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# Nitrous oxide flux from soil amino acid mineralization

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#### Abstract

Nitrous oxide (N<sub>2</sub>O) 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<sub>3</sub><sup>-</sup> content with a limited success, but predicting N<sub>2</sub>O 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<sub>2</sub>O flux from semi-arid soils. In laboratory incubations (-34 kPa moisture potential), soil mineralization of eleven AAs (100 µg AA–N g<sup>-1</sup> soil) promoted a wide range in the production of N<sub>2</sub>O (156.0±79.3 ng N<sub>2</sub>O-N g<sup>-1</sup> 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<sub>2</sub>-C determined that, with the exception of TYR, all of the AAs were completely mineralized during incubations, allowing for the calculation of a N<sub>2</sub>O-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<sub>2</sub> and N<sub>2</sub>O efflux. A model utilizing CO<sub>2</sub> respired from the three soils as a measure of organic matter C mineralization, a preincubation soil AA composition of each soil, and the N<sub>2</sub>O-N conversion rate from the AA incubations effectively predicted the range of N<sub>2</sub>O production by all three soils. Nitrous oxide flux did not correspond to factors shown to influence anaerobic denitrification, including soil NO<sub>3</sub><sup>-</sup> contents, soil moisture, oxygen consumption, and CO<sub>2</sub> respiration, suggesting that nitrification and aerobic nitrifier denitrification could be contributing to N<sub>2</sub>O production in these soils. Results indicate that quantification of AA mineralization may be useful for predicting N<sub>2</sub>O production in soils.

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Keywords: Nitrous oxide; Nitrifier denitrification; N Cycling; Greenhouse gas

### 1. Introduction

Nitrous oxide (N<sub>2</sub>O) 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<sub>2</sub>O has 100-year global warming potential that is about 310 times that of carbon dioxide (CO<sub>2</sub>) (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).

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Microbial transformations of soil N (nitrification and denitrification) contribute about 70% of the annual N<sub>2</sub>O 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<sub>3</sub> oxidation through formation of hydroxylamine (NH<sub>2</sub>OH) or NO<sub>2</sub><sup>-</sup> (Bremner et al., 1980; Chalk and Smith, 1983; Wrage et al., 2001). An extension of the nitrification pathway that can also produce N<sub>2</sub>O 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 NH3-oxidizers under aerobic conditions, whereas anaerobic denitrification is the stepwise reduction of  $NO_3^-$  to dinitrogen gas (N<sub>2</sub>) (Fig. 1) carried out

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Fig. 1. Pathways of nitrogen transformation in soil, showing nitrification (---), and nitrification (---), and nitrification (---).

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<sub>2</sub>O 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<sub>2</sub>O emissions from a sandy loam (Webster and Hopkins, 1996). Certain environmental conditions, including high soil NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup> content, low organic C content, low O<sub>2</sub> pressure, and possibly low pH are thought to be conducive to the production of N<sub>2</sub>O 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 N2O 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<sub>2</sub>O flux from soils, and that correspondingly, N<sub>2</sub>O 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<sub>2</sub>O 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 welldrained 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 goldeneve (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<sub>3</sub><sup>-</sup> 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 2000g (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:

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