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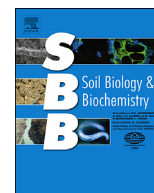

# Nitric oxide emission response to soil moisture is linked to transcriptional activity of functional microbial groups

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ABSTRACT

Numerous studies have shown that soil moisture controls NO flux from soils. Less is known, however, to what extent microbial N-cycling mediates this control. Does soil moisture control NO release primarily by affecting the physical gas exchange between soil and atmosphere, by modulating microbial activities involved in biotic NO turnover, or by both? Using a novel dynamic chamber system for high-resolution measurement of NO release, we found one or several soil-specific maxima of NO release during dry-out experiments in different soils. A mid-latitude arable soil displayed a single maximum at 0.10 water holding capacity (whc), whereas a dryland farming and a rice paddy soil showed two maxima at 0.65/0.10 and 0.90/0.10 whc, respectively. Transcription of *nirS* genes in the dryland soil at 0.65 whc was low, but larger than at 0.10 whc, while transcriptional activity of archaeal ammonia oxidizers showed the opposite pattern with higher activity at 0.10 whc, suggesting biogenic NO production at low soil moisture. Our study is a first attempt to link NO emission to soil moisture responses of different microbial groups.

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## 1. Introduction

Global sources of $NO_x$ ($NO + NO_2$) are estimated to be in the range of 42–47 Tg (N) $a^{-1}$ (Denman et al., 2007). Soils act as a significant source of NO and contribute about 9 Tg (N) $a^{-1}$ which ultimately can be ascribed to microbial N-transformations (Ciais et al., 2013; Homyak et al., 2017). $NO_x$ catalyzes the photochemical production of ozone ($O_3$) in the troposphere (Haagen-Smit, 1952) and thereby contributes to the greenhouse gas effect. Various laboratory incubation studies have found that the NO release rate, $J_{NO}$, follows an optimum curve over a range of soil moistures, which has been tentatively attributed to the soil moisture dependency of NO producing microbial activities (Yu et al., 2008; Ashuri, 2009; Laville et al., 2009).

Previously, the NO relationship to soil moisture was thought to be shaped by the tradeoff between restricted $O_2$ supply and reduced substrate diffusion in wet and dry soil, respectively (Skopp et al., 1990). Bargsten et al. (2010), studying NO response to soil moisture change under non-limiting diffusion conditions in the laboratory, claimed that the optimum soil moisture for NO emissions will depend on soil-specific gaseous diffusivity and Skiba et al. (1997) identified factors which regulate gaseous diffusivity, such as soil texture, bulk density and water content as central for NO flux in a study with temperate and tropical agricultural soils. Although taking account for denitrification and nitrification as distinct microbial sources for NO, none of the above studies considered the possibility that the soil moisture response of key-microbial groups controls the NO flux.

Nitric oxide (NO) in soils is produced and consumed predominately through the microbially mediated processes of nitrification and denitrification (Conrad, 1996). In unrestricted nitrification, ammonia ($NH_3$) is oxidized via hydroxylamine ($NH_2OH$) to nitrite ($NO_2^-$) and NO is released as intermediate in the second step

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(Koslowski et al., 2016). Nitrifiers and heterotrophic denitrifiers are taxonomically unrelated and have distinct physiologies. While aerobic ammonia oxidizers are autotrophs that form phylogenetically coherent groups within the beta- and gamma-proteobacteria (Purkhold et al., 2000) or the Thaumarchaeota (Pester et al., 2011), denitrifiers are phylogenetically diverse heterotrophs with the ability to respire nitrogeneous oxides in the absence of oxygen (Philippot, 2002; Wei et al., 2015).

There is some evidence that taxonomically distinct, but functionally redundant microbial groups such as AOB and AOA have distinct soil moisture optima. For instance, Adair and Schwartz (2008) found a higher abundance of ammonia oxidizing archaea (AOA) than ammonia oxidizing bacteria (AOB) in a semiarid soil which, unlike AOB abundance, was not related to soil temperature or precipitation. They interpreted these findings as indicative for a greater resistance of AOA to desiccation. Soil moisture affects the oxygen availability in soils and thereby the activity of different microbial functional groups (Gleeson et al., 2010). In the present study, we hypothezised that soil NO emissions at different soil moistures are determined by metabolic activities of taxonomically distinct microbial groups and that this should result in distinct maxima of NO flux over a range of soil moistures. Testing this hypothesis is timely as it would violate the assumption of one discrete maximum for NO flux over soil moisture, commonly used for upscaling NO fluxes (Meixner and Yang, 2006; Laville et al., 2009; Yu et al., 2008).

We tested our hypothesis by monitoring NO release of a dryland soil in a dry-out experiment. In addition, different fertilizers ($(NH_4)_2SO_4$ and $KNO_3$) were added to stimulate nitrification and denitrification. To link our results to microbial activities, we measured the relative expression of bacterial and archeal *amoA* (encoding ammonia monooxigenase) as well as *nirK* and *nirS* (encoding copper and cytochrome $cd_1$-containing nitrite reductase, respectively) at soil moistures showing maximum NO release. This approach is based on the assumption that an increase in the numbers of active microorganisms results from increasing ratios of mRNA over DNA copy numbers (Blazewicz et al., 2013; Rocca et al., 2015) and that the transcriptional activation of functional key genes can be used as a proxy for enzyme synthesis.

## 2. Material and methods

### 2.1. Experimental site description and soil properties

The principal soil used in this study, which was incubated under various conditions and analysed for functional gene abundance and community structure of bacterial and archaeal ammonia oxidizers and denitrifiers, was sampled from an agricultural jujube cotton field of Waxxari oasis (Xinjiang, P. R. China) in the Taklamakan basin (N 38.6778, E 87.3300). In this oasis mainly jujube trees (Chinese date), cotton or both are grown in dryland farming agriculture using flooding or dripping irrigation. The Taklamakan basin is characterized by a cold desert climate with a mean annual temperature of 14.3 °C (Ruoqiang oasis) and 22.9 mm annual rainfall (Mamtimin et al., 2011). Additional soils included in our study were sampled from a rice paddy soil in India (Ambai, India, N 8.7051, E 77.4629) and an agricultural maize field (Mainz, Germany, N 49.9856, E 8.1129). Both were studied for NO release only. The physical and chemical properties of the soils are given in Table S1 (Supplementary Material). Top soil (0–5 cm) was sampled within an area of 10 × 10 m at each 5 m grid point and mixed to a representative sample for each ecosystem. After sampling, the soil was air dried, sieved through a $2 \times 10^{-3}$ m mesh, and stored until laboratory analysis at 4 °C.

### 2.2. Laboratory incubation and NO release rate

To determine the NO release rate, sub-samples (0.06 kg) were re-wetted to field capacity and placed into a dynamic incubation system as described previously (Behrendt et al., 2014). The thickness of the soil layer was about 4 mm and therefore the system can be considered as non diffusion-limited. We investigated the moisture dependency of NO release in this soil under three different nitrogen regimes: an unfertilized control, a treatment with $KNO_3$ and a treatment with $(NH_4)_2SO_4$ fertilizer, both applied at a rate of 200 kg (N) ha$^{-1}$. The fertilizer was added as an aqueous solution immediately before starting the dry-out experiments. For molecular and soil chemical analyses, individual samples were removed from the incubator at gravimetric soil moistures ($\theta_g$) for which maximum NO release had been observed in preliminary experiments. Duplicate subsamples (0.5 g soil) were transferred into 2 mL vials, snap-frozen in liquid $N_2$, and stored at −80 °C before molecular analysis. Another 10 g soil was suspended in 50 mL of a 0.0125 M $CaCl_2$ solution for extraction of $NO_3^-$, $NO_2^-$ and $NH_4^+$ according to ISO/TS 14256–1 after 1 h shaking and 5 min centrifugation at 4000 rpm. The different inorganic N forms were analysed by photometrical method (UV–Vis, GBC, Australia).

During incubation, the soils were dried under a constant stream of synthetic air (0.5 L min$^{-1}$; Behrendt et al., 2014). The mixing ratios of NO and $H_2O$ were monitored continuously (every 30 s) in the inlet and outlet air of soil and control chambers, using a chemiluminescence $NO_x$ analyser (model 42i-TL, Thermo Fisher Scientific Inc., USA) and a non-dispersive infrared (NDIR) $CO_2/H_2O$ analyser (Model LI-COR 840A, LI-COR Biosciences Inc., USA), respectively. The $NO_x$ analyser was calibrated by help of a gas phase titration unit (model 146 C, Thermo Fisher Scientific Inc., USA) coupled to a NO standard gas bottle (5 ppm, Airliquide, Germany). While the soil was drying out at constant soil temperature, $T_{soil}$, the release rate of NO, $J_{NO}$ [ng kg$^{-1}$ s$^{-1}$], for a constant inlet mixing ratio, $m_{ref}$, was calculated as:

$$J_{NO}\left(m_{ref}, T_{soil}, \theta_g\right) = \frac{Q \cdot \left(m_{OUT} - m_{ref}\right) \cdot f_{NO}}{M_{soil}} \quad (1)$$

where Q is the purging rate through the chamber during measurement [2.5 L min$^{-1}$ or 4.16667 $10^{-5}$ m$^3$ s$^{-1}$ during measurement], $M_{soil}$ the dry mass of soil [kg] and $m_{ref}$ and $m_{OUT}$ are the NO mixing ratios at the inlet (measured from the reference chamber) and outlet of the chamber, respectively. Sterilization of the field soil using an autoclave (VX 95, Systec GmbH, Germany) resulted in a reduction of $J_{NO}$ of ≥80% over a large range of soil moisture (from field capacity to dry soil, data not shown) and hence confirmed that NO was predominately of biological origin. The gravimetric soil moisture content was calculated by mass balance based on the cumulative evaporation over time which was subtracted from the known initial soil moisture (Behrendt et al., 2014). The maximum water holding capacity (whc) was estimated based on the filter method (Whatman-filter paper no. 42). For normalization to equal matrix potentials, soil moisture was calculated as percent maximum water holding capacity, % whc.

### 2.3. Soil DNA and RNA extraction, functional gene expression and TRFLP analysis

Nucleic Acids were extracted from two 0.5 g aliquots of soil and resuspended in 1 mL extraction buffer according to a modified SDS-based protocol (Pratscher et al., 2011; Bürgmann et al., 2003). This protocol includes cell disruption by bead beating at 6 m s$^{-1}$ for 45 s and a phenol/chloroform/isoamyl alcohol extraction. After

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