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Abiotic nitrous oxide production from hydroxylamine in soils and their dependence on soil properties

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ABSTRACT

Despite the fact that microbial nitrification and denitrification are considered the major soil $N₂O$ emission sources, especially from agricultural soils, several abiotic reactions involving the nitrification intermediate hydroxylamine (NH₂OH) have been identified leading to N₂O emissions, but are being neglected in most current studies. Here, we studied N₂O formation from NH₂OH in cropland, grassland, and forest soils in laboratory incubation experiments. Incubations were conducted with and without the addition of $NH₂OH$ to non-sterile and sterile soil samples. N₂O evolution was quantified with gas chromatography and further analyzed with online laser absorption spectroscopy. Additionally, the isotopic signature of the produced N₂O (δ^{15} N, δ^{18} O, and 15 N site preference) was analyzed with isotope ratio mass spectrometry. While the forest soil samples showed hardly any N₂O evolution upon the addition of NH₂OH, immediate and very large formation of N₂O was observed in the cropland soil, also in sterilized samples. Correlation analysis revealed soil parameters that might explain the variability of NH₂OHinduced N2O production to be: soil pH, C/N ratio, and Mn content. Our results suggest a coupled biotic $-$ abiotic production of N₂O during nitrification, e.g. due to leakage of the nitrification intermediate NH2OH with subsequent reaction with the soil matrix.

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1. Introduction

Nitrous oxide (N_2O) is an important greenhouse gas. It has an about 300 times higher global warming potential than carbon dioxide $(CO₂)$ over a time frame of 100 years and contributes approximately 6% to anthropogenic radiative forcing, making it the third-most important contributor after $CO₂$ and methane ([WMO,](#page--1-0) 2013). Furthermore, N₂O is known to be partly responsible for the catalytic destruction of ozone in the stratosphere ([Crutzen, 1970\)](#page--1-0). While other historically dominant ozone depleting substances have been successfully regulated by the Montreal Protocol, N_2O is still unregulated and, if present trends continue, will become the dominant ozone depleting substance in the 21st century ([Ravishankara et al., 2009](#page--1-0)). The atmospheric mixing ratio of N_2O has increased by 20% from a pre-industrial level of

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270 ppb-325 ppb in 2012 at a rate of 0.80 ppb yr^{-1} over the last decade (WMO, 2013). The increase in atmospheric N₂O is tightly coupled to increasing anthropogenic nitrogen (N) fixation, mainly applied as fertilizer and manure on agricultural fields.

Soils have been identified as the major source of $N₂O$, contributing an estimated 50–60% to global N_2O emissions ([USEPA, 2010\)](#page--1-0). However, there is still a large uncertainty associated with estimates of global N₂O emissions from natural and anthropogenic sources, ranging from 8.1 to 30.7 Tg N yr^{-1} ([Ciais et al., 2013](#page--1-0)). This great range of estimated values is mainly a reflection of the great uncertainty of the individual source and sink strengths of the diverse processes involved in N_2O formation and consumption in soils ([Billings, 2008\)](#page--1-0).

Two microbial N transformation processes, autotrophic nitrification and heterotrophic denitrification, are considered the major N_2O sources, contributing an estimated 70% of the global N_2O emissions from soils ([Butterbach-Bahl et al., 2013\)](#page--1-0). N₂O release during both processes has been described by [Firestone and](#page--1-0) [Davidson \(1989\)](#page--1-0) in their conceptual 'hole-in-the-pipe' model, but N2O production in soils, especially during nitrification, is far from completely understood. The model attributes N_2O emissions from

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soils during nitrification and denitrification to leaks in the N transformation from ammonium to nitrate, and to the incomplete sequential reduction of nitrate via N_2O to elementary nitrogen (N_2) . However, this model is over simplistic, as it is known that there are a variety of processes and metabolic pathways involved in soil N_2O production. Because denitrification can both produce and consume N_2 O, an imbalance between N_2 O formation and reduction, depending on enzyme regulation, can make denitrifying bacteria net N2O producers or consumers. The fact that soils can, at least temporarily, function as significant N_2O sinks has been reported recently ([Chapuis-Lardy et al., 2007; Goldberg and Gebauer, 2009\)](#page--1-0).

Apart from soil bacteria, fungi can also denitrify, but largely lack N_2O reductase and therefore produce N_2O ([Laughlin and Stevens,](#page--1-0) [2002](#page--1-0)). Fungi are also involved in a hybrid reaction, called codenitrification, in which inorganic and organic N precursors lead to $N₂O$ formation [\(Spott et al., 2011\)](#page--1-0). Nitrifying bacteria produce $N₂O$ as a side product during the oxidation of $NH₂OH$, but can also reduce nitrite under oxygen-limiting conditions or at elevated nitrite concentrations in a process similar to denitrification known as nitrifier denitrification ([Poth and Focht, 1985; Wrage et al., 2001\)](#page--1-0). There are more alternative processes potentially involved in N_2O formation in soils, such as heterotrophic nitrification, dissimilatory nitrate reduction to ammonium (DNRA), nitrification by archaea, but also abiotic pathways [\(Bremner, 1997; Stevens et al., 1998;](#page--1-0) [Santoro et al., 2011](#page--1-0)). Abiotic N_2O formation pathways include (i) chemodenitrification, i.e., the decomposition of soil nitrite with NO as main product, but N_2O as minor product [\(van Cleemput and](#page--1-0) [Samater, 1996\)](#page--1-0), (ii) the abiotic decomposition of ammonium nitrate on reactive surfaces in the presence of light [\(Rubasinghege](#page--1-0) [et al., 2011](#page--1-0)), and (iii) the oxidation of the nitrification intermediate hydroxylamine ($NH₂OH$) that can be oxidized by several soil constituents to form N2O ([Bremner, 1997\)](#page--1-0).

Lately, stable isotope techniques have developed great potential for disentangling the variety of different N_2O formation processes; especially the intramolecular distribution of ^{15}N in N₂O, the socalled site preference (SP), has been in the focus of recent research ([Decock and Six, 2013](#page--1-0)). The site-specific isotopic signature of $N₂O$ produced by several microbial pathways has been studied ([Sutka et al., 2006, 2008; Well et al., 2006; Opdyke et al., 2009;](#page--1-0) [Frame and Casciotti, 2010; Wunderlin et al., 2013](#page--1-0)) as well as for abiotic N₂O production via NH₂OH oxidation [\(Heil et al., 2014\)](#page--1-0). However, until now it is impossible to unambiguously differentiate between N_2O production and consumption processes using SP information ([Ostrom and Ostrom, 2011](#page--1-0)).

For better N_2O mitigation strategies it is vital to understand the multitude of underlying microbial and abiotic processes of N_2O production in the terrestrial N cycle and their controlling factors, as it is likely that N_2O emissions from soils will increase at an ever growing rate due to an increasing demand for food, accompanied by an increased use of N fertilizer ([Ciais et al., 2013](#page--1-0)). A better understanding is also prerequisite for lowering the high model uncertainty related to $N₂O$ emissions that is caused by the multitude of simultaneous processes involved in $N₂O$ formation, but also by the high temporal and spatial variability of these processes.

The chemical oxidation of $NH₂OH$ in the presence of several transitions metals commonly found in soils was recognized more than 30 years ago ([Bremner et al., 1980](#page--1-0)), but is still neglected in most current N trace gas studies. The present study was designed to test for the potential of a coupled biotic-abiotic mechanism of N_2O production under aerobic conditions, in which NH2OH microbiologically produced during nitrification is leaking to a certain extent out of autotrophic and heterotrophic nitrifiers into the soil matrix, where it is readily oxidized to N_2O by transition metals, such as manganese or iron, or by nitrite, which is also excreted by ammonium oxidizers. To test for this potential mechanism, we added NH₂OH to soil samples from different ecosystems (forest, grassland, cropland), both under non-sterile conditions and after sterilization with three different sterilization methods. The guiding hypothesis of the study was that at least in some soils this coupled biotic-abiotic mechanism might play a significant role in aerobic N_2O formation during nitrification.

2. Material and methods

2.1. Sample collection

Soil samples were collected from three field sites (cropland, grassland, coniferous forest) that are part of the TERENO network, and additionally from a deciduous forest on the campus of Forschungszentrum Jülich (50°54'38″N, 6°24'44″E). The coniferous forest site (Wüstebach; 50°30′15″N, 6°18′15″E) was situated in the low mountain ranges of the Eifel National Park. The main vegetation at this site is Norway spruce (Picea abies (L.) Karst.). The soil type was a Cambisol with a loamy silt texture. The grassland site (Rollesbroich; 50°37′18″N, 6°18′15″E) was located in the Northern Eifel region. The soil type was also Cambisol. The agricultural site (Selhausen; 50°52′09″N, 6°27′01″E) was intensively used with regular lime and fertilizer applications. The soil type was classified as a Luvisol with a silty loam texture. At each site, soil samples were collected from the top 20 cm. At the coniferous forest site, the top 20 cm were divided into litter layer (L), organic topsoil horizon (Oh), and humic mineral topsoil layer (Ah) and collected separately. After collection, samples were transferred to the institute, where they were sieved to 2 mm and stored at 4 \degree C under well aerated conditions for further analysis.

2.2. Soil chemical analyses

Soil samples were analyzed for chemical parameters by the central analytical laboratory of Forschungszentrum Jülich. The total C and total N content were determined using an elemental analyzer (vario EL Cube, Elementar Analysensysteme, Hanau, Germany). For measurements, 20–50 mg sample material, in replicates of three, were analyzed. Concentrations of a range of elements were determined by ICP-OES. Sample extraction was done using lithium borate by extracting the mixture at 1000 \degree C for 30 min in a muffle furnace. The melt was dissolved in 30 mL HCl (5%) and filled up to a volume of 50 mL before analysis for total Ca, Cu, Fe, K, Mg, Mn, Na, and Sn content. Soil pH was determined in 0.01 M CaCl₂ solution. An overview of all soil chemical parameters determined can be found in Table 1.

2.3. Incubation experiments

To study the formation of $N₂O$ in the different soils in dependence of NH2OH content, incubation experiments were conducted

Table 1

Overview of the different soil samples and their chemical parameters that were used in this study.

Site	рH		N	C/N	Ca	K	Mε	Na	Fe	Mn
	-1	$\sqrt{8}$	[%]	$I-1$	[%]	[%]	[%]	[%]	[%]	[%]
Coniferous forest										
Litter	34			45.70 2.02 22.60 0.29		0.21	0.09	003 052 002		
Oh	29	29.30		1.43 20.50 0.11		0.73 0.13 0.10 2.05 0.00				
Ah	31			14.10 0.72 19.50 0.05 1.15 0.17 0.15 3.34 0.00						
Deciduous forest 3.6		12.60	0.63	19.90		0.27 1.00 0.16 0.43 1.23				008
Grassland	4.9	2.88	0.27	10.50	0.19	1.63 0.28		0.32 2.90		0.11
Cropland	6.4	1.09	0.11			9.60 0.37 1.46 0.33 0.57 2.25				0.09

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