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N_2O production by heterotrophic N transformations in a semiarid soil

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Abstract

Emissions of N_2O from soils of the Southwestern US are thought to result from the activity of anaerobic denitrifying bacteria, but the seasonal dryness and sandy texture of these soils are more conducive to the activities of aerobic microbes. Here, we present incubations of semiarid soils with added compounds known to stimulate the N-cycling processes ammonification (proteins, oligopeptides, and amino acids (AAs)), nitrification (NH_4^+ and NO_2^-), and denitrification ($NO_3^- \pm$ glucose). Non-flooded (−34 kPa) incubations with added organic N determined that oligopeptides (four AA in length) resulted in the highest potential N_2O flux over a 12-d incubation period (66 ng N_2O g^{-1} soil d^{-1}), three times that of proteins (21 ng N_2O g^{-1} soil d^{-1}) or AAs (24 ng N_2O g^{-1} soil d^{-1}). Initial N_2O production in incubations with added organic N decreased by more than 63% with addition of cycloheximide, an inhibitor of fungal activity, but additions of a bacterial inhibitor (streptomycin) increased N_2O flux by 100%. Additions of NH_4^+ and NO_2^- resulted in little NO_3^- production during the 12-d incubation, indicating that autotrophic N transformations were limited. Flooded soil (0 kPa) incubations with added NO_3^- and glucose resulted in considerable N_2O production by day 2 (200 ng N_2O g^{-1} soil d^{-1}), but 0 kPa incubations without glucose produced less than 10 ng N_2O g^{-1} soil d^{-1} revealing C, rather than water, limitations on denitrification in semiarid soils. Incubation of soils (−34 kPa) with ^{15}N-labeled substrates known to stimulate N mineralization and nitrification processes showed differences in $^{15}N_2O$ production after addition of glutamine (3 ng $^{15}N_2O$ g^{-1} soil d^{-1}), NH_4^+ (16 ng $^{15}N_2O$ g^{-1} soil d^{-1}), NO_2^- (26 ng $^{15}N_2O$ g^{-1} soil d^{-1}), and NO_3^- (1 ng $^{15}N_2O$ g^{-1} soil d^{-1}). All ^{15}N treatments produced similar native N_2O efflux of 12 ng $^{14}N_2O$ g^{-1} soil d^{-1} through the incubation period. The limitations of C and H_2O and minimal autotrophic N activity suggest that heterotrophic N-cycling processes may be responsible for most of the in situ N transformations and N_2O production in this system.
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Keywords: Nitrification; Denitrification; Heterotrophic nitrification; N mineralization; Proteins; Oligopeptides; Amino acids; Ammonium; Nitrite; Nitrate

1. Introduction

Microbial transformations of soil N via nitrification and denitrification contribute about 70% of the annual

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N_2O budget worldwide (Mosier, 1998). Until recently, nitrification and denitrification processes were regarded as separate, occurring in different layers of water, soils, and sediments. However, current research has revealed that these two processes can take place simultaneously, both in microbial communities and within single organisms (Kuenen and Robertson, 1994). Anaerobic denitrification is the anaerobic stepwise reduction of nitrate (NO_3^-) to N_2O and dinitrogen gas (N_2) by bacteria able to use NO_3^- in place of oxygen as an electron acceptor. Autotrophic nitrifiers catalyze the two-step oxidation of ammonium (NH_4^+) or ammonia (NH_3) to NO_3^- via nitrite (NO_2^-). This activity can also produce N_2O under suitable conditions (Poth and Focht, 1985), although the mechanism of N_2O production during nitrification processes is not completely elucidated. Heterotrophic nitrification is carried out by a variety bacteria and fungi that oxidize either NH_4^+ or organic N without energy gain from the process, but use organic C sources and aerobic respiration to generate energy (Kuenen and Robertson, 1994). Many heterotrophic nitrifiers also release N_2O aerobically, using the products generated during nitrification (NO_2^- and NO_3^-) as electron acceptors and reducing them to N_2O (Zhou et al., 2001).

Predicting gaseous loss of soil N as N_2O is difficult because of the complexity of N-cycling processes. Anaerobic denitrification activity in soils increases with increasing NO_3^- concentration, temperature, and moisture, and valid numerical models have been developed to estimate N_2O fluxes from some fertilizer-amended agricultural soils taking into account the presence of these strong driving factors (Bouwman, 1996; Smith et al., 1998). However, large-scale prediction of N_2O flux from unfertilized (low NO_3^-) soils has been exceptionally difficult, with the result that N_2O production from soil in undisturbed ecosystems is the least well-quantified of the known N_2O sources. Previous work with semiarid riparian soils revealed that measurement of soil organic N content and rate of organic matter mineralization show promise for predicting N_2O flux rates in laboratory incubations (McLain and Martens, 2005). The semiarid riparian soils studied were more than 80% sand with low moisture-holding capacity, properties that would preclude widespread formation of anoxic microsites in shallow (<0.5 cm) soil incubations. The strong correlations between C mineralization and N_2O production, together with the potential for high porespace oxygen tensions in incubations, raise the possibility that heterotrophic nitrification produces the majority of N_2O in this semiarid riparian soil.

Cooke and Rayner (1984) report that fungal populations are not restricted to soil water films as are bacterial populations, and thus, soil fungi are able to maintain growth and activity even under conditions of extreme dryness. The ability to tolerate conditions of low soil moisture may introduce an obvious advantage for fungal populations in semiarid soils. And yet, to our knowledge, fungal activity has not been considered as a potential pathway for N_2O production in semiarid soils.

Despite the large worldwide semiarid land mass (35%), little research has been conducted to characterize N transformations in semiarid soils. This study used incubations of semiarid soils and ^{15}N-tracer techniques to measure the potential mineralization, nitrification, and denitrification of N compounds representing successive steps in microbial N metabolism. We also partitioned N_2O production into fungal and bacterial components by the use of cycloheximide and streptomycin (Castaldi and Smith, 1998; Laughlin and Stevens, 2002), inhibitors of fungal and bacterial activity, respectively (Voet and Voet, 1995). Because our laboratory incubations were conducted at near in situ environmental conditions, our results are applicable to field settings and these incubation techniques can be used to identify N_2O production pathways and to quantify potential N_2O production in semiarid soils.

2. Materials and methods

2.1. Study site and soil

Soil (0–10 cm depth) was obtained in mid-monsoon season (July and August) from an alluvial terrace near the San Pedro River in southeastern Arizona, USA (31°40′N, 110°11′W; 1190 m elevation). Soils at this site are well-drained typic Torrifluvents of the Pima series. Vegetation is dominated by annual herbaceous plants, including peppergrass (*Lepidium thurberi*), Fremont's goosefoot (*Chenopodium fremontii*), and toothleaf goldeneye (*Viguiera dentata*). After transport to the laboratory, soil was sifted through a 2 mm sieve and refrigerated at 4 °C until analysis (total storage

period < 4 weeks). Soil texture was determined by hydrometer (Gee and Bauder, 1986) and pH was measured (2.5 g soil: 5 ml H_2O) using the method of Van Lierop (1990) with an Orion Model 310 meter (Thermo Orion, Beverly, MA) and an Orion electrode. Carbon and N content were determined by dry combustion interfaced with an Europa Hydra 20/20 IRMS (Europa Scientific, Crewe, UK).

Concentrations of N anions (NO_3^- and NO_2^-) were determined after DI water (5 ml) extraction of 1.0 g air-dried soil. The soil–water mixture was shaken for 30 min, followed by centrifugation at $2000 \times g$ (RSF) for 10 min. The supernatant was decanted into a clean vial, diluted to 10 ml, and analyzed for anions using a Dionex DX-500 ion chromatograph (Dionex Corp., Sunnyvale, CA) equipped with an AS-11 anion exchange column (Dionex Corp.). Anions were separated with an isocratic 10 mM NaOH gradient and detected with a Dionex ED-40 electrochemical detector set in the suppressed conductivity mode.

The air-dried and sieved soil had the following characteristics: 5.83 g kg^{-1} C, 0.63 g kg^{-1} N, 3.09 μg NO_3^-–N g^{-1}, pH 7.35, 830 g kg^{-1} sand, 130 g kg^{-1} silt, and 40 g kg^{-1} clay.

2.2. Laboratory incubations of field soil

Prior to incubation, the soil was leached with DI water to decrease native soil NO_3^- levels, reducing NO_3^-–N to 1.9 ± 0.2 μg g^{-1} soil. After air-drying, 20 g of soil were added to 250 ml Erlenmeyer flasks and sprinkled with DI water with and without added substrates (100 μg N added g^{-1} soil) to achieve moisture contents of 80% field capacity (−34 kPa). Control incubations (no N added) were performed using NO_3^--leached soils with water additions only, and N_2O produced in control flasks was subtracted from the total N_2O produced in the N-amended incubations during data analyses.

2.2.1. Experiment 1

The first set of incubations measured the N_2O production potential of soils with addition of substrates representing successive steps in soil organic N mineralization, including proteins, oligopeptides (four amino acids (AAs) in length), individual AAs (Table 1), and mineral N compounds (NH_4^+, NO_2^-, and NO_3^-). All incubations were performed at moisture levels of −34 kPa, with the exception of the incubations with added NO_3^-, which were performed at 0 kPa (flooded) and at −34 kPa, and with and without added glucose (1.0 mg glucose g^{-1} soil). Duplicate incubations of each treatment were performed. All substrates were purchased from Sigma (Sigma Chemical Company, St. Louis, MO).

Following substrate addition, flasks were tightly sealed with rubber stoppers equipped with an inlet and outlet port for syringe sampling and incubated at room temperature (22 ± 2 °C). Gas subsamples were removed from the incubation vessels using 3 ml syringes (Becton-Dickinson, Franklin Lakes, NJ) once every 24 or 48 h over a 12-d incubation period and analyzed within 2 h for N_2O concentration on a Shimadzu GC14-A gas chromatograph (Shimadzu Scientific Instruments, Columbia, MD) fitted with an electron capture detector and 80/100 HayeSep-Q column, 2 m × 3 mm i.d. (Supelco Inc., Bellefonte, PA) using N_2 as a carrier gas (flow rate, 40 ml min^{-1}), with a temperature program of 250 °C detector, 110 °C injector, and 45 °C column. Certified N_2O standards (Praxair Technology, San Ramon, CA)

Table 1
Properties of N substrates used in soil incubations

Substrate	Properties	Nitrogen content (mg g^{-1})
β-Casein protein		∼160
Carbonic anhydrase protein		∼160
Leucine peptide	Leu-Arg-Pro-Gly-NH_2	254.2
Arginine peptide	Arg-Gly-Glu-Ser	219.0
Asparagine		212.0
Glutamine	^{15}N labeled, 50% amide and 50% amine	191.7
NH_4^+	$^{15}NH_4Cl$ (99%)	261.9
NO_2^-	$Na^{15}NO_2$ (99%)	203.0
NO_3^-	$Ca(^{15}NO_3)_2$ (99%)	206.0

were used for calibration. Carbon dioxide concentrations were determined by flushing the gas from the incubation flasks through an S151 Infra-red gas analyzer (IRGA) (Qubit Systems, Kingston, Ont.).

2.2.2. Experiment 2

The contribution of fungal or bacterial activity to potential N_2O production of soils with added proteins, AAs, and NH_4^+ and NO_3^- with and without glucose additions was determined in incubations with added cycloheximide ($C_{15}H_{23}NO_4$) as an inhibitor of fungal activity or streptomycin ($C_{21}H_{39}N_7O_{12}$) as a bacterial inhibitor (Sigma Chemical Company Inc.). Cycloheximide was added to soils at 1.5 mg g^{-1} dry soil (Castaldi and Smith, 1998), while streptomycin was added at 3.0 mg g^{-1} dry soil (Laughlin and Stevens, 2002). As per Castaldi and Smith (1998), 24 h elapsed between addition of the antibiotics and addition of the C and N substrates. Following substrate addition, flasks were sealed and sampling proceeded as in Experiment 1.

2.2.3. Experiment 3

A third set of incubations was performed to quantify and separate N_2O production originating from an added ^{15}N substrate (glutamine, NH_4^+, NO_2^-, and NO_3^-) (Table 1) from microbial cycling of ^{14}N (hereafter, referred to as native N) stimulated by the N additions. Moisture, temperature, and sampling conditions were as outlined for Experiment 1. On each sampling day, additional gas subsamples from flasks with isotopically enriched substrates were drawn into evacuated exetainer vials (Labco Ltd., Buckinghamshire, UK) and analyzed for ^{14}N and ^{15}N composition of the N_2O produced on an ANCA TG-II GEO 20/20 isotope ratio mass spectrometer (PDZ Europa Ltd., Cheshire, UK).

2.3. Soil mineral nitrogen extraction

All incubated soils were air-dried and analyzed for extent of mineralization of the added N substrates (measured as NH_4^+, NO_2^-, and NO_3^-) at the end of the 12-d incubation period. The Experiment 1 incubations with added asparagine, NH_4^+, and NO_2^- were also performed in sufficient quantity to allow for analysis of soil mineral N in duplicate flasks for each of the first 4 d of the incubation period. Soil NO_2^- and NO_3^- concentrations were determined by DI water extraction and anion chromatography, described above, while soil NH_4^+ was extracted using 2 M KCl and concentrations were determined by steam distillation (Keeney and Nelson, 1982).

2.4. Statistical analysis

Differences in N_2O production between treatments over time were analyzed using a mixed linear model with repeated measures, with N treatment as the main effect, time (continuous) as a covariate, and with duplicates nested within the main treatment effect. Differences in N_2O production between treatments within a single time period were analyzed using one-way ANOVAs. A significance level of $\alpha = 0.05$ was used for all statistical tests. All statistical analyses were done using Minitab 13.32 Statistical Software (Minitab Inc., State College, PA).

3. Results

3.1. N transformations and nitrous oxide potential of organic and inorganic N amendments

For the first 3 d of the incubation period, N_2O production in all flasks with added organic N substrates was statistically equal, though protein-amended soils produced a high initial flush of N_2O from the β-casein treatment (Fig. 1). From days 4 to

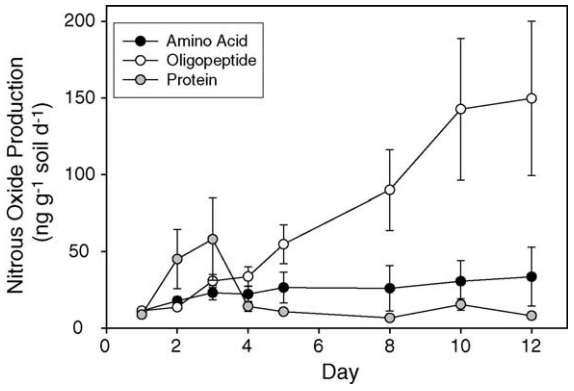

Fig. 1. Nitrous oxide production from duplicate incubations with added proteins, oligopeptides, and amino acids over 12 d period. Error bars represent mean of duplicates ± 1 S.D.

Table 2
Mineralization patterns in incubations with 100 μg substrate-N g^{-1} soil added

Substrate	Day of incubation	Added substrate present as (μg N g^{-1} soil)			Total mineral N (μg N g^{-1} soil)
		NH_4^+	NO_2^-	NO_3^-	
β-Casein	12	8 ± 4	0 ± 0	50 ± 3	58 ± 1
Carbonic anhydrase	12	7 ± 2	0 ± 0	68 ± 13	75 ± 14
Leucine peptide	12	55 ± 58	16 ± 23	63 ± 21	134 ± 78
Arginine peptide	12	93 ± 16	20 ± 2	93 ± 1	206 ± 18
Asparagine	1	70 ± 2	0 ± 0	2 ± 0	72 ± 2
	2	81 ± 5	0 ± 0	6 ± 0	88 ± 5
	3	77 ± 7	1 ± 1	10 ± 1	88 ± 7
	4	70 ± 11	1 ± 1	15 ± 1	86 ± 12
	12	7 ± 7	ND[a]	82 ± 3	89 ± 3
NH_4^+	1	87 ± 0	0 ± 0	3 ± 0	91 ± 0
	2	94 ± 7	0 ± 0	6 ± 0	100 ± 6
	3	93 ± 0	1 ± 2	7 ± 0	101 ± 1
	4	93 ± 8	0 ± 0	9 ± 0	102 ± 8
	12	61 ± 1	ND	8 ± 2	69 ± 2
NO_2^-[b]	1	ND	56 ± 4	12 ± 1	68 ± 3
	2	ND	48 ± 1	12 ± 1	60 ± 1
	3	ND	51 ± 1	13 ± 0	64 ± 0
	4	ND	55 ± 1	13 ± 1	68 ± 1
	12	ND	ND	9 ± 2	ND

[a] ND: not determined.
[b] NO_2^- analyses performed immediately following substrate addition recovered 47 ± 4 % of added N.

12, however, N_2O production from the oligopeptide incubations was the largest of the organic N amendments, averaging 94.1 ± 39.2 ng N_2O g^{-1} soil d^{-1}, significantly higher ($p < 0.01$) than production in the protein and AA flasks, which averaged 11.0 ± 2.1 and 27.7 ± 25.1 ng N_2O g^{-1} soil d^{-1}, respectively, during the same time period.

Mineralization of the added N as well as NO_3^- production was apparent in all organic N treatments, and by day 12, an average 71.2 μg NO_3^- was recovered (Table 2). In the oligopeptide incubations, however, the total inorganic N (NH_4^+, NO_2^-, and NO_3^-) measured at the end of the incubation period was 30–90% higher than the total organic N added (Table 2). The organic N substrates each induced CO_2 production 4–10 times greater than the control soils on day 2 (Table 3), but by day 8, CO_2 production in the protein and glutamine incubation flasks was equal to or below that of control soils. Carbon dioxide and N_2O production showed significant positive correlations over the 12-d incubation period in the flasks amended with proteins ($r = 0.84$, $p = 0.01$). In contrast, N_2O and CO_2 production in the flasks amended with oligopeptides ($r = -0.92$, $p < 0.01$) and AAs ($r = -0.95$, $p < 0.01$) had trends of increasing N_2O efflux and decreasing CO_2 production over time.

In contrast to the rapid production of NO_3^- by addition of organic N, addition of NH_4^+ and NO_2^- resulted in limited nitrification activity and on day 12, less than 10% of the added mineral N was recovered as NO_3^- (Table 2). Total N_2O production in the NO_2^- incubation flasks (20.6 ± 3.1 ng N_2O g^{-1} soil d^{-1}) was significantly higher ($p = 0.04$) than the NH_4^+ incubations (12.2 ± 1.0 ng N_2O g^{-1} soil d^{-1}) on days 2–6 but thereafter, total N_2O production in the NO_2^- and NH_4^+ incubations was equal ($p = 0.15$) (Table 3). All flasks amended with mineral N showed low CO_2 evolution during the incubation period (Table 3), and CO_2 and N_2O efflux were not significantly correlated in the NH_4^+ and NO_2^--treated flasks ($p = 0.27$ and 0.62, respectively).

Additional NH_4^+ incubations with added glucose showed N_2O efflux equal to that of control soils through the first 4 d of the incubation period. Thereafter, N_2O

Table 3
Nitrous oxide and carbon dioxide production in incubations with nitrogen substrates, with and without added inhibitors of fungal (cycloheximide) or bacterial (streptomycin) protein synthesis

Treatment	N_2O production (ng g^{-1} soil d^{-1})		CO_2 production (μg g^{-1} soil d^{-1})	
	Day 2	Day 8	Day 2	Day 8
Carbonic anhydrase	12 ± 1	4 ± 1	35 ± 2	5 ± 0
Plus cycloheximide	−71%	−80%	−15%	−28%
β-Casein	78 ± 12	9 ± 1	75 ± 13	8 ± 2
Plus cycloheximide	−78%	−84%	+15%	−22%
Plus streptomycin	+100%	−41%	−9%	+44%
Leucine peptide	13 ± 2	64 ± 69	57 ± 20	18 ± 7
Arginine peptide	15 ± 1	115 ± 29	70 ± 17	25 ± 7
Asparagine	30 ± 6	46 ± 31	46 ± 25	15 ± 9
Plus cycloheximide	−43%	−81%	+5%	−28%
Glutamine	21 ± 6	5 ± 0	46 ± 5	10 ± 1
Plus cycloheximide	−58%	−86%	+1%	−33%
NH_4^+	27 ± 4	26 ± 2	0 ± 0	2 ± 0
NH_4^+ plus glucose	10 ± 3	37 ± 9	80 ± 12	40 ± 2
Plus cycloheximide	−72%	−95%	+28%	+23%
NO_2^-	39 ± 5	35 ± 14	3 ± 0	3 ± 1
Control soil	6 ± 2	3 ± 0	8 ± 3	8 ± 2
Plus cycloheximide	−59%	+41%	−14%	+50%
Plus streptomycin	+100%	+25%	−15%	−5%

All incubations were performed at moisture potentials of −34 kPa.

efflux increased and on days 5–8, production in the glucose-amended flasks (37 ng N_2O g^{-1} soil d^{-1}) was greater than the NH_4^+ incubations (26 ng N_2O g^{-1} soil d^{-1}) without added glucose (Table 3).

Additions of cycloheximide resulted in 79 ± 11% lower N_2O production over the 8-d incubation period in all treatments, compared to N-amended flasks with no inhibitor added (Table 3). Control flasks with no added N showed a similar inhibition from days 1 to 4, but on days 5–8, the cycloheximide-treated control flasks produced 70% more N_2O than control flasks with no inhibitor. Carbon dioxide production in incubations with added cycloheximide showed no distinct patterns early in the incubation period (Table 3) but by day 8, CO_2 efflux in the flasks with added organic N substrates was reduced by 29% compared to controls, while the inorganic N plus cycloheximide incubation showed an increase in CO_2 production (Table 3).

Streptomycin additions to β-casein and control (no substrate added) flasks stimulated N_2O production by more than 100% through day 5. Thereafter, the increased N_2O production was only found in control flasks (Table 3). Streptomycin inhibited CO_2 production early in the incubation period, but by day 8, β-casein flasks amended with streptomycin produced 44% more CO_2 than N-amended flasks without added inhibitor (Table 3).

3.2. Denitrification potential in semiarid soils

On day 2, N_2O production (84 ng N_2O g^{-1} soil d^{-1}) in glucose-amended soils at 0 kPa was significantly higher ($p < 0.01$) than other NO_3^- treatments (Table 4), but this high N_2O production was reduced by 90% on day 4. Thereafter, N_2O efflux from all NO_3^- treatments was equal, averaging 2.2 ± 0.9 ng g^{-1} soil d^{-1}.

Regardless of moisture status, the NO_3^- plus glucose incubations respired CO_2 (35 μg g^{-1} soil d^{-1}) at a high rate through day 2. Thereafter, CO_2 efflux fell by 50% per day and on day 8, CO_2 production in all glucose-treated flasks averaged 4.5 μg g^{-1} soil d^{-1} (Table 4). Flooded, NO_3^--treated soils without glucose addition respired the lowest amounts of CO_2 throughout the 12-d incubation period.

Table 4
Nitrous oxide and carbon dioxide production in incubations using nitrate with and without glucose under two moisture regimes

Treatment	N_2O production (ng g^{-1} soil d^{-1})		CO_2 production (μg g^{-1} soil d^{-1})	
	Day 2	Day 8	Day 2	Day 8
NO_3^-, glucose (−34 kPa)	10 ± 3	4 ± 0	35 ± 0	5 ± 1
Plus cycloheximide	−71%	+72%	+100%	+300%
Plus streptomycin	−74%	+45%	+54%	+300%
NO_3^-, glucose (0 kPa)	84 ± 63	3 ± 0	35 ± 0	4 ± 1
NO_3^-, no glucose (−34 kPa)	6 ± 1	2 ± 0	21 ± 7	4 ± 6
NO_3^-, no glucose (0 kPa)	9.3 ± 1.6	2 ± 0	1 ± 4	0 ± 2

Inhibition experiments with added inhibitors of fungal (cycloheximide) and bacterial (streptomycin) protein synthesis were also performed on flooded treatments with glucose addition.

The addition of inhibitors of fungal and bacterial activity to the NO_3^- plus glucose incubations (−34 kPa) produced strong decreases in N_2O efflux from days 1 to 5, but from days 6 to 8, soils with added inhibitors produced larger amounts of N_2O than control flasks (Table 4). Addition of inhibitors increased CO_2 production in the NO_3^- plus glucose incubations throughout the 8-d incubation period.

3.3. Nitrous oxide production in incubations with ^{15}N-labeled substrates

To quantify N_2O production resulting from N substrate addition and separate it from that resulting from cycling of native soil N, incubations were performed with ^{15}N-labeled substrates (Table 1). Production of heavy isotope N_2O (molecular weight 45 and 46) was highest from flasks amended with mineral N forms. Nitrous oxide efflux from NO_2^--amended flasks was significantly ($p < 0.01$) higher than the other N treatments through day 6, after which it became equal to $^{15}N_2O$ production from NH_4^+ incubations (Fig. 2). In contrast, $^{15}N_2O$ production in glutamine incubations was lowest throughout the 12-d incubation period.

Production of N_2O from native soil N in flasks treated with ^{15}N-labeled glutamine, NH_4^+, and NO_2^- showed no significant differences ($p = 0.19$–0.53) during the 12-d incubation (Fig. 3). Native N_2O production in the NO_3^- plus glucose incubations on day 2 was similar to that from the glutamine, NO_2^-, and NH_4^+ incubations, but on days 4–8, the NO_3^- plus glucose incubations produced significantly ($p < 0.01$)

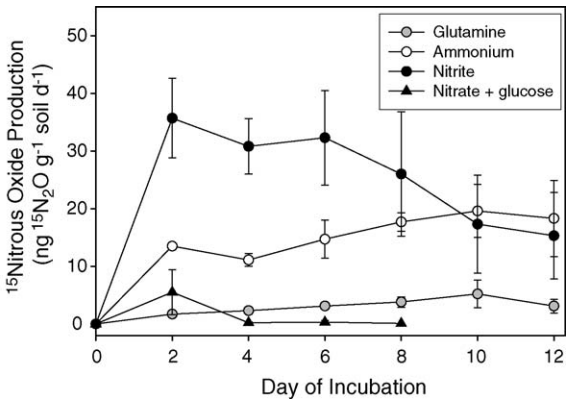

Fig. 2. Production of $^{15}N_2O$ in soil microcosms incubated at −34 kPa with different ^{15}N-labeled substrates. Error bars represent mean of duplicates ± 1 S.D.

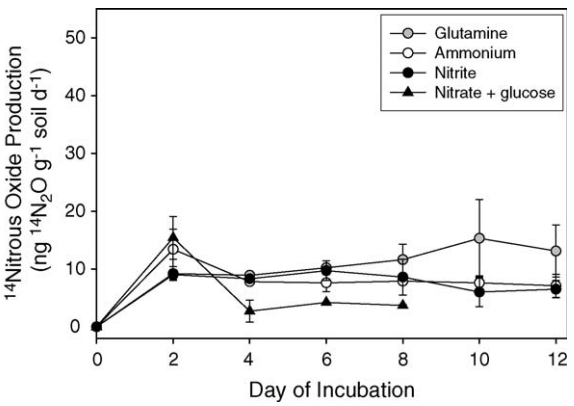

Fig. 3. Production of $^{14}N_2O$ in soil microcosms incubated at −34 kPa with different ^{15}N-labeled substrates. Error bars represent mean of duplicates ± 1 S.D.

less native N_2O than the other substrate additions (Fig. 3).

4. Discussion

4.1. Additions of organic N substrates

Because the incubations were intended to determine the factors influencing potential N-cycling in semiarid soils, the amount of N added to flasks (100 μg N added g^{-1} soil) was larger than native soil N levels to ensure that N would not limit microbial activity during the incubation period (Limmer and Steele, 1982). The protein incubations represented organic N forms resulting from the initial mineralization of plant biomass in soils, while oligopeptide incubations corresponded to N forms resulting from protein mineralization. Peptide decomposition produces AAs, represented in this study by asparagine and glutamine, two AAs with similar N contents that have been shown to play a pivotal role in N_2O production in soils (McLain and Martens, 2005).

Oligopeptide additions enhanced the N-cycling activity more than any other substrate added. The total soil N in mineral forms (NH_4^+, NO_2^-, and NO_3^-) on day 12 of the oligopeptide incubations was larger by 30–90% than the N initially added to the flasks, suggesting that a sizeable release of native soil N had also occurred during the incubation period. Research has suggested that oligopeptides are the prevalent organic N form in this soil (Martens and Loeffelmann, 2003) and such a priming effect may result from the seasonal extremes in dryness common to semiarid areas, where soil microbial communities must respond to sporadic moisture inputs with rapid increases in activity (Peterjohn and Schlesinger, 1991).

Although the added glutamine was labeled with more than 99% ^{15}N, native N_2O production in the glutamine incubations averaged 11.4 ng g^{-1} soil d^{-1}, more than three times the $^{15}N_2O$ production from the same treatment, suggesting that most of the added AA–N was incorporated into microbial cells rather than being directly mineralized to $^{15}NH_4^+$. Additional evidence exists for direct microbial uptake of AAs (Barraclough, 1997) followed by slow release of mineral N to the soil pool, as CO_2 and N_2O evolution were significantly negatively correlated in both the AA and oligopeptide incubations. Carbon dioxide evolution in these flasks was highest on day 2 but fell thereafter, a direct effect of rapidly declining AA–C in the soil (Müller et al., 2003), while during the same time period, N_2O evolution increased. Protein incubations also showed the highest CO_2 and N_2O evolution on days 2 and 3, but thereafter, flux of both gases decreased with time. In contrast to this pattern of stimulation of N_2O flux by protein–C mineralization, NH_4^+ additions with glucose promoted N immobilization and limited N_2O production for the first 4 d of the incubation period.

4.2. Additions of inorganic N substrates

Patterns of $^{15}N_2O$ production following additions of $^{15}NO_2^-$ and $^{15}NH_4^+$ to incubation flasks suggest a limitation of native mineral N on nitrifier activity in the semiarid soil. The production of $^{15}N_2O$ was evident 48 h after substrate addition, and it is possible that the addition of NH_4^+ prompted heterotrophic nitrifiers to increase NH_4^+ utilization (Barraclough and Puri, 1995). Nitrifiers have been shown to respond to mineral N input in other systems (Avrahami et al., 2002) and in general, heterotrophs are more competitive for NH_4^+ than autotrophic nitrifiers (Vitousek et al., 1982). Oxidation of NH_4^+ provides electron acceptors for aerobic or anaerobic denitrification, leading to higher overall N_2O emission rates (Avrahami et al., 2002).

The hypothesis for heterotrophic activity assumes that sufficient C-substrate exists to enable heterotroph activity (NH_4^+ immobilization) to dominate over that of the autotrophs (NH_4^+ oxidation). Under conditions of high C (NH_4^+ plus glucose), little N_2O production occurred until day 5 of the incubation period, indicating that heterotrophic activity was immobilizing the added N until the labile C was mineralized. Without labile C, the added mineral N cycled very slowly and more than 60% of the added NH_4^+ remained on day 12.

Nitrite added as a nitrifier substrate also cycled very slowly in the absence of added C, as on day 12, only 9% of the added NO_2^- was recovered as NO_3^-. However, NO_2^- analyses performed immediately after substrate addition recovered less than 50% of the added N, indicating that NO_2^- was unstable in the soil tested. Chemodenitrification or other possible

chemical reactions of NO_2^- with soil to produce N_2O can be excluded, as no N_2O was produced when NO_2^- was added to autoclaved soil (data not shown).

4.3. Patterns of N_2O production from NO_3^- incubations

The above results suggest the limited activity of autotrophic nitrifiers in this semiarid soil, and the NO_3^- incubations confirmed that anaerobic denitrifier activity is also limited, by moisture and C availability. Additions of glucose and sufficient water to flood NO_3^--incubated soils resulted in N_2O production 10-fold greater than soils moistened to −34 or 0 kPa soils with no added C, but $^{15}N_2O$ production in the $^{15}NO_3^-$-treated flasks ceased after the labile C was mineralized.

Surprisingly, additions of NO_3^- and glucose to soils at −34 kPa did not stimulate high N_2O production. Additions of labile C and water to semiarid soils with no N amendment have been shown to decrease rates of N_2O production, possibly due to microbial immobilization of available N under high C (Peterjohn and Schlesinger, 1991; Mummey et al., 1994). Our NO_3^- incubations were not N-limited, but it is likely that additions of labile C to the −34 kPa soils stimulated heterotrophic activity and immobilized native NH_4^+ for the length of the incubation period.

Nitrous oxide flux from semiarid soils following rainfall has been reported to result from anaerobic denitrification based on denitrification enzyme assays (DEAs) (Virginia et al., 1982; Peterjohn and Schlesinger, 1991). Although high rates of denitrification are more typical of wet, nutrient-rich environments, it has been suggested that frequent periods of rapid wetting and drying in desert environments may increase C availability, thus, decreasing soil O_2 concentrations and creating anaerobic microsites for denitrifiers (Peterjohn and Schlesinger, 1991). The −34 kPa moisture level selected for the laboratory incubations is the average moisture potential measured in field soils following significant (30 mm) rainfall, and thus, represents the upper limit for continuous soil moisture found in situ (data not shown). Continuous field monitoring showed that wet (moisture potentials above −25 kPa) soil conditions occurred only two times in 18 months, and field capacity (−10 kPa) moisture potentials were never measured, even following large (>40 mm) rainfall events (data not shown). Thus, the moisture conditions imposed in DEAs (0 kPa) are rare in situ in dry environments, and exposing semiarid soils to these conditions resulted in overestimations of denitrifier activity in this study.

4.4. Eukaryotic versus prokaryotic activity

The results of the cycloheximide incubations strongly suggest that soil fungi are a vital component of the N-cycling and N_2O production in this semiarid soil. Addition of cycloheximide was effective in inhibiting N_2O production for up to 8 d in incubations with added organic N compounds and NH_4^+ plus glucose. Streptomycin, however, was found to stimulate N_2O production. Similar patterns were observed by Laughlin and Stevens (2002) in a grassland soil, where cycloheximide additions caused an 89% inhibition in N_2O flux, leading to the conclusion that nearly 90% of the N_2O was produced by fungi. Using CO_2 evolution as a measure of microbial activity following inhibitor addition produced less equivocal results early in the incubation period. On day 2, organic N flasks treated with cycloheximide showed, on average, no change in production of CO_2, while additions of streptomycin decreased CO_2 production less than 20%. By day 8, cycloheximide additions to organic N flasks decreased CO_2 evolution by nearly 28%. Landi et al. (1993) reported that the addition of streptomycin or cycloheximide to a forest soil increased respiration and NH_4^+ concentration, and hypothesized that the surviving microorganisms were mineralizing the antibiotic-killed cells. In contrast, Laughlin and Stevens (2002) reported that during the lag phase of microbial growth, the average inhibition of respiration was 58% due to cycloheximide and 7% due to streptomycin.

4.5. Patterns of native N_2O production

Although added substrates were labeled with more than 99% ^{15}N, a steady proportion of the N_2O efflux from these incubations was derived from native soil N. The native N_2O production was statistically equal throughout the 12-d incubation period in the ^{15}glutamine, $^{15}NH_4^+$, and $^{15}NO_2^-$ incubations regardless of substrate addition. Martens and Dick (2003) also reported that $^{15}NH_4^+$ and $^{15}NO_3^-$ additions to coarse textured soils stimulated microbial activity to release

native soil N and the release of native N continued, while $^{15}NH_4^+$ and $^{15}NO_3^-$ remained in the soil.

McLain and Martens (2005) presented a model, utilizing soil AAs as a measure of organic N content and CO_2 evolution as a measure of organic matter mineralization, that showed promise for predicting N_2O flux rates from semiarid soils. At that time, the reasons for a strong predictive relationship between soil N content, CO_2 efflux, and N_2O production suggested involvement of nitrifier denitrification. However, the finding of stimulation of N_2O production in the presence of a C source (oligopeptide C, AA–C, or glucose–C), confirms that added C is enhancing the presence and activity of N_2O-producing microbial communities, suggesting that heterotrophic nitrification processes strongly impact N_2O production in these semiarid soils.

5. Conclusions

Emission of N_2O from semiarid soils is relatively small compared to wetlands and agricultural lands, but arid and semiarid ecosystems occupy nearly 35% of the terrestrial surface of the earth and thus, these soils may significantly impact atmospheric concentrations of N_2O. Results of this study suggest that anaerobic denitrification is not the dominant source of N_2O production in this semiarid soil. Rather, heterotrophic activity during decomposition of organic N is more likely a major contributor to in situ N_2O production. Further, N_2O production in this semiarid soil seems to be strongly correlated to the activities of soil fungi, rather than bacteria. Exposing laboratory soils to conditions closely replicating those found in situ allowed the identification of microbial pathways impacting N_2O production in this semiarid soil, information that will ultimately lead to an increased ability to model the contribution of semiarid soils to current and future global change.

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