Nitrification in adjacent cultivated and pasture landscapes - A comparison

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Abstract

Variability in nit&cation and autotrophic nitrifying communities were assessed at shoulder and footslope positions of a cultivated and a pasture landscape. The sampling design was a systematic grid design and all analysis were carried out for soils collected from 10 grid points at the shoulder and the footslope position of each landscape. Gross nitrification was measured in intact soil cores using ^{15}N isotope pool dilution technique. NH,' oxidizers were enumerated using the most probable number technique and Michaelis-Menten kinetics for NH_4^+ oxidation was determined in soil slurries. Gross nitrification did not vary between or within studied sites and remained less than 20% of the V_{max}. Population densities of NH_4^+ and NO_2^- oxidizing bacteria at the cultivated site were about 1 O-fold higher than those at the pasture site. The V_{max} for NH,' oxidation was 28 and 15 $\mu g\ g^{-1}$ soil d^{-1} at the cultivated and the pasture site respectively and varied significantly between sites. The apparent K_m for NH_4^+ oxidation varied from 5 to 8 μM at the cultivated site and they were about 10 to 1 OO-fold lower than those observed for the pasture site. Although in ***situ*** nitrification did not differ between sites, significantly higher V_{max} indicated that cultivation practices enhance nitrification by sustaining a larger nitrifying community having a higher substrate affinity. NH_4^+ oxidizing bacterial communities were different between sites with respect to the substrate affinity and the competitive ability for the substrate.

Introduction

Cultivation of undisturbed ecosystems results in changes in the physical and chemical nature of microbial habitats, and thereby the species diversity and the activity of microbial communities (Atlas and Bartha, 1993; Biederbeck et ***al.,*** 1995). As a consequence, differences in the microbially-mediated processes particularly associated

with nutrient cycling, are often observed between undisturbed and cultivated ecosystems (Schimel, 1986). Autotrophic nitrification is one such biological phenomenon change as undisturbed ecosystems are subjected to cultivation. It is carried out by an autotrophic nitrifying bacterial community, in which several bacterial spp. coexist competing for available substrates. However, the sensitivity of autotrophic nitrifiers toward environmental factors is varied among spp. and therefore different dominant nitrifyingg species could be established in various ecosystems. The dominant nitrifiers can be recognized by their nitrification rates and population densities as individual species are characterized by different growth and activity kinetics (Koops and Moller, 1992; Smorczewski and Schmidt, 1991). The objectives of this study were to quantify nitrification and to characterize the NH_4^+ oxidizing bacterial communities within an undisturbed (pasture) and a cultivated landscape.

Materials and methods

The study was conducted in cultivated and pasture (undisturbed) landscapes located at St Louis, Saskatchewan and the soil are characterized as clay loam Black Chemozem (Udic Boroll). Two landscape positions were ascertained: shoulder (upper slope positions) and footslope (lower slope positions) as described by Corre et al. (1996). The cultivated site was planted with pea (3 weeks old at the time of soil sampling) and urea was applied at a rate of 11 kg ha^{-1}. The shoulder of the pasture site was occupied with smooth bromegrass (Bromus *inermis.* Leyss) and the dominant vegetation at the footslope consists of aspen (Populaus *tremuloides* Michx.) and bromegrass. Measurements were made in July 1995, at randomly selected 10 grid points at each shoulder and footslope positions of both landscapes.

Gross nitrification was determined *in situ* using intact cores. Six metal cores (7.5 cm diameter x 10 cm height) were driven into the soils at each grid point. $K^{15}NO_3$ solution (Atom percent ^{15}N excess -70%) was injected into each core and three cores were collected from each grid point after 30 min. of isotope injection. At the site, soil was thoroughly mixed in a polythene bag and mixed with 2 M KC1 immediately. Following transport to the laboratory, soil mixtures were shaken and supematants were obtained. A similar extraction procedure was followed for the rest of the soil cores after

an incubation period of 24 h. Aliquotes of KC1 extractants were analysed for $NO_2^- + NO_3^-$ contents using a Technicon Autoanalyzer II System (Labtrronics Inc., Tarrytown, NY) and for $^{15}N/^{14}N$ ratio using a continuous flow, isotope ratio mass spectrometer followed by steam distillation. Gross nitrification and $NO_2^- + NO_3^-$ consumption rates were calculated as described by Kirkham and Bartholomew (1954).

Michaelis-Menten kinetics for NH_4^+ oxidation was determined by measuring net nitrification rates in soil slurries. Soil **(10 g)** was slurried in 50 ml of 1 *mM* phosphate buffer (pH~7.2) amended with different NH_4^+ concentrations and $CaCO_3$ (1 g L^{-1}) (Hart et ***al.,*** 1994; Verhagen et ***al,*** 1992). Slurries were shaken on an orbital shaker (180 rev min^{-1}) at a temperature of 24^0C (±1) for a period of 6 h. Aliqouts of 2 mL were removed from the slurry periodically and $NO_2^- + NO_3^-$ contents of supematants were measured as described previously. Apparent saturation constant (K_m) and maximum activities (V_{max}) for NH_4^+ oxidation were computed by double reciprocal plots.

The NH_4^+- and NO_2^- oxidizing bacteria were enumerated using the Most Probable Number (MPN) technique (Schmidt and Belser, 1994). Chloroform fumigation extraction method was used to determine soil microbial biomass N content (Voroney, 1993). Soil pH was measured in a 1: 1 water paste.

Results and Discussion

Soil properties

Selected soil properties of investigated sites are presented in Table 1. Moisture contents varied significantly between sites. Cultivation practices have increased bulk density by a factor of two compared with the pasture site. Although not significant, soil pH was apparently lower at the cultivated site perhaps due to the fertilizer application. Cultivation also resulted in a significant decrease in microbial biomass N contents. Moisture was the only factor that varied significantly within the cultivated site. Presence of a litter layer resulted in significantly higher moisture contents at the footslope of the pasture site compared to the shoulder. Differences in the soil characteristics within the pasture site resulted in approximately 2-fold higher microbial biomass N content at the footslope compared with that of the pasture site.

Table 1. Mean values (n= 10) for selected soil characteristics of cultivated and pasture sites.

Landform element	Moisture	Bulk density	pH	Microbial biomass N
	%	Mg m^{-3}		$\mu g\text{-}N\ g^{-1}$ soil
		Cultivated site		
Shoulder	20.4 (0.6)*	1.3 (0.1)	6.5 (0.1)	92 (7)
Footslope	26.0 (2.0)	1.4 (0.2)	6.6 (0.2)	105 (6)
		Pasture site		
Shoulder	19.2	0.77 (0.2)*	6.9 (0.1)	138 (14)*
Footslope	5 1.9 (6.0)	0.52 (0.2)	6.9 (0.2)	291 (41)
	t	t		t

* - Mean values are different between shoulder and footslope within site ($P \leq 0.05$).
t - Mean values are different (averaged over slope position) between sites ($P \leq 0.05$).
Values in parentheses are standard errors.

Gross nitrification and V_{max}

Gross nitrification of the studied landscapes ranged from 1.9 to 3.6 $\mu g\text{-}N\ g^{-1}$ soil and did not vary between or within sites (Table 2). The maximum carrying capacity of autotrophic nitrifiers as indicated by V_{max} varied significantly between sites and was about 2-fold higher at the cultivated site than that of the pasture site (Table 2). Since V_{max} was measured under optimum conditions for autotrophic nitrifiers, it can be considered as an indicator of the nitrifying enzyme content and the number of viable cells of autotrophic nitrifiers in the soil sample. Accordingly, V_{max} of about 7-fold higher than respective gross nitrification rates of both sites implies that a greater proportion of the nitrifying community did not nitrify *in situ* yet remain viable. Consequently, in the presence of a surplus amount of substrate and other optimal conditions, nitrification occurred without a lag phase. Despite the differences observed in V,, gross nitrification did not vary between sites indicating that differences may have been masked by the limited availability of the substrate or some other factor at the time of sampling.

Table 2. Mean values (n= 10) for gross nitrification, NO,' + NO_3^- consumption rates and V_{max} for NH,+ oxidation in cultivated and pasture sites.

Landform element	NH_4^+	Gross nitrification	V_{Max}	NO,- + NO,' consumption
	μg-N (g soil)$^{-1}$		μg-N g^{-1} soil d^{-1}	
		Cultivated site		
Shoulder	1.81 (0.19)	2.21 (0.5)	27.76 (7.4)	2.12 (0.86)
Footslope	2.22 (0.2.4)	3.62 (1.1)	28.32 (5.3)	4.16 (1.32)
		Pasture site		
Shoulder	0.68 (0.19) *	1.90 (0.3)	14.47 (2.2)	3.53 (0.47)
Footslope	6.19 (0.24)	2.28 (0.3)	16.49 (2.7)	5.43 (0.77)
	t		t	

* - Mean values are different between shoulder and footslope within sites ($P \leq 0.05$).
t - Mean values (averaged over slope position) are different between sites ($P \leq 0.05$).
Values in the parenthesis are standard errors.

Gross nitrifkation occurs at a rate of about 10% of the V_{max} generally indicates that the substrate availability for autotrophic nitrifiers is one-tenth of the apparent K_m value. NO_3^- consumption rates occurred at a comparable rate to nitrification rates suggests that NH_4^+ levels at the cultivated site were not high enough to fulfill the microbial demand for N. It also indicates that NH," levels at microsites where NO_3^- immobilization occurs were less than 0.1 μg mL^{-1} (Rice and Tiedje, 1989). Accordingly, such low NH_4^+ levels may have led to an intensive competition for NH,' among microbes in which, autotrophic nitrifiers are usually out-competed by heterotrophs due to their low affinity for NH_4^+ and slow growth rates (Verhagen et ***al.,*** 1992).

Autotrophic nitrifying communities at the pasture site were able to carry-out gross nitrification at a similar rate to that of the cultivated site regardless of several factors that could hamper their activity. Comparatively high biomass N contents indicated that there was a high microbial demand for NH_4^+ at the pasture site. As a result, intensive competition may have occurred in some microsites for NH_4^+ resulting in NH,' levels below 0.1 μg g^{-1} soil. In addition to the low substrate availability, the autotrophic nitrifkation was further restricted by the high apparent K_m values (ranged approximately

from 1.4 to 14.8 NH,'-N $\mu g\ g^{-1}$ soil) which were higher than respective KC1 extractable NH,' levels. These observations provide evidence that nitrification at the pasture site also restricted by the substrate availability similar to that of the cultivated site.

Autotrophic nitrifying communities

MPN counts indicated that population densities of NH,' and NO,' oxidizers were higher at the cultivated site than that of the pasture site but did not vary significantly within sites (Table 3). In general, NH,' oxidizers were more abundant than NO,' oxidizers at both sites. Although population densities of NH,'-oxidizers were 1 O-fold higher at the cultivated site than that of the pasture site, the V_{max} for NH,'-oxidation varied only by a factor of two between sites. Such differences could also occur due to differences in the dominant nitrifying spp. between sites and thereby a lack of correlation between activity and cell number is not surprising as nitrifying activity varies with the species (Belser and Schmidt, 1980; Prosser, 1989).

Table 3. Mean values (n=10) for NH_4^+ and MPN counts for autotrophic bacteria in cultivated and pasture sites.

Landform element	NH_4^+	NH_4^+- oxidizers	NO, -- oxidizers	K_m
	μg-N (g soil)$^{-1}$	cells (g soil)$^{-1}$		μM
		Cultivated site		
Shoulder	1.81 (0.19)	3.1 x 10^6 (8x10')	1.4 x 10^5 ($4x10^4$)	8 (1)
Footslope	2.22 (0.2.4)	3.7 x 10^6 ($9x10^5$)	2.7 x 10' ($4x10^5$)	5 (2)
		Pasture site		
Shoulder	0.68 (0.19) *	3.6 x 10^5 ($1x10^5$)	3.1 x 10^4 ($7x10^3$)	16 (3)*
Footslope	6.19 (0.24)	6.3 x 10^4 ($2x10^4$)	3.0 x 10^4 ($7x10^3$)	165 (3)
	t	†	t	

* - Mean values are different between shoulder and footslope within sites ($P \leq 0.05$).
t - Mean values (averaged over slope position) are different between sites ($P \leq 0.05$).
Values in parentheses are standard errors.

It is often considered that the K_m value for an enzyme is similar to the

physiological concentration of the substrate. Apparent K_m for NH,' oxidation at the cultivated site were 5 to 8 μM and they were significantly lower than the apparent K_m at the pasture site. The footslope of the pasture site exhibited about 1 O-fold higher apparent K_m compared with that of the shoulder. Hart *et al.* (1994) suggested that the apparent K_m for NH_4^+ oxidation *in situ* varies from 5 to 50 μM. The apparent K_m values at the cultivated site were within this range, and were significantly lower than those at the pasture site. If apparent K_m values represent physiological concentrations of the substrate available, it is evident that NH,+- oxidizers in the cultivated site have a higher substrate affinity than those at the pasture site. Substrate affinities are species specific (Prosser, 1989), and therefore difference in the apparent K_m values may reflect that a potential difference could exist in the species diversity within the nitrifying communities between sites.

At a low substrate concentration, enzymes having a comparatively high substrate, affmities permit nitrifiers to scavenge the substrate efficiently. Similarly, in this study, the apparent positive relationship between K_m values obtained for NH_4^+ oxidation and KC1 extractable NH,' levels at the pasture site (r^2=0.92). Thus, changes in the substrate affinity might be one of the survival mechanisms that autotrophic nitrifiers at the pasture site benefit from under low substrate concentrations. Although apparent K_m did not correlate with the KC1 extractable NH_4^+ at the cultivated site, it showed a negative relationship with V_{max} (r^2=0.70). This may indicate that at least one of the enzymes responsible for NH_4^+ oxidation may have mechanisms to alter its substrate affinity. This mechanism may provide a survival advantage for autotrophs in the cultivated site as they experience drastic substrate fluctuations during the growing season.

Conclusions

Cultivation practices enhance nitrification by sustaining a larger nitrifying population density which have a higher competitive ability for the substrate. NH_4^+ oxidizing bacterial communities were different between investigated cultivated and pasture landscapes with respect to their substrate affinities and adaptive mechanisms

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