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A highly sensitive method for the determination of hydroxylamine in soils

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ABSTRACT

Hydroxylamine (HA) is a crucial intermediate in the first step of nitrification, i.e., the oxidation of ammonium to nitrite. Due to its reactivity, HA may play a key role in soil N_2O emission. To determine soil HA concentrations and to explore the correlation between soil HA concentrations and N_2O emission rates, we developed a novel, extremely sensitive method based on fast extraction of HA from the soil at pH 1.7, oxidation of HA to N_2O with Fe^{3+} and analysis of the N_2O with gas chromatography (GC). The new method was tested on soil samples from a temperate Norway spruce forest, comparing soil HA concentrations with N_2O emission rates determined with microincubations of the soil samples in GC vials. Our results demonstrate that the new GC method is extremely sensitive, being able to detect soil HA contents as low as 0.3 μg N kg^{-1} dry soil. HA content in the forest soil samples ranged between 0.3 and 34.8 μg N kg^{-1} dry soil. Moreover, N_2O emission rates were significantly correlated with soil HA content ($r^2 = 0.80$), suggesting a key role of HA in N_2O formation under aerobic conditions.

1. Introduction

Hydroxylamine (HA) is a short-lived and reactive intermediate in the natural nitrogen cycle. It is formed during microbial nitrification, where ammonium (NH_4^+) is oxidized via HA to nitrite (NO_2^-) and nitrate (NO_3^-) (Lees, 1952). HA appears particularly interesting as it is not only an essential intermediate of nitrification but also a potential participant in soil N_2O formation (Bremner et al., 1980; Ritchie and Nicholas, 1972; Schreiber et al., 2012).

Certain nitrifiers, e.g., *Nitrosomonas europaea* and *Alcaligenes faecalis*, can produce N_2O during the oxidation of ammonia and HA (Otte et al., 1999; Ritchie and Nicholas, 1972). HA can also react with nitrite during denitrification and produce hybrid N_2O in denitrifiers, e.g., *Pseudomonas* sp. (Spott and Stange, 2011). Furthermore, large N_2O emissions from sterilized soil were observed after HA addition, indicating that chemical reactions between HA and other soil constituents may also play a crucial role in N_2O production (Bremner et al., 1980). In general, there are three chemical ways of HA oxidation to N_2O:

(i) The oxidation of HA by O_2:

$$2NH_2OH + O_2 \rightarrow N_2O + 3H_2O \quad (1)$$

(Bonner et al., 1978)

(ii) The reaction between HA and nitrite:

$$NH_2OH + NO_2^- \rightarrow N_2O + H_2O + OH^- \quad (2)$$

(Arnold, 1954)

(iii) The reactions between HA and metal ions or metal oxides:

$$4Fe^{3+} + 2NH_2OH \rightarrow 4Fe^{2+} + N_2O + H_2O + 4H^+ \quad (3)$$

$$2MnO_2 + 2NH_2OH \rightarrow 2MnO + N_2O + 3H_2O \quad (4)$$

(Bremner, 1997; Butler and Gordon, 1986).

Methods for HA determination have been developed since the 1950s. However, none of these methods have been widely accepted partly due to the inevitable disadvantages (Dias et al., 1979). An alternative approach, which involves oxidation of HA to N_2O by Fe^{3+} and the subsequent measurement of N_2O by gas chromatography with electron-capture detector (GC-ECD), was formerly developed for the determination of HA in seawater (Butler and Gordon, 1986; von Breymann et al., 1982). Compared to the former methods, this alternative approach is much more sensitive and can detect HA in water at concentrations as low as 5 nM, thereby exceeding the sensitivity of the spectrophotometric methods by at least an order of magnitude (Butler and Gordon, 1986; von Breymann et al., 1982). Until now, this GC method has been successfully used for the determination of HA in marine and pharmaceutical aqueous samples (Guzowski et al., 2003; Kock and

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Bange, 2013; Schweiger et al., 2007). Due to its high sensitivity, it appeared to be a very promising approach for the detection of HA in soils.

In contrast to water samples, soil is a much more complex matrix, containing potentially large amounts of organic matter, metal ions and, occasionally, nitrite, which could interfere with HA detection. As HA is highly reactive, the fast extraction of soil HA is crucial for reliable quantification of HA concentrations in soils. Different extraction conditions, such as temperature, pH, extraction method and time, may affect the determination of HA concentrations.

As no successful attempt to extract HA from natural soil samples has been reported until now, the first aim of the study was to test different methods for HA extraction from soils and identify the most suitable conditions for highest HA recovery from the soil samples. Another challenge was to minimize the potential interference of soil nitrite with N_2O formation from HA oxidation, as nitrite can artificially increase N_2O formation due to its reaction with HA by contributing one of the two nitrogen atoms of N_2O. Kock and Bange (2013) reported that already 5 μM nitrite could significantly bias HA analysis in water samples, but this bias could be eliminated by the use of 100 μM sulfanilamide (SA). Therefore, the second aim of this study was to explore the effect of nitrite at concentrations as high as 100 μM on HA detection via N_2O, as well as to identify the SA concentration sufficient for its elimination. Motivated by the hypothesis that there might be a close link between soil HA concentrations and N_2O formation in soils under aerobic conditions, the third aim of this study was to apply the new method to natural soil samples and compare their HA content with their N_2O emission rates.

2. Materials and methods

2.1. Soils

Soil samples were collected at 44 locations in a Norway spruce forest site (Wüstebach, 50° 30' 12'' N, 6° 20' 6'' E) in the Eifel National Park, Germany, which is part of the Terrestrial Environmental Observatories (TERENO) network (Bogena et al., 2013; Zacharias et al., 2011). At each sampling point, samples of organic (Oh) and mineral (Ah) horizons were collected between June 24 and 28, 2013. Soil of one of the sampling points was chosen for the development of soil HA analysis. At this location, also litter layer (L) was collected. Litter was cut with scissors, and all samples were passed through a 2-mm sieve. The sample material for test development was put into open plastic bags and stored in a refrigerator (4 ºC) until the beginning of the experiments. The other soil samples were stored in closed plastic bags in a freezer at −18 ºC until analysis with the final method. Before analysis, the frozen soil samples were taken out of the freezer, opened and kept at room temperature (21 ± 1 ºC, applies throughout the manuscript) for 3 d for reactivation of microbial activity. Soil samples were then passed through a 2 mm sieve. The basic properties of the soil and litter samples are shown in Table 1.

2.2. Principle of the assay

Hydroxylamine was determined using the method described by Butler and Gordon (1986), where HA was oxidized to N_2O by Fe^{3+} at acidic conditions according to Eq. (3). The final concentration of NH_2OH was calculated as follows (Gebhardt et al., 2004):

$$[NH_2OH] = 2 \cdot r^{-1} \cdot ([N_2O] - [N_2O]') \quad (5)$$

$$[N_2O] = (S \cdot N \cdot P \cdot V_{wp} + N \cdot P \cdot V_{hs}/RT)/V_{wp} \cdot 10^{-6} \quad (6)$$

where $[N_2O]$ is the concentration of N_2O produced by the reaction between NH_2OH and Fe^{3+} at a certain pH; $[N_2O]'$ is the background concentration of N_2O of the solution without HA and Fe^{3+} addition; r stands for the conversion factor, which is defined as the ratio of measured and theoretical HA concentration, determined by adding different known amounts of HA to deionized water samples; S is the solubility of N_2O (nmol L^{-1}) as a function of T and salinity of the sample at $1.01 \cdot 10^5$ Pa according to Weiss and Price (1980); N is the measured mole fraction of N_2O (ppb) in the headspace of vials; P is the pressure in the headspace ($1.01 \cdot 10^5$ Pa); V_{wp} is the volume of the water phase (mL); V_{hs} is the headspace volume (mL); R is the gas constant (8.31441 J K^{-1} mol^{-1}); and T is the equilibration temperature (room temperature) in Kelvin.

All samples were analyzed for their headspace N_2O concentrations using a static equilibration method and a GC-ECD system (Clarus 580, PerkinElmer, Rodgau, Germany), equipped with an automatic headspace sampler (TurboMatrix 110, PerkinElmer). Nitrogen (99.999%, Air Liquide, Germany) was used as carrier gas (flow rate 7 mL min^{-1}), and a mixture of argon/methane (90/10, Air Liquide, Germany) as ECD make-up gas (flow rate 25 mL min^{-1}). The samples were separated on a GC column (Elite-PLOT Q, 30 m, 0.53 mm ID and 20 μm df, PerkinElmer) isothermally at 30 °C, while the ECD was run at 375 °C. Signal processing and chromatogram integration were carried out with the GC-internal software (Totalchrom, PerkinElmer) software. The GC was calibrated with three different N_2O standard gas mixtures in the range between 240 and 746 ppb N_2O in nitrogen (99.999%), in which the detector showed a linear response ($r^2 > 0.99$). All experiments and analyses were carried out in 22-mL GC glass vials (VWR International, Darmstadt, Germany). For N_2O analysis of the headspace, the vials were crimped gas-tight with aluminum caps with butyl rubber seal (VWR International). If not indicated differently, the vials were then shaken on a rotary shaker at 250 rpm for 3 h. Preliminary experiments had shown that this time was sufficient for the reaction between HA and Fe^{3+} at pH 3 (see following section) and for full equilibrium between aqueous solution and headspace N_2O concentrations (data not shown).

2.3. Experimental design

2.3.1. Soil HA extractions

Four grams of fresh, field-moist soil or 2 g litter was first added to a 100-mL conical flask. Then, 25 mL of 2 mM SA solution in 0.02 M HCl (pH 1.7) and 0.002 M HCl (pH 2.7), respectively, was added. The extraction was tested at 4 °C and room temperature, respectively, using two different extraction types (magnetic stirring and shaking) and testing different extraction times. After extraction, the mixture of soil and extractant was centrifuged at 3500 rpm for 15 min in 50 mL

Table 1
Characteristics of the soil used in the experiments of this study ($n = 3$, ±SD).

Samples	C (%)	N (%)	Fe* (%)	Mn* (%)	Ca* (%)	K* (%)	Mg* (%)	pH
Litter	45.7 ± 0.1	2.02 ± 0.02	0.52	0.024	0.29	0.21	0.09	3.40 ± 0.06
Oh	29.3 ± 0.1	1.43 ± 0.03	2.05	<0.01	0.11	0.73	0.13	2.93 ± 0.06
Ah	14.1 ± 0.1	0.72 ± 0.011	3.34	<0.01	0.05	1.15	0.17	3.12 ± 0.05

* The relative error was 3% for values >1%, 20% for values <0.1% and 10% for the other values.