Biogeochemistry (2008) 87:17–27
DOI 10.1007/s10533-007-9165-4

SYNTHESIS AND EMERGING IDEAS

Fungal control of nitrous oxide production in semiarid grassland

C. L. Crenshaw · C. Lauber · R. L. Sinsabaugh · L. K. Stavely

Received: 20 February 2007 / Accepted: 22 October 2007 / Published online: 24 November 2007

Abstract Fungi are capable of both nitrification and denitrification and dominate the microbial biomass in many soils. Recent work suggests that fungal rather than bacterial pathways dominate N transformation in desert soils. We evaluated this hypothesis by comparing the contributions of bacteria and fungi to N_2O production at control and N fertilized sites within a semiarid grassland in central New Mexico (USA). Soil samples were taken from the rhizosphere of blue grama (*B. gracilus*) and the microbiotic crusts that grow in open areas between the bunch grasses. Soils incubated at 30% or 70% water holding capacity, were exposed to one of three biocide treatments (control, cycloheximide or streptomycin). After 48 h, N_2O and CO_2 production were quantified along with the activities of several extracellular enzymes. N_2O production from N fertilized soils was higher than that of control soils (165 vs. 41 pmol h^{-1} g^{-1}), was higher for crust soil than for rhizosphere soil (108 vs. 97 pmol h^{-1} g^{-1}), and increased with soil water content (146 vs. 60 pmol h^{-1} g^{-1}). On average, fungicide (cycloheximide) addition reduced N_2O production by 85% while increasing CO_2 production by 69%; bactericide (streptomycin) reduced N_2O by 53% with mixed effects on CO_2 production. N_2O production was significantly correlated with C and N mineralization potential as measured by assays for glycosidic and proteolytic enzymes, and with extractable nitrate and ammonium. Our data indicate that fungal nitrifier denitrification and bacterial autotrophic nitrification dominate N transformation in this ecosystem and that N_2O production is highly sensitive to soil cover, N deposition and moisture.

Keywords Nitrous oxide · Fungi · Semiarid grasslands · Sevilleta LTER · Denitrification · Extracellular enzyme activity

C. L. Crenshaw · C. Lauber · R. L. Sinsabaugh (✉) · L. K. Stavely
Biology Department, University of New Mexico, Albuquerque, NM 87131, USA
e-mail: rlsinsab@unm.edu

Introduction

Where precipitation rates are high, the production and decomposition of organic matter are closely integrated, and most of the nitrogen (N) needed to sustain the carbon (C) cycle is generated by the mineralization of accumulated soil organic matter (Asner et al. 1997). In semiarid ecosystems, this integration is less defined (Austin et al. 2004; Huxman et al. 2004). Sporadic moisture inputs of varying amplitude combined with widely fluctuating surface temperatures impose a pulsed pattern on biotic activity that may reduce the temporal integration of plant and microbial metabolism (Loik et al. 2004; Belnap et al. 2004). Although soil N levels are generally low in semiarid ecosystems (Zak et al. 1994; Welter et al. 2005), steady inputs of inorganic N from aeolian and

atmospheric deposition, combined with episodic biotic activity and limited hydrologic export, can lead to inorganic N accumulation in soil and subsoil horizons (Walvoord et al. 2003; White 2006) even at relatively low rates of N deposition (Fenn et al. 2003).

Despite low nutrient concentrations and temperature, moisture and pH conditions that often reach extremes, recent evidence indicates that microbial diversity in semiarid grasslands is comparable to that of mesic ecosystems. Fierer and Jackson (2006) reported that bacterial diversity varied among biomes in relation to soil pH, with the greatest values occurring in desert grassland and shrubland. High values of fungal diversity have also been reported for desert grassland (Porras-Alfaro et al. 2007).

The combination of high microbial diversity, restricted biotic opportunity and weak integration of ecological processes make if difficult to resolve the contributions of specific pathways and populations to N cycling. Most studies in arid soils have highlighted the role of water content and carbon availability in controlling rates of N_2O production and assumed that bacteria were the principal agents of nitrification and denitrification (Virginia et al. 1982; Peterjohn and Schlesinger 1991; Davidson et al. 1993). However, McLain and Martens (2005, 2006) conducted a series of microcosm manipulations on soils from an alluvial terrace in southeastern Arizona and concluded that most of the N_2O flux was generated by fungal mineralization of amino acids (heterotrophic nitrifier denitrification). Bacterial production contributed relatively little to N cycling because soil moisture was rarely high enough for dissimilatory denitrification and autotrophic nitrifiers were not as effective as heterotrophs in competing for NH_4.

To assess the effects of N deposition on N cycling in semiarid soils, we began a series of studies at experimental grama grassland sites located in the Sevilleta National Wildlife Refuge in central New Mexico. A comparative survey of soil enzyme activities in rhizosphere and cyanobacterial crust soils (Stursova et al. 2006) found that aminopeptidase potentials were an order of magnitude higher than those of temperate soils, a result that is consistent with McLain and Martens (2006) findings on the importance of amino acid metabolism by fungi to N_2O production. Stursova et al. (2006) also reported that inhibition of nitrous oxide reductase with acetylene had comparatively little effect on N_2O production. This observation suggests a significant fungal N_2O contribution because many fungi lack this enzyme, making N_2O the principal product of their denitrification (Shoun et al. 1992; Zhou et al. 2001). To further evaluate the role of fungi in the N cycle of desert grassland, we conducted a series of microcosm studies to compare N_2O and CO_2 production from rhizosphere and cyanobacterial crust soils in relation to water availability and biocide treatments.

Methods

Site description and sample collection

Soil samples were collected from experimental sites located in the Sevilleta National Wildlife Refuge (SNWR) in central New Mexico, the site of the Sevilleta Long-Term Ecological Research (LTER) program. The SNWR contains extensive semiarid grassland dominated by C4 perennial grasses (*Bouteloua gracilis*, *B. eriopoda*, *Sporobolis* spp., *Hilaria jamesii*, and *Muhlenbergia* spp.). In 1995, an N-addition experiment was established within the grama grassland biome (McKenzie Flats, N 34°24′, W 106°41′, elevation 1630 m). The experiment includes twenty 5×10 m plots: ten of which are untreated controls and ten are fertilized with $NH_4^+NO_3^-$ at a rate of 100 kg N ha^{-1} $year^{-1}$ (Johnson et al. 2003). Vegetation cover averages 60% with open areas between plants colonized by light cyanobacterial crusts, dominated by *Microcoleus* spp. (Porras-Alfaro and Lipinski, unpublished).

This site receives approximately 250 mm of precipitation annually, although annual precipitation and its distribution throughout the year vary widely (Pennington and Collins 2007). Atmospheric N deposition is approximately 2 kg ha^{-1} $year^{-1}$(Báez et al. 2007). Belnap (2002) estimated rates of N_2 fixation by crusts on the Colorado Plateau at 1.4 kg ha^{-1} $year^{-1}$. Soil and vegetation maps and meteorological data are available at http://sev.lternet.edu.

In August 2005, rhizosphere soil samples (0–10 cm depth) were collected with a trowel beneath three blue grama (*B. gracilus*) tussocks in each of the 20 experimental plots. Crust soil (0–2 cm depth) was collected from three locations per plot by scraping the soil surface with a trowel. The crusts are located in shallow depressions between the bunch grasses and easily

delineated by pigmentation of the soil. The rhizosphere and crust soil samples from the 20 plots were combined within treatment to yield ~10 kg composite samples of rhizosphere and crust soil from control and N-amended treatments. These four composite soil samples were sieved through 2 mm mesh to remove large roots and rocks.

Soil properties

The experimental sites are located on fine-grained soils of the Turney loamy sand series, formed by aeolian and alluvial deposition. Gravimetric water holding capacity (WHC), was 20 g/100 g air-dried soil. Bulk density was 1.6 g/cm^3. Soil pH, determined by equilibrating 100 ml of deionized water with 40 g of air-dried soil was 8.5 ± 0.2 units. The composite soil samples for the four treatment-location combinations were analyzed for extractable N, %C and %N, following Mulvaney (1996). Extractable N was determined by extraction with 2 M KCl followed by analysis for NH_4–N using Technicon Industrial Method 98–70 W, Ammonia in Water (Oct. 1973) and NO_3–N using Technicon Industrial Method 100–70 W, Nitrate and Nitrite in Water (Sep. 1973) using a Technicon AutoAnalyzer. %N and %C were determined by high temperature combustion using a ThermoQuest CE Instruments NC2100 Elemental Analyzer.

Subsamples of the four composite soil samples were used in a series of experiments to quantify N_2O and CO_2 flux from fungi and bacteria under different moisture regimes. The experiments followed a complete block design with two levels of moisture availability (30% and 70% WHC 6 g and 14 g water per 100 g soil, respectively and three biocide treatments (control, cycloheximide ($C_{15}H_{23}NO_4$) at 150 mg/100 g soil and streptomycin sulfate ($C_{42}H_{84}N_{14}O_{36}S_3$) at 300 mg/100 g soil). The concentrations of cycloheximide (fungicide) and streptomycin (bactericide) followed protocols used in previous studies (Castaldi and Smith 1998; Laughlin and Stevens 2002; McLain and Martens 2006).

For each treatment, 100 g soil was added to three replicate 250 ml serum bottles. Deionized water, with or without dissolved biocide, was added to each vial to attain 30% or 70% WHC and target biocide concentration. The vials were shaken to mix the water, sealed with butyl rubber stoppers, and incubated in the dark for 48 h at 20°C.

N_2O and CO_2 flux

After approximately 48 h, gas subsamples were removed from the incubation vials using gastight syringes and stored in evacuated 12 ml serum vials. Samples were analyzed for N_2O within 24 h of collection; CO_2 concentrations were measured at the end of the experiment (2 weeks or less). The N_2O was quantified using a Shimadzu GC14-B gas chromatograph fitted with an electron capture detector (at a temperature of 320°C) and a 80/100 mesh HayeSep-Q column, 2 m × 3 mm ID (Supleco, Inc., Bellefonte, PA) at 45°C using ultra high purity N_2 as a carrier gas. The CO_2 was analyzed on a Buck 610 gas chromatograph fitted with a thermal conductivity detector (100°C) and a 80/100 mesh HayeSep-A column, 2 m × 3 mm ID (Supleco, Inc., Bellefonte, PA) at 50°C using helium as a carrier gas (flow rate 10 ml/min). The total quantities of N_2O and CO_2 in each vial after 48 h were converted to production rates with units of pmol and nmol h^{-1} g^{-1} dry soil, respectively.

Because the grassland soil contain carbonates, the biocide additions have the potential to release inorganic CO_2 resulting in overestimates of microbial respiration. The pH of the streptomycin and cycloheximide solutions was 5.4 and 4.6, respectively. Titration with 0.5 M NaOH yielded a total base neutralizing capacity of 75 and 10 µmol, respectively, which could inflate apparent microbial respiration rates by 8.0 and 1.0 nmol h^{-1} g^{-1}, respectively. The values were subtracted from the gross CO_2 flux in our calculation of respiration rates for the biocide treatments.

Extracellular enzyme assays

To compare functional responses among treatments, we measured soil extracellular enzyme activity (EEA) at the conclusion of the 48 h incubations. The soils were assayed for the potential activities of alkaline phosphatase (AP), β-glucosidase (βG), N-acetyl-glucosaminidase (NAG), cellobiohydrolase (CBH), and L-leucine aminopeptidase (LAP). The assays were performed on soil suspensions in bicarbonate buffer (pH 8.2) using methylumbelliferyl-linked substrates, following protocols in Stursova et al. (2006). Potential activities were expressed as nmole of substrate hydrolysed per hour per g soil (nmol h^{-1} g^{-1}).

Statistical analyses

N_2O, CO_2, and EEA data were LN transformed to normalize the distributions and compared by N treatment (control vs. N-amended plots), soil cover (grass vs. crust), WHC (30% vs. 70%) and biocide amendment (non-amended, cycloheximide, streptomycin) using a four factor, fixed effects ANOVA. Because the activities of the five enzymes assayed were correlated, principal components analysis was used to reduce the EEA data to a single factor. All statistical analyses were conducted using SPSS 11.04.

Results

N_2O flux was significantly higher from soil collected in the N-amended plots relative to the control plots (means for non-biocide treatments: 165 vs. 41 pmol h^{-1} g^{-1}), in cyanobacterial crust soils compared to rhizosphere soils (means for non-biocide treatments: 108 vs. 97 pmol h^{-1} g^{-1}), and at 70% WHC relative to 30% WHC (means for non-biocide treatments: 146 vs. 60 pmol h^{-1} g^{-1}) (Fig. 1, Table 1). Five of the six two-factor interactions and three of the four three-factor interactions were also statistically significant, highlighting that effects of cover, N availability and water on N_2O production were non-additive (Table 1). For non-biocide treatments, the highest N_2O production (295 pmol h^{-1} g^{-1}) was associated with rhizosphere soil from the N treatment plots at 70% WHC. In contrast, the lowest value (4.4 pmol h^{-1} g^{-1}) was measured in rhizosphere soil from control plots at 30% WHC.

Across treatments, cycloheximide addition reduced N_2O flux by 85 ± 15% (range 52–97%). The largest effects (91–97% reduction) occurred in soils at 70% WHC. At 30% WHC, reductions were significantly lower for soils from control plots (66%) relative to those from the long term N treatment plots (84%). The effects of streptomycin on N_2O production were more variable with reductions of 53 ± 31% (range +11% to −85%). The greatest reductions (84%) occurred in soils from the control plots at 70% WHC. Flux from N treatment plot soils at 70% WHC was reduced by 61%. At 30% WHC, N_2O production declined by 54% in soils from the N treatment plots, but only by 13% in control plot soils. In only one case, rhizosphere soil from control plots at 30% WHC, was streptomycin addition associated with a positive (non-significant 11%) increment in N_2O production (Fig. 1).

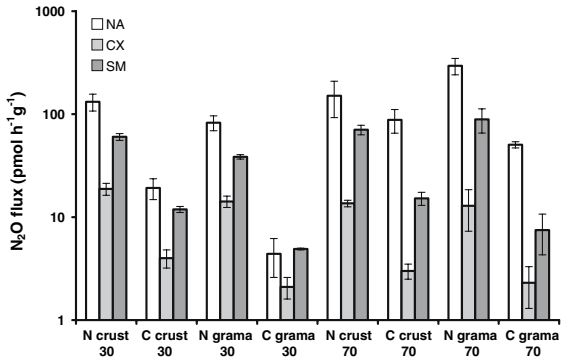

Fig. 1 Rates of N_2O production (±s.d.) by semiarid grassland soils incubated for 48 h at 20 C. Soil from the rhizosphere of grama grasses (grama) and cyanobacterial crusts (crust) was collected from control plots (C) and N-amended plots (N). Incubations were conducted at water holding capacities of 30 and 70% with or without biocide additions. NA, no biocide; CX, cycloheximde; SM, streptomycin

The magnitude of the biocide effects on N_2O production was not significantly correlated with N_2O production rates from the non-amended soils. However, there was a strong correlation (r = 0.97) between soil response to cycloheximide and response to streptomycin, suggesting that the relative contributions of fungi and bacteria to N_2O production were similar regardless of cover, N treatment and water availability. The regression, cycloheximide response (%) vs. streptomycin response (%), has a slope of 2.03 (r^2 = 0.95, n = 8) indicating that fungal contributions to N_2O flux were approximately twice that of bacteria.

CO_2 production was less variable than N_2O (Fig. 2). Respiration rates for cyanobacterial crust soils were significantly greater than those of rhizosphere soils (means for non-biocide treatments: 51 vs. 31 nmol h^{-1} g^{-1}). Water content was only a marginal effect (p = 0.07) and N treatment had no effect (Table 1). Four of the six two-factor interactions were statistically significant, indicating strong non-additive relationships in relation to biocide responses. For soils not amended with biocide, the greatest respiration rate (62 nmol h^{-1} g^{-1}) was recorded for crust soil from control plots at 70% WHC; the lowest rate (29 nmol h^{-1} g^{-1}) was associated with rhizosphere soil from control plots at 70% WHC.

Table 1 ANOVA results for N_2O and CO_2 flux from grama grassland soils in relation to N treatment (control vs. N-amended plots), soil cover (grass vs. crust), water holding capacity (WHC, 30% vs. 70%) and biocide amendment (non-amended, cycloheximide, streptomycin)

Factor	N	N_2O flux		CO_2 flux	
		F	p	F	p
WHC	43	97	<0.001	3.44	0.069
N Trt	43	1,168	<0.001	0.03	0.860
Cover	43	64	<0.001	4.17	0.046
Biocide	28	621	<0.001	21.6	<0.001
WHC × N Trt		16	<0.001	0.50	0.481
WHC × cover		29	<0.001	1.51	0.224
N Trt × cover		46	<0.001	10.0	0.002
WHC × biocide		72	<0.001	12.1	0.001
N Trt × biocide		1.86	0.160	4.80	0.012
Cover × biocide		0.52	0.600	9.48	<0.001
WHC × N Trt × cover		0.93	0.340	0.663	0.419
WHC × N Trt × biocide		20	<0.001	0.184	0.833
WHC × cover × biocide		6.2	0.004	1.50	0.232
N Trt × cover × biocide		5.5	0.006	1.75	0.183

The R^2 values were 0.97 for the N_2O model and 0.67 for the CO_2 model

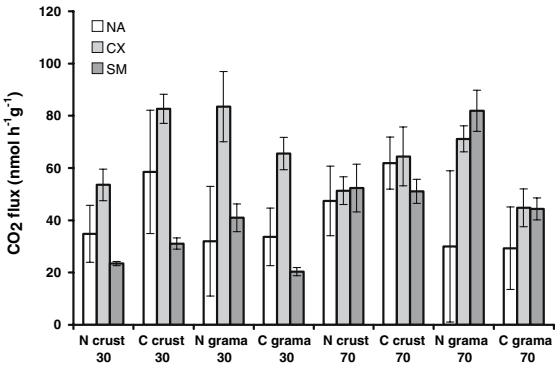

Fig. 2 Rates of CO_2 production (±s.d.) by semiarid grassland soils incubated for 48 h at 20 C. Soil from the rhizosphere of grama grasses (grama) and cyanobacterial crusts (crust) was collected from control plots (C) and N-amended plots (N). Incubations were conducted at water holding capacities of 30 and 70% with or without biocide additions. NA, no biocide; CX, cycloheximde; SM, streptomycin

In contrast to N_2O, cycloheximide addition increased rates of CO_2 production by an average of $69 \pm 57\%$ (range 4–161%) (Fig. 2). The largest responses occurred in rhizosphere soils from the N treatment plots (161% and 137% at 30% and 70% WHC, respectively). The smallest responses were measured for crust soils at 70% WHC (4% and 8% for soil from control and N treatment plots, respectively).

The effects of streptomycin amendment on respiration were mixed (range −47% to 173%). Respiration rates generally declined for soils at 30% WHC. The exception was rhizosphere soil from the N treatment plots where respiration increased by 28%. In contrast, respiration rates generally increased for soils at 70% WHC. The exception was crust soil from the control plots where respiration decreased by 18% (Fig. 2). Unlike N_2O, there was no statistically significant relationship between soil response to cycloheximide and response to streptomycin.

The molar ratio of CO_2:N_2O flux varied by nearly three orders of magnitude across treatments (Fig. 3). Soils from the control plots, without biocide addition, had a mean CO_2:N_2O ratio of 3080; and showed a large response to WHC (3050–7980 at 30% and 576–724 at 70% WHC). Soils from the N-amended plots, without biocide addition, had a mean CO_2:N_2O ratio of 286 with little variation in relation to WHC (272–399 at 30% and 94–377 at 70% WHC). Because cycloheximide depressed N_2O production and increased CO_2 production, CO_2:N_2O ratios jumped to 25,600 (range 21,300–36,000) for soils from control plots and 4,660 (range 2,870–6,120) for soils from N-amended plots. The effects of streptomycin were less dramatic: mean CO_2:N_2O ratio increased to 4290 (range: 2,620–7,000) for control plot soils and 793 (range: 391–1,070) for soil from N-amended plots.

C, N and P concentrations did not vary significantly among the four soil types (Table 2), but there were differences in the concentration of extractable NO_3^- and NH_4^+ that correlated with N_2O flux rate.

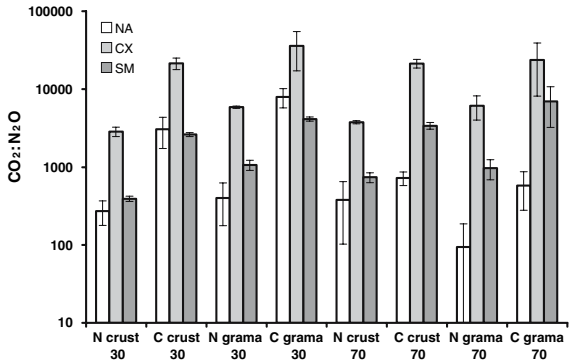

Fig. 3 Molar ratios of CO_2:N_2O production (±s.d.) by semiarid grassland soils incubated for 48 h at 20 C. Soil from the rhizosphere of grama grasses (grama) and cyanobacterial crusts (crust) was collected from control plots (C) and N-amended plots (N). Incubations were conducted at water holding capacities of 30 and 70% with or without biocide additions. NA, no biocide; CX, cycloheximde; SM, streptomycin

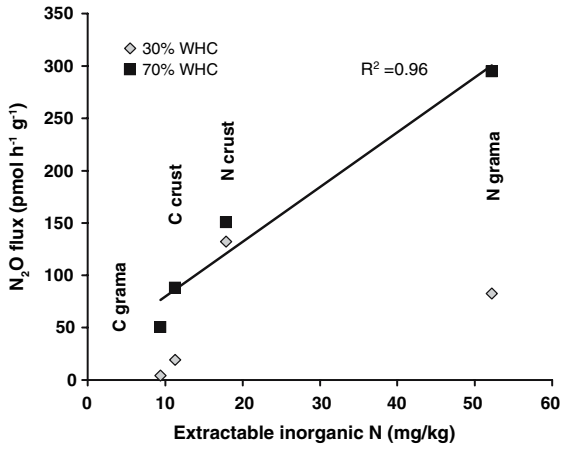

Fig. 4 Rates of N_2O production by semiarid grassland soils as a function of extractable inorganic N concentration. Soil from the rhizosphere of grama grasses (grama) and cyanobacterial crusts (crust) was collected from control plots (C) and N-amended plots (N). Incubations were conducted at water holding capacities of 30 and 70%. The regression is only for soils incubated at 70% WHC

For soils not amended with biocides, the correlations with NH_4^+ were stronger than those with NO_3^- and in both cases the correlations were stronger when WHC was 70% vs. 30%: for NO_3^- and N_2O, $r = 0.31$ for soils at 30% WHC and $r = 0.94$ ($p < 0.05$) for soils at 70% WHC; for NH_4^+ and N_2O, $r = 0.64$ for soils at 30% WHC and $r = 0.99$ ($p < 0.05$) for soils at 70% WHC (in all cases $n = 4$). The rhizosphere soil collected from the N-amended plots had the highest concentrations of inorganic N and the greatest increment in N_2O flux when moisture was increased from 30% to 70% WHC (Fig. 4).

In addition to soil organic matter, the biocides used to inhibit bacterial and fungal activity are also potential substrates for microbial metabolism. For cycloheximide, 64% C and 5% N by mass, the biocide amendment added 96 mg C and 7.5 mg N to a soil pool of approximately 500 mg C and 50 mg N (Table 2). On average, cycloheximide addition reduced N_2O flux by 85%, suggesting that little of the biocide N was mineralized. The increase in CO_2 production, 69% on average, was probably the result of bacterial metabolism of lysed fungal biomass, but it is possible that some of the biocide C was oxidized, adding to the respiration increment. For streptomycin, 34% C and 13% N, the amendments added 103 mg C and 40 mg N. To the extent that fungi were able to mineralize the biocide N, the 53% average reduction in N_2O flux that resulted overestimates bacterial contribution to N transformation. However, respiration rates, averaged across treatments, did not increase, suggesting that metabolism of biocide C was minimal.

In general, all five soil enzyme activities showed statistically significant responses to the four ANOVA factors, but only mean values for the non-biocide treatments are presented (Table 3). Because individual enzyme responses were correlated, the EEA data

Table 2 Nutrient concentrations for grama grassland soils in relation to N treatment (control vs. N-amended plots) and soil cover (grass vs. crust)

Soil type	%C	%N	%P	C:N	C:P	NH_4–N	NO_3–N
Control grama	0.469	0.0452	0.0167	12.1	73.7	4.34	5.04
Control crust	0.539	0.0464	0.0212	13.6	66.7	5.91	5.36
N-amended grama	0.504	0.0483	0.0220	12.2	60.1	20.4	31.8
N-amended crust	0.498	0.0469	0.0224	12.4	58.4	11.4	6.48

NH_4 and NO_3 values are given in units of mg N/kg soil

Table 3 Mean (±s.d.) enzyme activities (nmol h^{-1} g^{-1}) for grama grassland soils in relation to N treatment (control vs. N-amended plots) and soil cover (grass vs. crust)

Soil type	βG	CBH	NAG	LAP	AP
Control grama	36.3 ± 4.5	11.4 ± 1.6	3.0 ± 0.5	60.8 ± 6.1	33.9 ± 3.6
Control crust	38.9 ± 14.9	9.0 ± 0.5	5.2 ± 3.8	78.5 ± 5.6	33.7 ± 4.8
N-amended grama	47.3 ± 7.1	14.3 ± 1.7	4.9 ± 4.2	66.6 ± 5.5	35.4 ± 3.6
N-amended crust	31.9 ± 3.1	8.1 ± 1.4	2.5 ± 0.3	71.2 ± 7.4	28.9 ± 1.1

Key: βG, β-glucosidase; CBH, cellobiohydrolase; NAG, N-acetyl-glucosaminidase; LAP, L-leucine aminopeptidase; AP, alkaline phosphatase

were reduced to a single PCA factor that represented 56% of the variance (EEA factor loadings: bG 0.90, CBH 0.88, NAG 0.60, LAP 0.44, AP 0.82). The EEA factor did not correlate with N_2O or CO_2 production for soils incubated at 30% WHC, but at 70% WHC, EEA and N_2O production were significantly correlated (Fig. 5).

Discussion

N_2O is a product of the cycling of N through redox pathways. In semiarid soils, prevailing conditions favor aerobic pathways, i.e. heterotrophic nitrifier denitrification, codenitrification, and autotrophic nitrification; and fungi may account for a large portion of N transformation because of their capacity to metabolize at low water potentials (McLain and Martens 2005, 2006). At the SNWR, fewer than 10% of precipitation events are large enough (>10 mm) to saturate soil surfaces, and thereby create transient conditions suitable for dissimilatory denitrification. We attempted to simulate both "extended" and "transient" conditions by adjusting soil moisture to 30% and 70% WHC, respectively, and choosing a 48 h incubation period to approximate the longest window of response that might accompany a precipitation event.

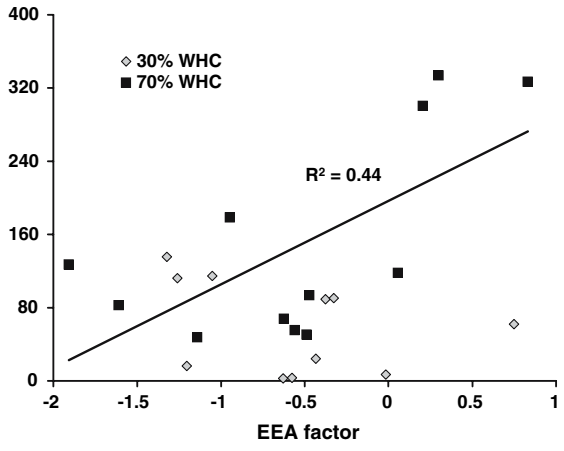

Fig. 5 Rates of N_2O production by semiarid grassland soils as a function of soil enzyme activity. Enzyme activity is represented by a factor generated from principal components analysis of five enzyme variables. Activity is related to N_2O production only for soils incubated at 70% WHC

N_2O production rates for soils not amended with biocides were similar to those reported in a previous study of microbial activities at the Sevilleta long term N deposition sites (Stursova et al. 2006). In the earlier study, initial rates of N_2O production measured at 50% WHC averaged 95 pmol h^{-1} g^{-1} for grama-associated soil and 112 pmol h^{-1} g^{-1} for cyanobacterial crust soil. The addition of acetylene to inhibit nitrous oxide reductase (and thereby dissimilatory denitrification to N_2) increased N_2O flux by 35% for soils from control plots and decreased flux by 28% for soils from N-amended plots. These weak responses, which were not statistically significant, suggested that autotrophic nitrification or heterotrophic nitrifier denitrification pathways played a larger role in N transformation than dissimilatory denitrification.

In this study, we used biocides to compare the relative contributions of fungi and bacteria to N_2O production. On average, cycloheximide additions reduced N_2O production rates by 85%, compared to 53% for streptomycin, indicating that fungal and bacterial contributions to N transformation were both important. The long term N addition treatment did not alter the 2:1 ratio of fungal to bacterial contribution, but it did narrow the range of response in relation to soil cover and water content: cycloheximide depressed N_2O production by 89 ± 6% for soils from the N-amended plots compared to 81 ± 21% for

control soils, for streptomycin the corresponding numbers were 58 ± 8% for N-amended soils and 54 ± 36% for control plot soils. The greater variation observed for control soils was largely associated with water content: both biocides produced significantly larger effects at 70% WHC than at 30% WHC. The mechanisms for this water effect, which was not observed in the N treatment plot soils, are unclear, but they may be related to low N availability in the control soils, i.e. the pool of bioavailable N was larger at 70% WHC for control plot soils relative to 30% WHC while the higher N availability of N plot soils mitigated the water content effect. For control plot soils, the CO_2:N_2O production ratios decreased by an order of magnitude when water content was increased from 30% to 70% WHC, a response not observed in the soils from the N addition treatments.

The increments in CO_2 production, 69% on average, in cycloheximide amended soils suggested that fungi dominated microbial biomass as well as N cycling, assuming that the CO_2 increments were the result of bacterial metabolism of labile carbon released by fungal lysis. The CO_2 responses also show that there was an active bacterial community poised to consume the newly available carbon. However, these consumers apparently were not major contributors to soil denitrification because N_2O production decreased by 85%.

Compared to cycloheximide, the effects of streptomycin on CO_2 production were modest and more variable. Respiration rates generally declined for soils at 30% WHC and increased for soils at 70% WHC. The results suggest that bacteria were a relatively small component of microbial biomass, although still significant contributors to N transformation. There was no correlation between respiratory responses to streptomycin addition, both positive and negative, and reductions in N_2O production. For both biocides, any potential redirection of C or N from bacterial to fungal, or *vice versa*, pathways had no positive effects of soil denitrification.

In general, our findings are consistent with those reported by McLain and Martens (2005, 2006) for desert grassland and shrubland soils in Arizona. For unamended soil collected from open areas they reported CO_2 and N_2O production rates equivalent to 6–19 nmol h^{-1} g^{-1} and 1.2–3.2 pmol h^{-1} g^{-1}, respectively (CO_2:N_2O ratio 4,900–5,900), at 80% WHC. Values for mesquite soils, which had extractable N concentrations comparable to those measured in the grama soil from our N treatment plots (Table 1), were equivalent to 11–21 nmol h^{-1} g^{-1} for CO_2 and 1.6–6.3 pmol h^{-1} g^{-1} for N_2O (CO_2:N_2O ratio 3,300–6,800). These production rates are lower than those we measured for unamended Sevilleta grassland soils (means: 46 nmol h^{-1} g^{-1} for CO_2, 41 pmol h^{-1} g^{-1} for N_2O, CO_2:N_2O ratio 3,800), probably because McLain and Martens (2005) measured gas production over a 12 days period, while we used a 2 days incubation. The lower rates could be partly a result of substrate depletion during extended incubation.

In a subsequent study, McLain and Martens (2006) reported that cycloheximide reduced N_2O generation from unamended semiarid soils by 63%; reduction rates increased to 79% for soils amended with N substrates, with little effect on respiration. Laughlin and Stevens (2002) reported that cycloheximide reduced N_2O production by 89% in soil from a perennial ryegrass site in Northern Ireland, accompanied by a significant decrease in respiration. Similar levels of N_2O suppression have been reported for other fungicides. Kinney et al. (2004) reported decreases in N_2O production of 47% and 40% for tilled agricultural soils treated with the fungicides mancozeb and chlorothalonil, respectively. The same treatments in no-till soils reduced N_2O production by 80% and 48%. In all cases, the fungicide additions led to a decrease in soil respiration.

These N_2O results are comparable to the 85% reduction observed for Sevilleta soils, however at our sites fungicide addition led to increased CO_2 production. One potential explanation is CO_2release from soil carbonates. However, the base neutralizing capacity of cycloheximide is low, equivalent to 1% of the observed CO_2flux rate. An alternative hypothesis is that rapid mineralization of fungal biomass is facilitated by the extremely high oxidative and proteolytic enzyme activity associated with our soils (Stursova et al. 2006; Stursova and Sinsabaugh 2007). High soil pH optimizes these activities creating reaction potentials (1–5 mmol h^{-1} g^{-1} soil organic matter for oxidative activities and 5–10 μmol h^{-1} g^{-1} soil organic matter for peptidase activities) that exceed those of other soils by more than an order of magnitude. The aridity of the soils also promotes stabilization of extracellular oxidative enzymes to the extent that autoclaving has no effect on activity (Sursova and Sinsabaugh 2007).

Reported N_2O responses to streptomycin are more variable than responses to fungicides. McLain and Martens (2006) reported a 100% increase in N_2O production with streptomycin, indicating that resource consumption by bacteria was impeding soil denitrification. The 23% reduction in N_2O reported by Laughlin and Stevens (2002) was more comparable to the response of Sevilleta soils, which had a mean decline of 53%. In all these systems, including ours, the effects on respiration were small, presumably because bacterial biomass was not a large component of soil organic matter.

Although the number of studies is small, it appears that fungal metabolism controls N_2O production in grassland and shrubland ecosystems under most conditions, supplemented by bacterial contributions that vary considerably with environmental conditions. In the Arizona soils, McLain and Martens (2006) concluded that most N_2O was produced through fungal nitrifier denitrification pathways (Wrage et al. 2001) that were fueled by the mineralization of protein. Laughlin and Stevens (2002) concluded that fungal metabolism controlled both nitrification and denitrification in ryegrass soil and that all the N_2O was produced by reduction of nitrate.

Within the grama grassland ecosystem at Sevilleta, it appears that fungal and bacterial contributions to N transformation are co-dominant. Because most fungi lack nitrous oxide reductase (Shoun et al. 1992; Zhou et al. 2001), N_2O is the principal product of fungal denitrification, and of heterotrophic nitrifier denitrification in general (Wrage et al. 2001). The major product of dissimilatory denitrification is N_2, so inhibition of bacteria by streptomycin should have a comparatively small effect on soil N_2O flux even if the quantities of NO_3 processed through bacterial and fungal pathways are comparable. In fact, N_2O production might even increase in response to bactericides, as McLain and Martens (2006) observed, if the shutdown of bacterial denitrification redirects N into fungal denitrification pathways. Conversely, even if fungicide increased the availability of NO_3 for bacterial denitrification, net N_2O production may still decline because N_2O is not the principal product of the pathway. Consequently, the most probable mechanism for the substantial declines in N_2O production that we observed with streptomycin addition is inhibition of bacterial nitrification. If rates of autotrophic and heterotrophic nitrification are similar, streptomycin addition should reduce the supply of NO_3 available for fungal denitrification and codenitrification leading to net reductions in N_2O production (Fig. 6).

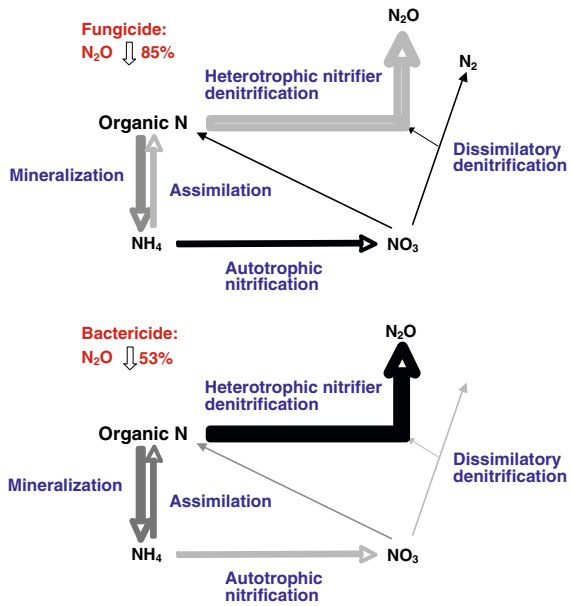

Fig. 6 Hypothetical flow diagram for N cycle of semiarid grassland. Arrow thickness indicates the relative magnitude of N flow through the indicated pathway. The partial inhibition of N flow through a pathway as the result of biocide treatment is indicated by lightening the arrows from black to gray

Other studies have shown comparable results. Phillips et al. (2001) measured N_2O fluxes for 2 years on intact soil cores from a *Pinus taeda* forest in a laboratory incubation; the rates reported for ambient treatments (110–570 pmol h^{-1} g^{-1}) were similar to our values for semiarid grassland. When given additional N, there was more denitrification compared to water addition alone. The authors also found that there was more N_2O produced through nitrification, rather than denitrification, at 94% WFPS. Mummey et al. (1994) found that in semiarid shrub steppe grassland soils nitrification accounted for 61–98% of the N_2O produced from soil at water contents below saturation while denitrification was the primary source of N_2O under saturated conditions.

For Sevilleta soil collected from control plots, the mean ratio of CO_2:N_2O production was about 3,000. Ratios calculated from data presented by Mclain and Martens (2005, 2006) for grassland and shrubland soils in Arizona are similar. With a mean production rate of 46 nmol h^{-1} g^{-1} and a soil C content

of approximately 0.5% (Table 2), about 2% of the C in control plot soils was converted to CO_2 during our 48 h incubations. With a mean N_2O production rate of 41 pmol h^{-1} g^{-1} and a soil N content of 0.046% (Table 2), about 0.012% of soil N was released. If this value is tripled to allow for potential NH_3 volatilization and N_2 production by dissimilatory denitrification, then about 0.04% of soil N was vented, yielding a C:N ratio of approximately 50 for gas export. The C:N ratio of the soil is about 13 (Table 2), so under these assumptions the average N atom gets cycled about three times relative to the average C before it is exported, assuming that respiration and denitrification are the only significant C and N outputs from arid soils. For soils collected from the N addition plots, the mean CO_2:N_2O production ratio was approximately 300, with rates of gas production that averaged 36 and 165 pmol h^{-1} g^{-1}, respectively. Over 48 h, about 1.5% of soil C was respired and 0.048% of soil N was emitted as N_2O. Tripling this estimate to allow for N_2 and NH_3 release, raises the N loss estimate to 0.15% and yields a C:N ratio for gas export of 10, compared to a value of 12 for soil C:N. Thus, relative to C, N retention by control plot soils is 3–4 greater than that of soils from the N addition plots. This scenario may explain why the soil C and N content of the control and N treatment plots remains similar even after 15 years of heavy fertilization.

Modeling N_2O production rates across ecosystems using commonly measured environmental variables has been problematic, presumably because of the diversity of N transformation pathways. Within ecosystems, production rates may be predicted by regressions that include measures of environmental conditions and nutrient concentrations. McLain and Martens (2005), for example, predicted N_2O production in arid soils from measures of amino acid concentration and respiration, assuming that mineralization of amino acids by fungi was the principal source of N_2O (McLain and Martens 2005, 2006) and that amino acids were the most common form of organic N in their soils (Martens and Loeffelmann 2003). As described previously, the Sevilleta grassland soils have high aminopeptidase activity (Table 3) indicating a high potential for rapid protein degradation (Stursova et al. 2006). Based on these relationships, we should be able to predict N_2O production from our enzymatic measures of C and N mineralization potential. Our PCA-derived enzymatic factor does show a modest relationship to N_2O generation at high soil water contents (Fig. 5), but overestimates N_2O fluxes from soils from control plots and underestimates N_2O fluxes from N addition plots. The residuals are correlated ($r = 0.76$), with extractable inorganic N, which has a stronger overall relationship with N_2O than EEA potential (Fig. 4). These regressions, considered with the results from biocide treatments and earlier acetylene block manipulations (Stursova et al. 2006), indicate that N_2O generation in these desert grassland soils, while primarily aerobic, is the product of nitrification and denitrification by both fungi and bacteria (Fig. 6).

Arid and semiarid ecosystems represent about 30% of terrestrial environments. The soil N cycle in these ecosystems is complex and varies with vegetation cover, N availability and soil water content. Of particular interest is the role of cyanobacterial crusts, which are not well developed in semiarid grasslands compared to other arid ecosystems (Bowler et al. 2002), but nonetheless have C and N mineralization rates that exceed those of rhizosphere soil. The sensitivity of N cycling processes combined with an ecosystem structure of intercalating grass and crust patches suggests that regional alterations in precipitation or N deposition patterns could significantly alter both C and N cycling.

Acknowledgements This work was supported by a grant from the National Science Foundation, Ecosystem Science Program (DEB 0516113) and the Sevilleta LTER program.

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