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Moss-nitrogen input to boreal forest soils: Tracking ^{15}N in a field experiment

Kathrin Rousk [a,b,c,*], David L. Jones [a], Thomas H. DeLuca [a,d]

[a] School of the Environment, Natural Resources, & Geography, Bangor University, Deiniol Road, Bangor, Gwynedd LL57 2UW, UK
[b] Department of Biology, Section of Microbial Ecology, Lund University, Lund, Sweden
[c] Department of Biology, Terrestrial Ecology Section, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark
[d] School of Environment and Forest Sciences, University of Washington, Box 352100, Seattle, WA, USA

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ABSTRACT

Cyanobacteria living epiphytically on mosses in pristine, unpolluted areas fix substantial amounts of atmospheric nitrogen (N) and therefore represent a primary source of N in N-limited boreal forests. However, the fate of this N is unclear, in particular, how the fixed N_2 enters the soil and becomes available to the ecosystem. In this study, we applied ^{15}N-ammonium chloride (^{15}N-NH_4Cl) onto carpets of the feather moss *Pleurozium schreberi* and traced the ^{15}N label into green (living) and brown (senescent) moss and into the upper soil layer over time. Further, we placed filters between moss and soil to assess the role of moss-associated fungi for N-transfer to the soil. The experiment was conducted at endpoints of a N_2 fixation gradient in Northern Sweden. Feather moss retained the applied N in the green moss parts for up to 1 year and no increase of excess ^{15}N was found in the brown moss parts or in the soil within that same time frame. The filter treatment did not alter the ^{15}N-distribution in moss or soil. Nitrogen retention in the moss was similar regardless of position along the N_2 fixation gradient. Our results suggest that mosses represent a short-term inorganic N sink and that transfer of N to the soil is not facilitated by fungal hyphae.

1. Introduction

Moss-cyanobacteria associations represent a significant nitrogen (N) source in N-limited boreal ecosystems due to the ability of the cyanobacteria to fix atmospheric N_2 (DeLuca et al., 2002a; Gundale et al., 2011). In these environments, atmospheric N deposition is low (1–2 kg N ha^{-1} yr^{-1}), resulting in low rates of total N input to these systems (Tamm, 1991; Gundale et al., 2011). Given the abundance and biomass of mosses in boreal forests (Oechel and Van Cleve, 1986) and their N_2 fixation capacity, which equals the N input via deposition (>2 kg N ha^{-1} yr^{-1}) (DeLuca et al., 2002a), their contribution to the N-pool via N_2 fixation could constitute a major component of the N cycle.

Mosses are efficient at absorbing aerial deposited N and in recycling and retaining trapped N (Aldous, 2002; Friedrich et al., 2011). Thus, mosses create a regulating layer between atmospheric-N and soil-N, likely representing a short-term N sink (Friedrich et al., 2011). Since mosses capture and retain significant amounts of N from throughfall and host N_2 fixing cyanobacteria, it has been suggested that forest ecosystems are dependent on N release from moss carpets (Weber and Van Cleve, 1984; Oechel and Van Cleve, 1986; Carleton and Read, 1991), especially in low-N deposition areas where N_2 fixation rates in mosses are high (Gundale et al., 2011). However, no attempt has been made to relate N_2 fixation in mosses with their N retention abilities.

Mosses' connection to soil is limited to non-vascular rhizoids that reach only surface layers of soil. However, studies have shown that mosses grow in association with different fungi (Kauserud et al., 2008; Davey et al., 2009), which could function in the transfer of N from moss to soil via fungal hyphae. In spite of numerous studies on feather mosses and the role they play in ecosystem N dynamics, there is currently little known about the amount and extent in which the N_2 fixed by cyanobacteria is transferred to the soil. Thus, the role and importance of feather mosses in the boreal N cycle, as a long-term N source, or short-term N sink remains unresolved.

Moss biomass as well as N_2 fixation in feather moss-cyanobacteria associations is negatively affected by increased N

* Corresponding author. Department of Biology, Terrestrial Ecology Section, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark. Tel.: +46 705290367.
E-mail address: kathrin.rousk@gmx.net (K. Rousk).

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inputs, which can result in the total reduction of N_2 fixation (Zackrisson et al., 2004; DeLuca et al., 2008; Ackermann et al., 2012). The purpose of the study reported herein was to assess the fate of NH_4^+-N within the moss layer as an indication of the fate of the cyanobacterial fixed N_2, in particular the transfer to the soil and to evaluate the role of rhizoids in this process. A field experiment was conducted to assess the N-transfer from moss to soil in the context of the combined observations (a) N_2 fixation inhibition by N input and (b) moss-rhizoid-extensions via associated fungi. We hypothesize that (1) cutting the moss above the humus layer reduces the transfer of N to the soil, (2) the moss retains acquired N for several months, and (3) the transfer from moss to soil is higher close to busy roads given higher exogenous N input, which likely exceeds the moss' N demand.

2. Material and methods

2.1. Study sites

The sites are located in forests in Northern Sweden between latitude 64–66°N, longitude 18–19°E and between 230 and 540 m above sea level and have been described previously in Ackermann et al. (2012). This study was conducted near three busy, paved road segments (Borup (65.0060°N, 19.2509°E); Nyvall (65.2308°N, 19.2632°E); Strömsforsheden (65.0813°N, 18.5179°E)). The vegetation at all sites was dominated by Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* L. (Karst)), feather moss carpets (*Pleurozium schreberi*, *Hylocomium splendens* (Hedw.)), shrubs (e.g. *Vaccinium vitis-idea* (L.), *Vaccinium myrtillus* (L.), *Empetrum hermaphroditum* (Hagerup)). The soils were classified as Typic Haplocryods (USDA, 2003). All forest stands were of mid- to late succession status.

2.2. ^{15}N application experiment and sampling

The experiment was established in June 2011 at 0 m and 150 m distance from the road edge, representing the endpoints of a N_2 fixation gradient, in which N_2 fixation increases with distance from the roads due to road-derived N input (Ackermann et al., 2012). We measured a number of factors (total carbon and N in soil, heavy metal concentration in moss tissue, moss ground cover) along these gradients but found that only N deposition decreased significantly with distance from the roads. Four treatments with three replicate plots (1 m^2 each) per site per distance were established. The treatments were as followed: (1) control (100 ml distilled water) with filter barrier; (2) control without filter; (3) 0.5 g N m^{-2} as ^{15}N-NH_4Cl (99% enriched) with- and (4) 0.5 g N m^{-2} as ^{15}N-NH_4Cl without filter. We used NH_4^+ as N source to mimic the N-species that is the most common form of N transferred between cyanobacteria and colonized plants (Rai et al., 2000). The ^{15}N solutions were sprayed on top of the moss carpets, that is, on the green moss parts where the cyanobacteria are present to match the location of N_2 fixation within the moss. In the boreal forest, 5 kg of N addition ha^{-1} is not an unrealistic amount of N to be experienced by moss carpets, mosses experience total N inputs of 5–10 kg N ha^{-1} yr^{-1} (Rousk et al., 2013). Also, cyanobacterial N_2 fixation can reach levels >5 kg N ha^{-1} yr^{-1} (e.g. Lindo et al., 2013).

To assess the role of fungi in the N transfer to the soil, we cut the moss above the humus layer prior to the ^{15}N application and placed a filter between moss and humus. The moss was subsequently placed back on the filter (Munktell Analytical Filter papers 3, 15 cm diameter, 90 g m^{-2}, pore size >10 μm, cellulose paper with 0.06% ash content). The solutions were evenly sprayed on the feather moss carpets. At different time points after the applications (2 h, 2 weeks, 2 months, 1 year), moss and soil samples were collected. The moss shoots were separated into brown (senescent and dead) and green (alive) parts. Soil samples were collected beneath the moss in each treatment with a 2.5 cm diameter stainless steel soil core to depth of ~10 cm. Although more than half of the depth of the soil cores was composed of humus, we refer to these samples as soil samples throughout the manuscript. Three replicate soil and moss samples were collected in each plot from each treatment. Moss and soil samples were dried at 80 °C for 24 h, ground and analyzed for total N (TN) and ^{15}N by oxidative combustion using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (IRMS) (Sercon Ltd., Cheshire, UK).

2.3. Statistical analyses

^{15}N data are represented as excess ^{15}N (atom %) calculated by subtracting the ^{15}N values (in atom %) of the control samples from the ^{15}N values (in atom %) of the treated samples. $\partial^{15}N$ (‰) values of moss tissue are given in Table S1. To test for significant differences in excess ^{15}N and total N between the samples (green moss, brown moss and soil), between times after application of ^{15}N-NH_4Cl, and between the N input effects (no filter, 0 m from road; with the filter, 0 m from road; no filter, 150 m from road; with the filter, 150 m from road), we used 2-way-ANOVA approaches followed by Tukey's Post Hoc Test. Road site was used as a random factor (block). All data analyses were performed in R 2.14.0 (2011).

3. Results

3.1. Excess ^{15}N in moss and soil

Two hours after the ^{15}N application, the green moss showed higher ^{15}N excess than the brown moss parts and the soil (0.54 (mean) ±0.13 (SE), 0.1 ± 0.04, −0.0006 ± 0.001 for green moss, brown moss and soil, respectively) ($F = 69.7$; $p < 0.0001$) (Fig. 1). The distribution of excess ^{15}N along the moss-soil-profile was similar 2 weeks after the ^{15}N addition (Fig. 1), the green moss had higher ^{15}N excess than the brown moss and the soil ($F = 44.0$; $p < 0.0001$). The same pattern was also seen 2 months after the ^{15}N application, in which the green moss still had the highest excess ^{15}N (0.54 ± 0.1, 0.12 ± 0.06; 0.0009 ± 0.0 for the green moss, brown moss and soil, respectively) ($F = 27.2$; $p < 0.0001$) (Fig. 1). This same pattern held even one year after the ^{15}N application, the green moss parts still showed the highest excess ^{15}N (at %) ($F = 143$; $p < 0.0001$) and no more enrichment was found in the brown moss parts and soil after 1 year than after only a few hours after application of the tracer. Excess ^{15}N did not change with time in moss or soil samples. Neither the filter treatment nor the position along the N_2 fixation gradient resulted in differences in excess ^{15}N in soil samples at any time after labeling (Fig. 1).

3.2. Total N in moss and soil

Total nitrogen (TN) in moss tissue and soil samples was similar in the control and ^{15}N plots (Table 1, Table S2). Cutting the moss and insertion of filters did not result in differences in TN in moss or in soil samples (Table 1). Total N in soil samples collected at 150 m away from the road was approximately 2 mg g^{-1} soil higher than collected close to the road ($t = -4.2$; $p < 0.001$) (Table 1). No consistent differences in moss tissue-TN over time or between the two distances were found. However, TN in moss and soil samples were significantly lower 1 year after the application than at all other time points ($F = 25.0$; $p < 0.001$) (Table 1). Soil-TN values from the 2 weeks sampling point were lost and therefore excluded.