Soil Biology and Biochemistry 123 (2018) 74–79

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Are nitrous oxide emissions and nitrogen fixation linked in temperate bogs?

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ARTICLE INFO

Keywords:
Acetylene reduction
Diazotrophs
Nitrogen fixation
Nitrous oxide
Pleurozium schreberi
Sphagnum

ABSTRACT

A pre-requisite for denitrification and nitrification is the availability of inorganic nitrogen (N). In many natural ecosystems, atmospheric N deposition as well as moss-associated N_2 fixation are the main sources of 'new' N to the ecosystem N pool and could provide inorganic N to sustain N_2O production. While a link between N_2 fixation and N_2O emissions is plausible, hardly any attempts have been undertaken to test this link in areas with a moss-dominated understory. Here, we report results from a combined field and laboratory study in which we assessed N_2 fixation, net N_2O emission under different conditions, and potential nitrification and denitrification in three moss species from a *Sphagnum*-moss dominated, temperate bog. The three moss species were chosen to represent a gradient of N_2 fixation activity. *Sphagnum* mosses emitted less N_2O than the other two moss species (*Pleurozium schreberi*, *Hypnum cupressiforme*), but at the same time, showed the highest N_2 fixation activity. The lack of a link between N_2 fixation and net N_2O emissions in three abundant and common moss species indicates that N transformation processes may be decoupled within the moss carpet. This raises new questions on N supply for N_2O production and the fate of fixed N_2 in moss-dominated systems.

1. Introduction

One pre-requisite for nitrous oxide (N_2O) production is the availability of inorganic nitrogen (N). In natural, pristine ecosystems that do not receive N from fertilizer application, N_2O production may be stimulated by the fixation of atmospheric N_2. Nitrogen cycling processes are closely linked to each other, and increased N_2 fixation rates could result in higher soil N, stimulating N transformation processes such as N mineralization, nitrification and denitrification. Higher soil ammonium content can increase rates of nitrification (Robertson and Vitousek, 1981; Norton and Stark, 2011), and subsequent production of nitrate, which can stimulate N_2O emissions.

Mosses account for a large fraction of the groundcover in heath and forest ecosystems (e.g. Oechel and Van Cleve, 1986; Street et al., 2013), and most of the dominant mosses are colonized by N_2 fixing bacteria (diazotrophs). Together, they can contribute up to 50% to total ecosystem N input in pristine systems (e.g. DeLuca et al., 2002; Rousk and Michelsen, 2017). Further, N_2 fixed by moss-associated diazotrophs has recently been shown to be transferred to soil, soil microorganisms and vascular plants within 3 days of fixation (Rousk et al., 2017a). Thus, N_2 fixation in mosses may directly influence N_2O emissions by increasing the pool of available soil N, and higher N_2 fixation rates could translate into larger N_2O emissions. Yet, to date, no studies comparing N_2O emissions between mosses with different N_2 fixation potentials have been reported.

Different moss species can vary several orders of magnitude in N_2 fixation by their associates. For instance, *Sphagnum* mosses show generally higher N_2 fixation rates than feather mosses like *Hylocomium splendens* (Hedw.) Schimp. and *Pleurozium schreberi* (Brid.) Mitt. (e.g. Rousk et al., 2015). This may be due to different water holding capacities (Elumeeva et al., 2011), different diazotroph species colonizing, as well as different localization of the associated diazotrophs. *Sphagnum*-associated diazotrophs are found within hyaline cells of the moss (Bragina et al., 2012a), and are predominantly alphaproteobacteria (Bragina et al., 2012b; Leppänen et al., 2014). In contrast, diazotrophs associated with feather mosses such as *P. schreberi* and *H. splendens* are found epiphytically on the moss leaves, and are predominately cyanobacteria (Ininbergs et al., 2011; Leppänen et al., 2013). Thus, moss-specific differences may control the link between N_2 fixation and N_2O emissions. Mosses with high N_2 fixation potential may also support nitrifiers and denitrifiers by providing substrate, as mosses have been found to create soil microsites with high ammonium concentrations (Sedia and Ehrenfeld, 2005). Further, mosses contribute to stable temperature and moisture conditions in soils (e.g. Blok et al., 2011), promoting processes such as N_2 fixation and nitrification.

Nitrogen fixation in mosses may directly feed N_2O emissions by

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https://doi.org/10.1016/j.soilbio.2018.05.002
Received 30 January 2018; Received in revised form 17 April 2018; Accepted 2 May 2018

providing inorganic N (see above). In addition, denitrification is regulated by oxygen availability, pH, temperature and carbon to nitrogen ratio; these factors also influence N_2 fixation. Hence, a link, either directly or indirectly, between N_2 fixation and N_2O emissions in moss-dominated habitats seems plausible. Bog habitats in temperature regions provide waterlogged, anoxic conditions that could promote N_2 fixation by moss-associated diazotrophs as well as denitrification. Such sites are reported to range between consumption and release of N_2O with an average of -6.8 mg N_2O-N m^{-2} $year^{-1}$ (Beyer and Höper, 2015). Similar N_2O uptake activities were reported for calcareous fens in Ireland showing -0.04 mg N_2O-N m^{-2} day^{-1} in the summer season when N is limiting (Kang et al., 1998).

To assess if differences in moss-associated N_2 fixation rates translate into changes in moss-N_2O emissions, we performed a field as well as a laboratory study with mosses from a temperate bog. For this, we firstly measured net N_2O emissions and N_2 fixation *in situ* in a *Sphagnum* dominated bog. Secondly, we collected the three most common moss species (*Sphagnum* sp., *Pleurozium schreberi*, *Hypnum cupressiforme* Hedw.) in this habitat. These three mosses have different N_2 fixation potentials, and thereby represent a gradient of N_2 fixation activity with the highest activity in *Sphagnum* followed by *P. schreberi*. The third moss species, *H. cupressiforme*, has not been reported previously to be associated with diazotrophs. We measured N_2O emissions and uptake, N_2 fixation, denitrification and nitrification in these mosses under controlled conditions in the laboratory to assess if the N_2 fixation gradient translates into an N_2O emission gradient via moss-species specific differences in nitrification and denitrification.

2. Materials and methods

2.1. Site description

Our study site is a *Sphagnum*-dominated bog close to Copenhagen (55°49′33′N, 12°33′48′E), Denmark, with a moist-temperate climate. The bog is in the early development into a raised bog from a lake overgrown with bryophytes, dominated by a thick *Sphagnum* moss layer. This suggests that groundwater input to the upper layer is minimal, which characterizes the raised bog. Atmospheric N deposition is around 15 kg N ha^{-1} yr^{-1} (envs.au.dk/luft, Aarhus University, 2015). The concentration of phosphate-P (P), ammonium (NH_4^+) and nitrate (NO_3^-)-N in the upper 10 cm bog water are 0.2 mg phosphate-P, 0.6 mg NH_4^+- and 0.1 mg NO_3^--N l^{-1} bog water. These values are within the range of values reported previously for temperate *Sphagnum*-bogs (e.g. Bragazza and Gerdol, 2002). Bog water NH_4^+-N and phosphate-P were analysed on a Hitachi U 2010 spectrophotometer using the indophenol and molybdenum blue method (Allen, 1989), respectively. Nitrate-N in bog water was analysed using the cadmium reduction method (Allen, 1989) on a FIASTAR 5000 analyser (Foss Tecator, Sweden).

2.2. Field measurements of net N_2O production and consumption

The field measurements were performed in June 2016. To quantify net N_2O emissions under field conditions, we established four plots within a homogenous cover of *Sphagnum* sp., with at least 1 m distance between the four plots. Due to the large groundcover of *Sphagnum* and the patchy occurrence of *P. schreberi* and *H. cupressiforme*, we focused on *Sphagnum* mosses for our field measurements of N_2O emission and N_2 fixation. Transparent plastic chambers (0.25 m × 0.25 m) were pressed into the moss carpet so that the whole bottom edge of the boxes was water-covered to ensure gas tight conditions. Gas samples were taken through a rubber septum just after the chambers were placed on the moss (0 min) as well as 15 and 30 min after chamber placement. The gas samples were stored in 6 ml pre-evacuated vials (Labco, Ceredigion, UK) and analysed for N_2O within 2 days of sampling using a gas chromatograph with an EC detector operated at 330 °C after separation on a 1.8 m Haysep Q column at 40 °C (SRI 8610C, Instruments, Torrance, California, USA).

2.3. Field measurements of N_2 fixation

To measure N_2 fixation in the field, we used the acetylene reduction assay (ARA) as in Rousk and Michelsen (2017) in the same plots described above, shortly after the N_2O measurements. The nitrogenase enzyme reduces N_2 to ammonia, as well as acetylene to ethylene, which is assessed with ARA. The ARA is therefore a measure of the nitrogenase enzyme activity. For ARA, we placed a 20 ml vial containing calcium carbide in the chamber and added water through the septa to induce the development of 10% (vol.) acetylene in the chambers. After 1 min and after 2 h of incubation with acetylene, 6 ml gas samples were taken and transferred into 6 ml pre-evacuated, airtight vials (Labco, Ceredigion, UK). Gas samples were analysed for acetylene and ethylene on a gas chromatograph (SRI 310C, SIR Instruments, CA, Torrance, USA) shortly after sampling. The gas chromatograph was equipped with a flame ionization detector with injector, column and detector temperature at 250, 60 and 120 °C, respectively, using He as carrier gas.

2.4. Collection and handling of moss samples

The three most dominant moss species at our study site –*Pleurozium schreberi, Hypnum cupressiforme, Sphagnum sp.* – were collected just after the field measurements from six areas within the bog that were at least 1 m apart from each other. Soil temperature at the time of sampling was 15 °C (4–5 cm depth), and 8 °C at 2 cm depth within the moss carpet. Several moss patches were taken from each sampling area and combined to one composite sample, totalling 18 samples (n = 6 per moss species). The moss samples were transported in plastic bags to the laboratory at the University of Copenhagen and sorted into 117 ml glass flasks for the different assays on N transformation processes (see below). One ml double distilled water was added to *P. schreberi* and *H. cupressiforme* samples prior the assays to ensure sufficient moisture conditions for the assays. The adjusted moisture content was similar to field moisture as assessed visually from presence of a few droplets of free water on the moss surface. *Sphagnum* samples were sufficiently moist and were not watered.

2.5. N_2O production and consumption under oxic and anoxic conditions

To assess gas exchange between moss and atmosphere, several moss shoots were added to glass flasks (117 ml) corresponding to 3–30 mg dry weight (dw) (5–6 replicate flasks per moss species). We placed the flasks in a growth chamber at 10 °C with a light/dark cycle of 16/8 h. After one day to obtain gas equilibrium between the flask atmosphere and the mosses, the flasks were sealed with rubber septa and one ml gas was removed from the headspace two times during the first hour to assess N_2O production under oxic conditions. N_2O content in the gas sample was analysed on a gas chromatograph with the specifications described above. Following the assessments of net N_2O emission under oxic conditions, the atmospheric air in the flasks was replaced with N_2 by evacuating and refilling to 2 bar with N_2 from a tank, repeated three times and finally left with N_2 at 1 bar. Three ml N_2 was additionally supplied to the flasks to compensate for gas sampling. Nitrous oxide in the flask headspace was measured 2 and 3 h after the sealing of the flasks to quantify net N_2O production under anoxic conditions.

To simulate field conditions (larger moss biomass, larger incubation vials, higher moisture content compared to the previous incubations), we conducted another set of experiments in which we incubated a larger biomass of moss for 3 days before measuring N_2O emissions. The gas exchange from the upper layer of the moss carpets was measured on 8, 8 and 25 g moss (dw average) for *P. schreberi* and *H. cupressiforme* and for *Sphagnum sp.*, respectively, in water-saturated conditions in 1 l sealed jars.