Sp act (10^{-16} ; g of N min $^{-1}$ cell $^{-1}$)
38	CT-1	0.169 (0.044)	2	4.52 (0.385)	2	50.6
	AZ	0.243	1	5.69	1	5.0
65	NT-1	0.456	1	3.03	1	4,300.0
	AZ	1.230	1	6.61	1	3.1
65	NT-2	0.280	1	3.37	1	1,190.0
	AZ	1.376	1	6.09	1	11.2
66	CT-3	0.017 (0.004)	3	3.93 (0.455)	3	20.0
	AZ	0.170 (0.098)	3	7.30 (0.771)	2	0.1
89	NT-3	0.986	1	3.23	1	5,814.0
	AZ	1.039	1	6.00	1	10.5
89	NT-4	1.065	1	3.60	1	2,708.0
	AZ	0.406	1	4.68	1	84.2

^a AZ, activity zones; NT, no-tillage soil.

soil. The activity zone in core NT-1 (Table 3), which represented 11.13% of the total core dry weight, contained 99.79% of the total culturable denitrifying bacteria within the whole core and exhibited a specific activity in the range of values reported for pure cultures. Natural populations of bacteria in denitrification activity zones can exhibit specific activities similar to values reported for pure cultures of denitrifying bacteria added to soil (Table 3).

Part of the discrepancy between specific activity measurements made in pure cultures and those made in soil may be due to the presence of denitrification activity zones. In core NT-1 (Table 3), the bulk soil (62.6 g of dry soil) had a specific activity of $4,300 \times 10^{-16}$ g of N min^{-1} cell^{-1} and the established activity zone (7.9 g of dry soil) had a specific activity of 3.1×10^{-16} g of N min^{-1} cell^{-1} . A homogeneous subsample from core NT-1 would have had a specific activity of 10.0×10^{-16} g of N min^{-1} cell^{-1} . This sample would overestimate the specific activity of the activity zone by 3-fold and underestimate the nominal specific activity of the bulk soil by 430-fold. This sampling regimen would ignore the distinct spatial heterogeneity within the core and would not detect any difference in specific activity between the activity zone and the bulk soil.

Martin et al. (5) interpreted the difference between specific activity values of pure cultures of denitrifying bacteria added to soil and those of in situ denitrifier populations as evidence for the existence of a large population of denitrifying bacteria that cannot be isolated but do contain functional denitrifying enzymes. Specific activity values for bulk soil for four of the cores (NT-1 to NT-4) in Table 3 are an order of magnitude larger than values reported by Martin et al. (5). The large specific activity values are associated with low levels of recoverable denitrifier biomass (approximately 10^3 cells g of dry soil $^{-1}$) and indicate that in some soils the MPN technique may underestimate denitrifier density by as much as 3 orders of magnitude. The difference in specific activity values between the two studies could be related to seasonal or site differences between the soils (moisture, carbon availability, or temperature) or soil handling (the soils used by Martin et al. [5] were sieved prior to assay).

There are three possible explanations for the occurrence of higher levels of denitrifier biomass in the established activity zones. The glucose-and-nitrate enrichment could have stimulated the growth of indigenous organisms or enhanced the recovery of organisms, or elevated levels of denitrifier biomass could have been present in the area prior to the enrichment.

It is not possible to sample the same area of a soil core for DEA and MPN measurements before and after the establishment of activity zones because both measurements require destructive sampling. We have demonstrated that the technique for establishing activity zones can increase whole-core denitrification rates between 19- and 68-fold and that the increase in denitrification rate occurs in the area of the core that receives the nutrient input (Table 1). If the bulk soil contained previously existing activity zones, then the bulk soil sections (Table 1) would exhibit high rates of denitrification. Moreover, if the bulk soil contained large areas of elevated denitrifier biomass prior to our treatment, we would not expect to see the large differences in MPN biomass and specific activity that occur between established activity zones and the bulk soil (Tables 2 and 3).

Given the low background rate of denitrification in the soil ($0.040 \mu\text{g}$ of $\text{N}_2\text{O-N}$ core h^{-1}) and bulk soil (Table 1) and the

very patchy distribution of denitrification hot spots (1, 2, 7, 8) in agricultural soils in general, it is extremely unlikely that the denitrification activity zones in the six soil cores evaluated in Table 3 contained areas of elevated denitrification activity and biomass prior to nutrient enrichment. In the unlikely event that we augmented previously existing areas of elevated denitrifier biomass when activity zones were established, this occurrence would only emphasize the importance of spatial heterogeneity in the in situ distribution of microbial populations.

Tables 1, 2, and 3 clearly demonstrate that spatial heterogeneity in the distribution of DEA and denitrifier biomass can occur in agricultural soils and that populations of denitrifying bacteria in soil can exhibit specific activities in the range of values reported for pure cultures of denitrifying bacteria added to soil. Measurements of denitrifier specific activity must be carried out in a manner which takes into account the potential heterogeneous distribution of denitrifying bacteria in soil. Measurements made from a mixed and homogenized soil sample obtained from a soil core containing a zone of elevated denitrification activity will not provide an accurate measurement of the physiological status or the population densities of all of the denitrifying bacteria within the soil core. This difficulty has probably been at least partially responsible for the generally poor correlations between nitrogen gas loss, DEA, and MPN population measurements observed in field studies (5, 9).

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