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Nitrous oxide flux from soil amino acid mineralization

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Abstract

Nitrous oxide (N_2O) is a greenhouse gas produced during microbial transformation of soil N that has been implicated in global climate warming. Nitrous oxide efflux from N fertilized soils has been modeled using NO_3^- content with a limited success, but predicting N_2O production in non-fertilized soils has proven to be much more complex. The present study investigates the contribution of soil amino acid (AA) mineralization to N_2O flux from semi-arid soils. In laboratory incubations (-34 kPa moisture potential), soil mineralization of eleven AAs (100 μg AA–N g^{-1} soil) promoted a wide range in the production of N_2O (156.0 ± 79.3 ng N_2O-N g^{-1} soil) during 12 d incubations. Comparison of the $\delta^{13}C$ content (‰) of the individual AAs and the $\delta^{13}C$ signature of the respired AA–CO_2-C determined that, with the exception of TYR, all of the AAs were completely mineralized during incubations, allowing for the calculation of a N_2O-N conversion rate from each AA. Next, soils from three different semi-arid vegetation ecosystems with a wide range in total N content were incubated and monitored for CO_2 and N_2O efflux. A model utilizing CO_2 respired from the three soils as a measure of organic matter C mineralization, a preincubation soil AA composition of each soil, and the N_2O-N conversion rate from the AA incubations effectively predicted the range of N_2O production by all three soils. Nitrous oxide flux did not correspond to factors shown to influence anaerobic denitrification, including soil NO_3^- contents, soil moisture, oxygen consumption, and CO_2 respiration, suggesting that nitrification and aerobic nitrifier denitrification could be contributing to N_2O production in these soils. Results indicate that quantification of AA mineralization may be useful for predicting N_2O production in soils.
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Keywords: Nitrous oxide; Nitrifier denitrification; N Cycling; Greenhouse gas

1. Introduction

Nitrous oxide (N_2O) is a greenhouse gas that affects atmospheric chemistry and has been implicated in global climate warming (Dickinson and Cicerone, 1986; Finlayson-Pitts and Pitts, 2000). N_2O has 100-year global warming potential that is about 310 times that of carbon dioxide (CO_2) (Finlayson-Pitts and Pitts, 2000; IPCC, 1996) and has an atmospheric lifetime of approximately 120 years (IPCC, 1996). Nitrous oxide is chemically inert in the troposphere, but readily diffuses to the stratosphere, where it becomes involved in a series of photochemical reactions that induce the destruction of ozone (Crutzen, 1970).

Microbial transformations of soil N (nitrification and denitrification) contribute about 70% of the annual N_2O budget worldwide (Davidson, 1991; Gödde and Conrad, 2000; Mosier, 1998). Nitrification is a three-step oxidation of ammonium (NH_4^+) or ammonia (NH_3) to nitrate (NO_3^-) via nitrite (NO_2^-) carried out by two groups of soil microorganisms (Fig. 1). Nitrous oxide can also be produced during NH_3 oxidation through formation of hydroxylamine (NH_2OH) or NO_2^- (Bremner et al., 1980; Chalk and Smith, 1983; Wrage et al., 2001). An extension of the nitrification pathway that can also produce N_2O is nitrifier denitrification (Fig. 1), in which the oxidation of NH_3 to NO_2^- is followed by the reduction of NO_2^- to N_2O (Wrage et al., 2001). Nitrifier denitrification reactions are carried out by autotrophic NH_3-oxidizers under aerobic conditions, whereas anaerobic denitrification is the stepwise reduction of NO_3^- to dinitrogen gas (N_2) (Fig. 1) carried out

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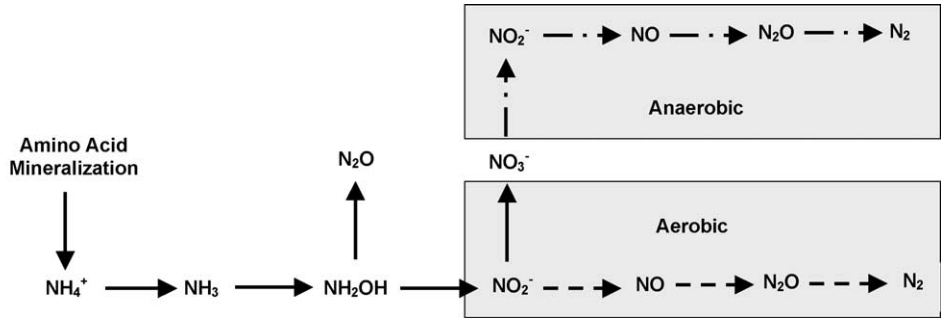

Fig. 1. Pathways of nitrogen transformation in soil, showing nitrification (——►), anaerobic denitrification (– - –►), and nitrifier denitrification (– – –►).

by denitrifying bacteria that are able to use NO_3^- in place of oxygen as an electron acceptor.

Very little is known about the ecological significance of the nitrifier denitrification pathway in soils (Wrage et al., 2001). The amount of N_2O produced by soils via this pathway has been estimated to range from insignificant in a loamy forest soil (Robertson and Tiedje, 1987) to 85% of total N_2O emissions from a sandy loam (Webster and Hopkins, 1996). Certain environmental conditions, including high soil NH_3/NH_4^+ content, low organic C content, low O_2 pressure, and possibly low pH are thought to be conducive to the production of N_2O by nitrifier denitrification (Wrage et al., 2001).

In soil, organic N sources (90–98% of total soil N content) include proteins released from plant residues and products of protein mineralization, peptides and amino acids (AAs). Mineralization of the soil organic N produces NH_4^+ and NO_3^- for plant use; these ions are also utilized by microbes in the pathways of N_2O production. The key role of AAs in soil biogeochemical cycling of C and N has received much recent research attention, and the direct use of AAs as a N source by both plants and soil microbes has also been well established (Jones and Hodge, 1999; Owen and Jones, 2001; Vinolas et al., 2001a). Microbial utilization of N from AAs occurs via the direct route in which organic N compounds are assimilated by soil microorganisms followed by the release of excess N as NH_4^+ (Barak et al., 1990; Barraclough, 1997; Gibbs and Barraclough, 1998). Incorporation of hydrolyzed AAs is essential for microbial cell survival as AAs serve as sources of energy and as the building blocks for cellular proteins.

Despite the key function of AAs to provide nutrients to plants and microorganisms, AA mineralization kinetics have not been widely used to explore or to quantify ecosystem N cycling or trace gas flux. The present study examines the possibility that soil AAs provide the N substrates for the nitrifier denitrification pathway for N_2O flux from soils, and that correspondingly, N_2O production by the nitrifier denitrification pathway can be predicted from the AA composition of a soil. This paper thus represents the first attempt to quantify the direct contribution of soil AA mineralization to the nitrifier denitrification N_2O production pathway.

2. Materials and methods

2.1. Study site and soils

Soils were obtained 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 were well-drained Typic Torrifluvents of the Pima series (Table 1). Soils were collected from three vegetation types, the first (open site) was dominated by annual herbaceous dicots, including peppergrass (*Lepidium thurberi*), Fremont's goosefoot (*Chenopodium fremontii*), and toothleaf goldeneye (*Viguiera dentata*). The second soil (mesquite site) was dominated by velvet mesquite (*Prosopis velutina*), a leguminous tree, and the third (sacaton site) was populated by sacaton (*Sporobolus wrightii*), a perennial bunchgrass. After transport to the laboratory, soils were sifted through a 2 mm sieve and refrigerated at 4 °C until analysis. Soil texture was determined using the hydrometer method (Gee and Bauder, 1986) and pH was measured using the using the method of van Lierop (1990) with an Orion Model 310 meter (Thermo Orion, Beverly, MA) and an Orion electrode. Carbon and nitrogen content and isotope composition of the soils and AAs studied (Sigma Chemical Co., St Louis, MO) were determined by a dry combustion analyzer interfaced with a Europa Hydra 20/20 IRMS (Europa Scientific, Crewe, UK).

Soil NO_3^- concentrations were determined by deionized (DI) water (5 ml) extraction of 1.0 g air-dried soil (shaken for 30 min), followed by centrifugation at 2000*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. Anions were separated with an isocratic 10 mM NaOH gradient and detected with a Dionex ED-40 electrochemical detector set in the conductivity mode.

2.2. Properties of added AAs

The 20 polymeric AAs can be grouped into five logical units based on their synthesis mechanisms:

Table 1
Properties of San Pedro soils used in this study

Soil	Organic C ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	C/N	pH	NO_3^--N ($mg\ kg^{-1}$)	Sand ($g\ kg^{-1}$)	Clay ($g\ kg^{-1}$)
Open	5.83	0.63	9.2	7.35	1.53	830	40
Mesquite	29.90	3.08	9.7	6.55	71.80	829	63
Sacaton	17.61	1.81	9.7	6.05	7.23	754	59

the alpha-ketoglutarate AAs (Group 1), the oxaloacetate-aspartate AAs (Group 2), the pyruvate-derived AAs (Group 3), the 3-phosphoglycerate-derived AAs (Group 4), and the aromatic AAs (Group 5) (Coruzzi and Last, 2000). The 11 AAs used for the incubations in this study (Table 2) included at least two representative AAs from each group. Each of the 20 polymeric AAs has a common core structure to which is attached a variable R-group, a side chain that defines the AA and bestows unique chemical (e.g. acidity, polarity) and structural (e.g. shape) properties. Five types of R-group side chains were represented by AAs in this study: aliphiatic (ALA, LEU, GLY), aromatic (PHE, TYR), non-aromatic hydroxyl-containing (SER, THR), basic (ARG, LYS), and acidic (ASP, GLU). The size and polarity of individual AAs also allow for additional grouping according to hydrophobicity. The AAs in this study included hydrophobic, non-polar AAs (ALA, LEU, PHE), hydrophilic, polar AAs (ASP, GLU, GLY, SER, THR, TYR), and hydrophilic, basic AAs (ARG, LYS). Nitrogen-containing carbohydrates, or amino sugars (AS), can also be significant sources of organic N in soil (Martens and Loeffelmann, 2003) and thus, a representative AS, glucosamine, was also included in soil incubations.

2.3. Laboratory incubations of field soils

Soils from the three vegetation types were very similar in physical properties, varying only in C and N (Table 1). Only the soil lowest in C and N (open soil) was used for the AA incubations to reduce the levels of background N_2O efflux not originating from the mineralization of the added AA. In addition, soils from each of the three vegetation types were incubated at 80% field capacity to quantify N_2O efflux from unamended soils. Prior to incubation, all soils were leached with DI water to remove excess soil NO_3^-. Following washing and air-drying, 20 g of soil were added to 250 ml Erlenmeyer flasks and sprinkled with DI water with and without AA (100 μg AA–N added g^{-1} soil) to achieve moisture contents of 80% field capacity (−34 kPa). Flasks were then tightly sealed with rubber stoppers with an outlet

Table 2
Properties of AAs and amino sugar used in this study

	C ($g\ kg^{-1}$)	N ($g\ kg^{-1}$)	C/N	$\delta^{13}C$ (‰)
Alpha-ketoglutarate family (Group 1)				
Glutamine (GLU)	410.9	191.7	2.14	−26.27
Arginine (ARG)	413.7	321.6	1.29	−13.93
Average, Group 1	412.3 (2.0)	256.6 (91.8)	1.71 (0.61)	−20.10 (8.72)
Oxaloacetate-aspartate family (Group 2)				
Asparagine (ASP)	363.6	212.0	1.71	−26.73
Threonine (THR)	403.3	117.6	3.43	−27.93
Lysine (LYS)	493.0	191.6	2.57	−11.30
Average, Group 2	420.0 (66.3)	173.7 (49.7)	2.57 (0.86)	−21.99 (9.27)
Pyruvate-derived family (Group 3)				
Alanine (ALA)	404.4	157.2	2.57	−22.67
Leucine (LEU)	549.4	106.8	5.14	−29.05
Average, Group 3	476.9 (102.5)	132.0 (35.6)	3.86 (1.82)	−25.86 (4.51)
3-Phosphoglycerate family (Group 4)				
Serine (SER)	342.9	133.3	2.57	−36.88
Glycine (GLY)	320.0	186.6	1.71	−34.68
Average, Group 4	331.4 (16.2)	160.0 (37.7)	2.14 (0.61)	−35.78 (1.55)
Aromatic AAs (Group 5)				
Tyrosine (TYR)	596.6	77.3	7.72	−21.36
Phenylalanine (PHE)	654.4	84.8	7.72	−11.05
Average, Group 5	625.5 (40.9)	81.0 (5.3)	7.72 (0.00)	−16.20 (7.29)
Average, all AAs	422.9 (131.9)	161.2 (70.2)	3.51 (2.32)	−23.80 (8.79)
Amino sugar				
Glucosamine (GLUx)	402.2	78.2	5.14	−19.18

The valve in paranthesis indicates the standard deviation of the mean.

port for syringe sampling and incubated at room temperature (~20 °C). Duplicate incubations of each AA treatment were performed.

Gas subsamples were removed from the incubation vessels using gastight syringes once every 24 h and analyzed for N_2O on a Shimadzu GC14-A gas chromatograph (Shimadzu Scientific Instruments, Columbia, MD) fitted with an electron capture detector and a 80/100 mesh HayeSep-Q column, 2 m × 3 mm ID (Supelco, Inc., Bellefonte, PA) at 45 °C, using N_2 as a carrier gas (flow rate, 40 ml min^{-1}). The injector and detector were maintained at 110 and 250 °C, respectively. Certified N_2O standards (Praxair Technology, San Ramon, CA) were used for calibration. Carbon dioxide and O_2 concentrations were determined by flushing the gas from the incubation flask through an O_2 sensor (Qubit Systems, Kingston, Ontario) and an S151 infra-red gas analyzer (IRGA) (Qubit Systems).

After passage through the IRGA, headspace gas from each incubation vessel was bubbled into a flask containing a mixture of 2 ml saturated $SrCl_2$ (Sigma Chemical Co.) and 20 ml of 250 mM NaOH, trapping CO_2 as $SrCO_3$ (Harris et al., 1997). Following repeated washings with DI water to remove excess NaOH, dried carbonate samples were analyzed for ^{13}C isotope composition by dry combustion with a Europa Hydra 20/20 IRMS. After gas collections and analyses, incubation vessels were flushed with CO_2-free air and re-sealed. Daily net gas production rate in each incubation vessel was calculated by subtracting background CO_2 and N_2O production from control flasks containing unamended open area soil. Total incubation time was 12 days.

2.4. Soil organic N extraction and identification of AA and AS

To extract and quantify organic N from field soils, the procedure of Martens and Loeffelmann (2003) was used. Briefly, soil samples (100 mg) were treated with 2 ml of 4 M methanesulfonic acid and autoclaved for 60 min at 136 °C (112 kPa). After sample cooling, centrifugation and collection of the supernatant, the residue was washed with two aliquots of DI water (1 ml), centrifuged between each addition, and the three supernatants combined for AA analysis. All combined supernatants were titrated to pH 4–5 with 5 M KOH, centrifuged to remove precipitate and diluted (0.25–1.00 ml aliquot diluted to 10 ml) for analysis.

The AAs released as hydrolysis products of acid digestion were separated on a Dionex DX-500 (Dionex Corp.) ion chromatograph equipped with a AminoPac PA10 column (2 mm ID). Separation was achieved with a tertiary water, NaOH (5–80 mM) and sodium acetate (125–200 mM) gradient. Pulsed amperometric detection was by a Dionex ED-40 electrochemical detector set in the integrated pulsed mode with a gold working electrode. AA standards were obtained from Sigma (Sigma Chemical Company, St Louis, MO).

2.5. Calculations and statistical analysis

Isotope composition of the $SrCO_3$ collected from headspace gas and concentrations of respired CO_2 in the control and AA-addition flasks were used to calculate the AA–C contribution to CO_2 in incubation flasks. These calculations were done using a mixing equation (Gearing, 1991)

$$(X + Y)(\delta^{13}C_{mix}) = (X)(\delta^{13}C_x) + (Y)(\delta^{13}C_y)$$

where X is the amount (μg) of C respired from soil (unamended control flasks); Y, amount (μg) of C respired from soil AA incubations; $\delta^{13}C_{mix}$, isotope ratio of total CO_2 respired (AA–C + control soil-C); $\delta^{13}C_x$, isotope ratio of soil C respired (unamended control soils); $\delta^{13}C_y$, isotope ratio of AA–C respired.

All statistical analyses were done using Minitab 13.32 Statistical Software (Minitab, Inc., State College, PA).

3. Results and discussion

3.1. Amino acid incubations: N_2O production

Following AA addition to the incubated soils, N_2O production was low in all flasks for the first 24 h, ranging from 2.7 to 10.8 ng N_2O g^{-1} soil day^{-1}. Nitrous oxide production rates increased by 48 h, ranging from 5.0 to 25.8 ng N_2O g^{-1} soil day^{-1}. Continued AA mineralization promoted N_2O flux in three general patterns (Table 3; Fig. 2). Seven of the AA incubations (GLU, ARG, LYS, ALA, LEU, GLY, and PHE) showed small but steady N_2O production (9.7 ± 3.9 ng N_2O g^{-1} soil day^{-1}) from days 3 to 12 (Fig. 2a). The remaining four AAs, ASP, THR, SER, and TYR, showed a trend of increasing N_2O production from day 1 to day 5. Thereafter, rates of N_2O efflux increased in ASP flasks by an average of 12% per day, showing production of 56.6 ± 14.8 ng N_2O g^{-1} soil day^{-1} on day 12. From days 5 to 12, N_2O production by THR, SER, and TYR remained steady, averaging 17.9 ± 2.3 ng N_2O g^{-1} soil day^{-1} (Fig. 2b). Overall, these three AAs had significantly ($\rho < 0.001$) higher N_2O production through the 12-day incubation period (188.3 ± 18.7 ng g^{-1} soil) compared to the seven lower-production AAs (106.7 ± 23.7 ng g^{-1} soil) (Fig. 2a). However, ASP produced significantly more N_2O (453.0 ± 63.0 ng N_2O g^{-1} soil) during the 12-day incubation than the other 10 AAs tested.

There were small, non-significant differences in the chemical properties of the seven AAs with low N_2O flux vs the four AAs with higher N_2O flux (Table 2). The four AAs that produced the most N_2O (ASP, THR, SER, and TYR) were slightly lower in organic C content (426.6 ± 107.5 g kg^{-1}) than the seven low-flux AAs (463.7 ± 106.7 g kg^{-1}). However, the correlation between N_2O produced and AA–C content over all incubations was negative, indicating that the AAs with higher C content

Table 3
Gas production and consumption in incubations with added AAs and amino sugar

	CO_2 production ($\mu g\ g^{-1}$ soil)	CO_2-C conversion (%)	N_2O production ($ng\ g^{-1}$ soil)	N_2O-N conversion (%)	NO_3^--N ($\mu g\ g^{-1}$ soil)	O_2 consumption ($\mu g\ g^{-1}$ soil)
Glutamine (GLU)	336 (28)	42.8 (3.6)	110.8 (17.7)	0.07 (0.01)	72.9 (15.4)	528 (91)
Arginine (ARG)	292 (2)	61.9 (0.3)	150.8 (63.2)	0.10 (0.04)	58.2 (9.1)	457 (34)
Average, Group 1	**314 (31)**	**52.4 (11.2)**	**130.8 (28.3)**	**0.08 (0.03)**	**65.5 (10.4)**	**492 (70)**
Asparagine (ASP)	436 (164)	25.7 (4.5)	453.0 (63.0)	0.29 (0.04)	81.9 (3.2)	708 (249)
Threonine (THR)	512 (49)	40.7 (3.9)	205.6 (27.1)	0.13 (0.02)	41.9 (3.4)	803 (69)
Lysine (LYS)	384 (13)	40.7 (1.3)	101.4 (21.2)	0.06 (0.01)	77.1 (5.1)	667 (34)
Average, Group 2	**447 (64)**	**35.7 (8.2)**	**253.3 (49.3)**	**0.16 (0.06)**	**67.0 (21.8)**	**726 (85)**
Alanine (ALA)	378 (51)	40.2 (5.4)	73.5 (17.3)	0.05 (0.01)	75.6 (15.9)	659 (81)
Leucine (LEU)	450 (31)	23.8 (1.6)	106.2 (25.4)	0.07 (0.02)	20.3 (1.1)	709 (5)
Average, Group 3	**414 (50)**	**32.0 (10.0)**	**89.8 (23.1)**	**0.06 (0.02)**	**47.9 (39.2)**	**684 (35)**
Serine (SER)	479 (28)	50.8 (2.9)	190.7 (17.6)	0.12 (0.01)	55.7 (3.8)	733 (74)
Glycine (GLY)	352 (66)	56.0 (10.5)	112.3 (30.4)	0.07 (0.02)	67.4 (14.8)	558 (123)
Average, Group 4	**415 (89)**	**53.4 (7.0)**	**151.5 (25.4)**	**0.10 (0.03)**	**61.6 (8.3)**	**645 (94)**
Tyrosine (TYR)	685 (8)	24.2 (0.3)	168.5 (41.6)	0.11 (0.03)	37.0 (2.2)	1244 (96)
Phenylalanine (PHE)	486 (31)	17.2 (1.1)	80.7 (9.3)	0.05 (0.01)	38.1 (1.7)	859 (44)
Average, Group 5	**585 (141)**	**20.7 (4.1)**	**124.6 (32.1)**	**0.08 (0.04)**	**37.5 (0.8)**	**1051 (73)**
Average, all AAs	**436 (108)**	**38.5 (14.4)**	**156.0 (67.3)**	**0.09 (0.04)**	**56.9 (20.2)**	**712 (232)**
Glucosamine (GLUx)	404 (67)	21.4 (3.5)	117.9 (20.0)	0.08 (0.01)	43.6 (2.5)	612 (122)

The valve in parenthesis indicates the standard deviation of the mean.

tended to produce less N_2O, although this relationship was not significant ($\rho = 0.103$). The four high-flux AAs had a lower total N content ($135.0 \pm 52.3\ g\ kg^{-1}$) compared to the seven low-flux AAs ($177.2 \pm 73.6\ g\ kg^{-1}$) and as a result, the high-flux AAs had a higher average C/N ratio (3.86 ± 2.47) than the low-flux AAs (3.31 ± 2.21).

The hydrophilic, non-polar AAs, a group which included the four high-flux AAs and also GLU and GLY, had significantly ($\rho = 0.048$) higher N_2O production over the 12 day incubation ($206.8 \pm 126.9\ ng\ g^{-1}$ soil) than the hydrophobic, non-polar AAs (ALA, LEU, PHE) ($86.8 \pm 17.2\ ng\ g^{-1}$ soil). This might be expected, given that hydrophilic AAs would tend to interact with the aqueous environment and would more readily go into solution, perhaps becoming more available to microbial populations. The hydrophilic, basic AAs (ARG, LYS) were intermediate in N_2O production ($126.1 \pm 34.9\ ng\ g^{-1}$soil). The AA synthesis mechanism group (Group 1–5, Table 2) and R-side chain properties were not significant predictors of N_2O production in the incubated soils (data not shown).

Soil analysis at the end of the 12-day incubation period indicated that much of the added AA–N had been mineralized to NO_3^- (Table 3), with NO_3^--N recoveries averaging $55.8 \pm 19.6\%$ across all treatments. The four high-flux AAs and the seven low-flux AAs had similar soil NO_3^- concentrations at the end of the incubation (high: $54.15 \pm 18.80\ \mu g\ g^{-1}$ soil; low: $58.51 \pm 22.24\ \mu g\ g^{-1}$ soil), and regression analysis confirmed that the NO_3^- contents of the soil on day 12 were not a significant predictor of total N_2O production in these incubations ($\rho = 0.34$).

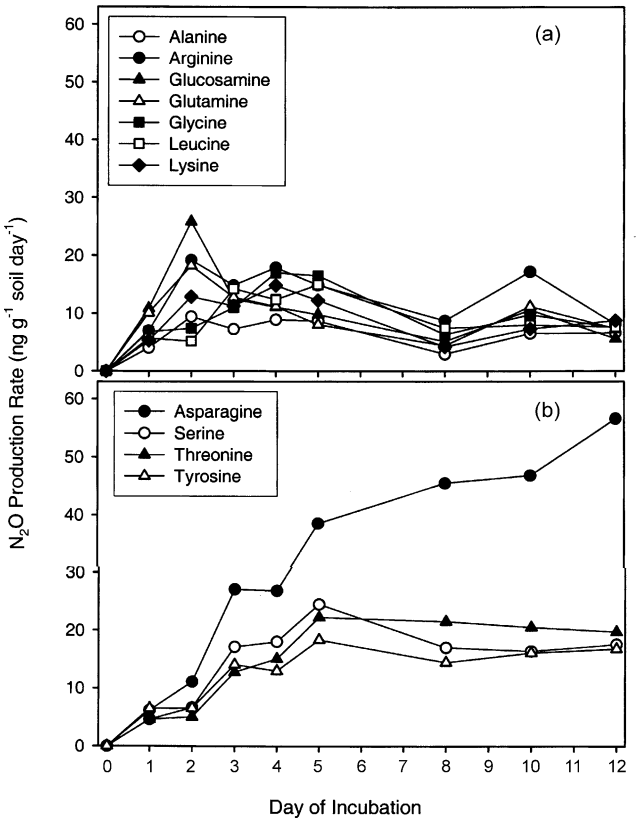

Fig. 2. Nitrous oxide production rate by 7 'low flux' substrates (a) and 4 'high flux' substrates (b) in incubation flasks with added amino acids during 12-day incubation period.

3.2. Amino acid incubations: CO_2 production and O_2 consumption

The total CO_2 respired from all AA incubations over the 12-day incubation period averaged 432.8 ± 103.1 μg CO_2 g^{-1}soil. The wide variation in CO_2-C efflux from the AA incubations (Table 3) is not surprising. Additions of AAs to incubation flasks were determined only by AA–N content and thus, C additions to flasks ranged from 2.6 (ARG) to 15.4 mg AA–C (PHE).

The AAs which had a higher C content produced more total CO_2 ($\rho = 0.021$) over the 12 d incubation period. The C content of the individual AAs accounted for 53% of the variability in CO_2 production, with the amount of C added to the soil (0.13–0.77 mg AA–C g $soil^{-1}$) as the strongest predictor of percentage organic C conversion to CO_2. Net CO_2 efflux has been shown in other studies to be a poor indicator for quantification of AA mineralization (Jones, 1999; Vinolas et al., 2001b). Jones (1999) observed a weak correlation between addition of a mixture of 15 AAs to incubation flasks and respiration in both topsoils ($r^2 = 0.34$) and subsoils ($r^2 = 0.15$), and reported that AA–C was predominantly used in the production of new cell biomass ($66 \pm 2\%$) rather than respiration ($34 \pm 2\%$). Similarly, our study found an average contribution of added AA–C to respired CO_2 of $35.9 \pm 14.4\%$ over the 12-day incubation period.

The CO_2 respired from AA incubations was significantly ($\rho = 0.002$) higher from TYR and PHE, the aromatic side chain, high C, Group 5 AAs (Table 2). Other R-side chain properties did not significantly influence CO_2 production (data not shown). In addition, there were no significant differences in CO_2 production between the hydrophilic AAs (average production 431.1 ± 117.4 μg g^{-1}) and the hydrophobic AAs (437.99 ± 54.6 μg g^{-1}).

Although the high N_2O flux AAs and the low N_2O flux AAs were essentially equal in C content, CO_2 production in the four high N_2O flux AAs (527.9 ± 109.1 μg g^{-1}) was significantly ($\rho = 0.046$) higher than production in the seven low N_2O flux AAs (386.2 ± 66.9 μg g^{-1}), suggesting that AAs with low N_2O flux were either mineralized at a slower rate than the AAs giving higher N_2O flux, or had a higher incorporation rate into microbial biomass. These variations in CO_2 production were not the result of different amounts of AA–C added to the incubation flasks, as the total AA–C added to the high N_2O flux incubations was not significantly higher ($\rho = 0.191$) than the AA–C added to the low N_2O flux incubations.

Regression analysis showed that the relationship between AA–N content and CO_2 efflux was significant ($\rho < 0.001$) and negative, indicating that the incubations with lower N-content AAs (Table 2) produced more CO_2. The experimental design for the incubations included the addition of equal amounts of AA–N (100 μg N g^{-1} soil) to each flask. Thus, AAs with lower N content were added to flasks in larger amounts. However, the AA–N content and total C added to the incubation flasks were negatively correlated (data not shown), indicating that the higher production of CO_2 did not result from more AA–C added to flasks.

In addition to N_2O and CO_2 production, consumption of O_2 in all flasks was monitored throughout the 12-day incubation period (Table 3). Oxygen consumption and CO_2 production showed similar patterns, as the incubations with the four high N_2O flux AAs consumed more O_2 (872.4 ± 251.3 μg g^{-1} soil) than the seven incubations with low N_2O production (631.2 ± 123.4 μg g^{-1} soil), though these differences were not significant ($\rho = 0.149$). In addition, incubations with aromatic side chain Group 5 AAs consumed significantly ($\rho < 0.001$) more O_2 during mineralization. Flasks with hydrophilic AAs added consumed more O_2, but these differences were not significant ($\rho = 0.134$).

Overall, N_2O production in AA-amended soils was not significantly correlated with CO_2 production ($r = -0.06$, $\rho = 0.577$) (Fig. 3a) nor was the percentage of added N converted to N_2O related to the percentage of added C respired ($\rho = 0.901$). Carbon dioxide production was, however, significantly correlated with O_2 consumption ($r = 0.92$, $\rho < 0.001$) (Fig. 3b). Thomson et al. (1997)

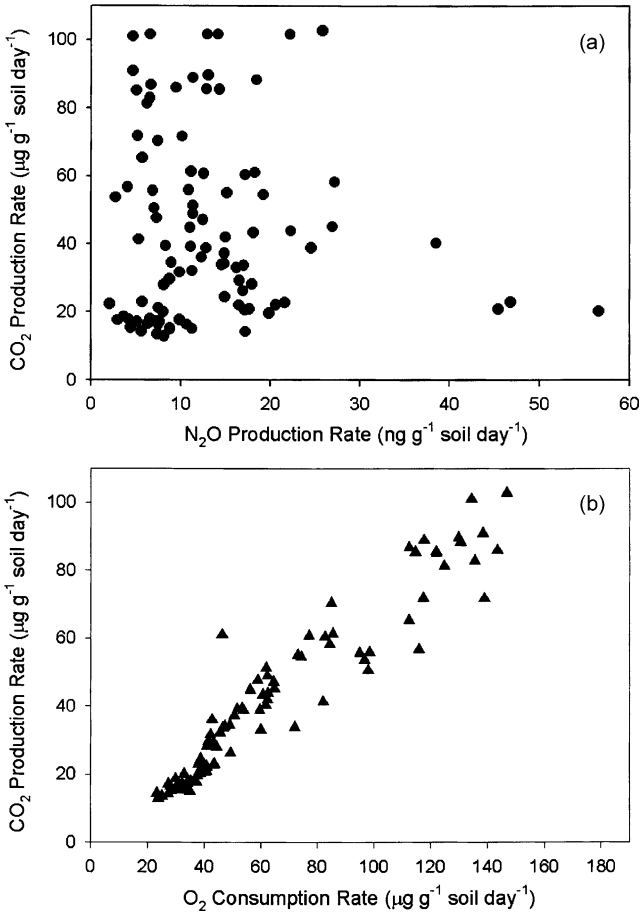

Fig. 3. Correlation of carbon dioxide production rate with nitrous oxide production rate (a) and oxygen consumption rate (b) in incubation flasks with added amino acids during 12-day incubation period.

noted a strong correlation between CO_2 production from added plant residues (grass-clover mixture) and N_2O flux ($r^2=0.85$), which they related to increased formation of anaerobic microsites due to microbial activity. Anaerobic microsite formation in the present study was thought to be minimal for several reasons. The sandy (83% sand) open soil was maintained at moisture levels below field capacity throughout the incubation period, and soil amounts (20 g per flask) used resulted in only a very thin (<0.5 cm) soil layer in each incubation flask, where headspace O_2 levels never dropped below 18.9% during the incubation period.

3.3. ^{13}C analysis of CO_2-C: contribution of AA to CO_2 signature

The isotopic signature of respired CO_2 collected from flasks on the first 5 days of the incubation period was used to quantify the contribution of AA–C to the total amount of CO_2 respired, and thus monitor levels of AA mineralization throughout this period. On day 1 of the incubations, CO_2 produced by the AA mineralization averaged 47.9 ± 10.5 μg CO_2 g^{-1} soil day^{-1} and AA mineralization contributed an average 69.6 ± 15.8% to the isotopic signature of the CO_2 respired. By Day 5, the AA contribution to CO_2 production was more variable, averaging 7.6 ± 6.9 μg CO_2 g^{-1} soil day^{-1} and 25.8 ± 23.4% of total CO_2 produced. During the same 5-day period, CO_2 production in the unamended control soils was constant, averaging 34.5 ± 7.5 μg CO_2 g^{-1} soil day^{-1} with a $\delta^{13}C$ signature of -21.4 ± 1.0. All of the soil incubations showed significant ($r^2>0.9$) exponential decreases in the percentage of AA–C contributing to the respired CO_2 over time and thus, it was possible to use the time series equation derived from the first 5 days of incubations to predict the percentage contribution from each AA to the CO_2 pool on the last day of the incubations, day 12 (Table 4). Mineralization of free soil AAs has been shown to proceed very quickly in soils (Jones, 1999; O'Dowd et al., 1999; Vinolas et al., 2001a), and AA mineralization in the present study, revealed by contribution of signature ^{13}C to respired CO_2, was complete by the end of the incubation period (Table 4). The lone exception was TYR, which continued to contribute more than 70% of the total C to the respired CO_2 through day 12 of the incubation period. This suggests a short AA lifetime in our soils, in agreement with the work of other researchers who have reported mean residence times of AAs in soils ranging from less than 4 h for a mixture of 15 AAs added to a sandy loam (Jones and Shannon, 1999) to several hours to 1 day for GLY and GLU added to a loamy alpine meadow soil (Lipson et al., 1999).

3.4. Incubation of unamended field soils

The use of environmental variables to model N_2O emissions from non-agricultural soils has been largely unsuccessful (Baumgärtner and Conrad, 1992; Robertson and Klemedtsson, 1996; Veldkamp et al., 1999), in part due to extremely complex biochemical reactions governing N_2O production. Gödde and Conrad (2000) standardized temperature and soil moisture in laboratory incubations to decrease the overall variability of N trace gas turnover, and reported that NO_3^-, NO_2^-, pH, soil texture, and nitrification rates each contributed to regulation of N_2O production. Some studies have reported that N_2O flux in non-agricultural soils was most limited by soil moisture and available N and that large pulses of N_2O were produced following wetting of dry soil (Davidson, 1992; Hutchinson et al., 1993; Mummey et al., 1994), possibly related to the rapid release of C and N from microbial cells and physically protected soil organic matter following wetting (Mummey et al., 1994). Results from the present study indicate that pulses of N_2O are directly related to, and perhaps can be quantified from, the release of organic N from the soil N pool via AA mineralization.

To test the hypothesis that mineralization of soil organic N may be a direct source of N_2O-N in these soils, samples from the 0 to 5 cm mineral layer from the open, mesquite, and sacaton vegetation areas were first analyzed for AA

Table 4
Percentage of respired carbon dioxide derived from added AAs over 12-day incubation period

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 12
Glutamine (GLU)	46.8	20.7	12.0	10.6	3.6	0.0
Arginine (ARG)	61.7	23.7	6.3	0.0	0.0	0.0
Asparagine (ASP)	71.6	45.4	34.6	29.2	12.6	0.0
Threonine (THR)	77.4	73.8	68.3	50.8	28.6	0.0
Lysine (LYS)	100.0	100.0	84.5	56.1	48.3	0.0
Alanine (ALA)	80.3	81.2	39.9	14.9	13.4	0.0
Leucine (LEU)	49.3	60.5	57.2	41.4	26.0	0.0
Serine (SER)	67.3	61.2	35.2	27.8	17.5	0.0
Glycine (GLY)	62.7	51.7	27.7	10.9	8.7	0.0
Tyrosine (TYR)	59.2	92.2	83.7	84.7	81.5	71.7
Phenylalanine (PHE)	91.4	100.0	100.0	52.8	49.2	0.0
Glucosamine (GLUx)	68.1	76.7	57.7	23.2	20.5	0.0

Table 5
Average (± standard deviation) AA and amino sugar composition of study soils

AA	Open soil (mg kg^{-1})	Mesquite soil (mg kg^{-1})	Sacaton soil (mg kg^{-1})
Arginine	1268 (301)	2400 (372)	916 (197)
Lysine	163 (32)	1013 (296)	531 (98)
Galactosamine	526 (136)	1384 (144)	416 (70)
Glucosamine	773 (197)	2186 (220)	772 (120)
Alanine	874 (319)	1203 (118)	737 (109)
Threonine	490 (212)	1121 (119)	480 (91)
Glycine	1009 (428)	1225 (77)	840 (83)
Valine	602 (376)	1041 (52)	335 (42)
Serine/proline	1042 (333)	1983 (98)	943 (204)
Isoleucine	264 (192)	598 (18)	223 (53)
Leucine	461 (266)	1171 (135)	553 (124)
Methionine	63 (18)	131 (32)	28 (14)
Histidine	199 (71)	253 (42)	260 (73)
Phenylalanine	328 (123)	632 (89)	271 (10)
Glutamine	856 (406)	1915 (109)	604 (112)
Asparagine	877 (472)	1848 (56)	709 (261)
Cysteine	82 (45)	212 (14)	78 (21)
Tyrosine	258 (110)	618 (60)	245 (62)
Total (gkg $^{-1}$)	**10 (4)**	**21 (1)**	**9 (1)**

content and composition (Table 5). The AA profiles of all three sites were strikingly similar, but differed in the total amount of AAs present (Table 5, Fig. 4). Overall, ARG and GLUx were the most prevalent, each accounting for 8–12% of total AAs in all three soils (Fig. 4).

One complicating factor in the identification and quantification of the N_2O-producing pathway in this soil system is the spatial variability in availability of NO_3^-. Typically, desert soils contain only 1–2 mg NO_3^--N kg^{-1} soil (Virginia and Jarrell, 1983). The open soil of the present study falls within this range, with an NO_3^--N content of 1.5 ± 0.7 mg kg^{-1} soil and the sacaton soils have only slightly higher NO_3^--N, averaging 7.2 ± 1.9 mg kg^{-1} soil. However, the mesquite soil contains significantly higher NO_3^--N, 71.8 ± 22.2 mg kg^{-1}. Such extremes in NO_3^- concentration are typical of arid soils and have confounded predictions of N_2O flux from desert ecosystems. In addition, high NO_3^- concentrations and increases in N_2O production following rain events in semi-arid soils dominated by mesquite have led researchers to identify denitrification as the sole microbial process producing N_2O (Virginia et al., 1982; Westerman and Tucker, 1978).

To reduce the confounding effects of NO_3^--N in incubations, soil samples were first washed several times with DI water. Assays performed before and after washing determined that soil NO_3^- concentrations fell by 91% in the open soils, 85% in the mesquite soils, and 50% in the sacaton soils, showing that the washing was effective in reducing the substrate pool for anaerobic denitrification. After water additions to achieve a moisture potential of -34 kPa, incubations of soils from the 0 to 5 cm mineral layer of all three sites were then performed and N_2O and CO_2 production and O_2 consumption were monitored over a 12-day period (Table 6). The mesquite soil with the higher total N and AA–N contents produced N_2O at a rate twice that of the open soil and nearly three times the rate of the sacaton soil. Nitrous oxide flux from the three soils was not significantly correlated with total N content ($r=0.32$, $\rho=0.53$), total C content ($r=0.39$, $\rho=0.44$) or NO_3^- content after washing ($r=0.52$, $\rho=0.29$). Carbon dioxide production by the incubated soils was significantly correlated ($r=0.83$, $\rho=0.03$) with C content of the soils, but CO_2

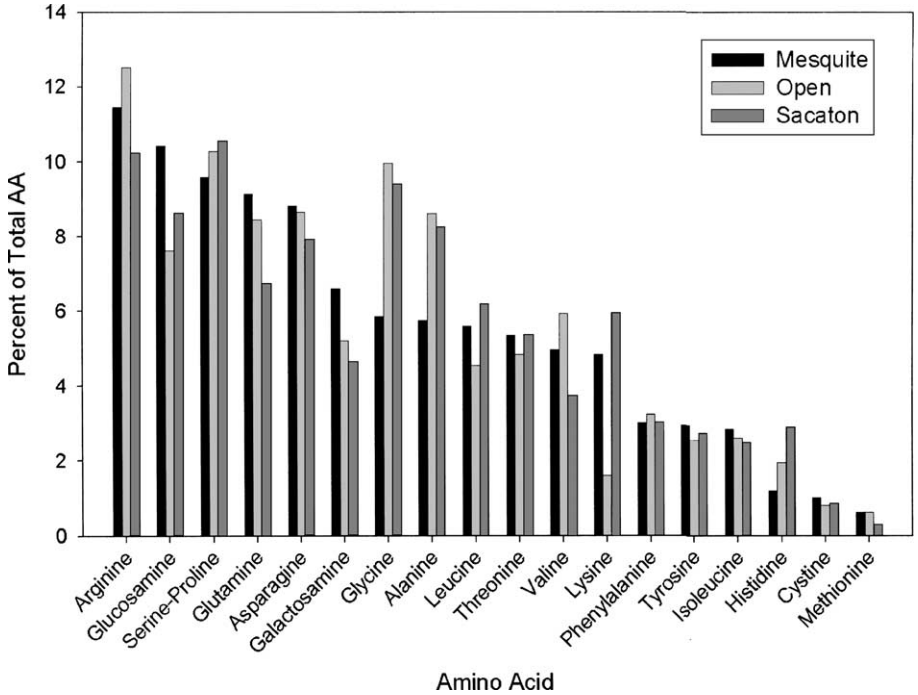

Fig. 4. The contribution of individual amino acids to total amino acid pool in unamended field soils.

Table 6
Nitrous oxide and carbon dioxide production in unamended field soils from San Pedro Riparian Area

	CO_2-C production ($\mu g\ g^{-1}$ soil)	Carbon mineralized (% of total C)	Actual N_2O production ($ng\ g^{-1}$ soil)	Modeled N_2O production ($ng\ g^{-1}$ soil)
Open	73–236	1.25–4.05	15.6–40.1	12.8–38.2
Mesquite	133–270	0.44–0.90	19.6–80.2	17.0–58.0
Sacaton	120–126	0.68–0.72	17.5–17.8	15.1–26.6

production and N_2O production were not correlated with one another ($r = -0.08$, $\rho = 0.88$).

3.5. Modeling of N_2O production in unamended field soils

To test if the production of N_2O in incubated field soils can be attributed to AA mineralization, a predictive model was developed based on initial soil AA composition (Table 5), AA conversion to N_2O (Table 3) and percentage of organic C mineralized during incubation, measured as CO_2 respired. In the first step, the percentage of organic C mineralized in each incubation flask was calculated by dividing the total CO_2-C released by the organic C content of each soil (Table 1). Dividing this number by the C/N ratio of the soil (Table 1), gave an estimate of the total N mineralized during the incubation period. Next, the AA profile of each of the three soils (Fig. 4) was used to calculate the percentage of the total soil N represented by each AA, and these percentages were multiplied by the total N mineralized during the incubation period to determine the total AA–N released. In the final step, the N_2O production (Table 3) from each of the AA incubations (ng N_2O per μg AA–N) was multiplied by the AA–N released in the unamended soil incubations to predict the N_2O emissions from each AA, and the sum of these values determined the total expected N_2O production from each unamended soil incubation. Nitrous oxide production by AAs that were identified during the soil AA analyses (Table 5), but not used in incubations were estimated from AAs from the same group. For example, valine N_2O production was predicted from the average of the Group 3 AAs incubated (ALA and LEU). Application of this model, which utilized CO_2 efflux as a proxy for organic N mineralization, the N_2O-N conversion percentage for the AAs, and initial soil AA composition, accurately predicted the N_2O production by the open, mesquite, and sacaton soils during the 12-day incubation period (Table 6).

A model with the ability to predict N_2O flux from unfertilized soils, such as described above, could be a valuable resource to soil scientists and modelers attempting to quantify soil inputs to global trace gas production. A considerable portion of soil N_2O production arises from nitrification and denitrification of fertilizers in agricultural soils throughout the world, and several studies have reported significant mathematical relationships between microbial N_2O production in agricultural soils and environmental factors including soil moisture, temperature, and NO_3^- content (Dobbie and Smith, 2003; Skiba et al., 1998; Smith et al., 1998; Weitz et al., 2001). In contrast, quantifying N_2O flux from non-agricultural ecosystems has proven to be much more complex, in part because the soil properties and microbial interactions that regulate the production of N_2O in soils are still largely unknown (Gödde and Conrad, 2000). At present, the model described above, utilizing AA composition and C mineralization rates to predict N_2O efflux, has yet to be tested in systems other than in vitro incubations of soils from this semi-arid riparian area. Yet this model shows great promise in furthering our understanding of quantification of N_2O flux from unfertilized soils.

3.6. Contribution of nitrifier denitrification to N_2O production

Data from the AA incubations suggests that nitrifier denitrification may be a significant pathway for N_2O production in these semi-arid soils. The unsaturated soil conditions and the extremely sandy texture of the incubated soils may have precluded the widespread formation of anoxic microsites necessary for anaerobic denitrification. Several studies have indicated that nitrification dominates N_2O production in dry soils, whereas anaerobic denitrification dominates in wetter soils (Davidson et al., 1993; Kester et al., 1997; Robertson and Tiedje, 1987) because high soil moisture promotes the formation of anoxic microsites. In a study of nitrifier denitrification in a sandy loam soil (60% sand), Webster and Hopkins (1996) found that nitrifier denitrification was the main source of N_2O from a dry soil (matric potential −100 kPa), whereas anaerobic denitrifiers were identified as the dominant producers of N_2O from a wetter soil (matric potential −10 kPa). In that study, of a total of 62 pmol $N_2O\ g^{-1}$ soil h^{-1} produced from denitrification sources, 55 pmol $N_2O\ g^{-1}$ soil h^{-1} (89%) were generated by nitrifier denitrification in the drier soil. In soils where anaerobic denitrification is the dominant pathway for N_2O production, some studies have found that CO_2 and N_2O efflux are closely correlated (Linn and Doran, 1984; Thomson et al., 1997). In the present study, N_2O and CO_2 production were not significantly correlated in incubations with AA additions ($r = 0.14$, $\rho = 0.15$). Carbon dioxide and N_2O production were also independent of one another ($r = -0.08$, $\rho = 0.88$) in the unamended soil incubations.

Presently, only limited techniques exist to assess the ecological significance of nitrifier denitrification in soils. Therefore, the importance of this pathway in different soils under a range of conditions is still a matter of speculation. Preliminary results of the present study suggest that this pathway could be a significant contributor to N_2O flux in semi-arid soils. Additional research using isotopically labeled substrates is presently underway to further elucidate

the contribution of nitrifier denitrification to overall N_2O emissions in this system.

4. Conclusions

Numerical models have been developed to predict the flux of N_2O from agricultural soils taking into account the presence of strong driving factors, including NO_3^- concentration and moisture. Conversely, prediction of N_2O flux from soils in natural settings has been exceptionally difficult for researchers. Even with our understanding of the biology and chemistry of soils of natural systems, models still fail to reliably simulate N_2O emissions over a wide range of field conditions suggesting that either presently identified mechanisms have been imperfectly modeled or that unidentified mechanisms still remain to be discovered. In the present study, AA mineralization has been shown to proceed quickly in soils, as evidenced by contribution of the AA isotopic signature to respired CO_2. AA mineralization in soils, and the subsequent release of organic N into the soil N pool, contributes to N_2O production in soil incubations. The N_2O production rates from individual AA incubations can then be incorporated into models and used to predict net N_2O production in unamended field soils. The ability to model N_2O production in soils based on AA composition and organic C mineralization rates suggests that these environmental parameters will aid in future modeling of soil N_2O production.

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