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Feather moss nitrogen acquisition across natural fertility gradients in boreal forests

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ABSTRACT

Feather mosses utilize various sources of nitrogen (N): they absorb N deposited on leaf tissue, they host N_2 fixing cyanobacteria, and they are able to take up N directly from soil. In addition to their importance as primary producers in boreal ecosystems, feather mosses play a significant role in N cycling. However, estimates of their ability to take up N from soil *in situ* are scarce. Further, connecting uptake of N from soil with N_2 fixation could significantly improve our understanding of their role in ecosystem N cycling, but to date this issue has not been addressed. We report results from an uptake experiment in which we tracked ^{13}C-carbon (C), ^{15}N-alanine and ^{15}N-ammonium chloride (NH_4Cl) into feather moss (*Pleurozium schreberi* (Brid.) Mitt.)-soil cores taken along natural fertility gradients in Northern Sweden. The varying fertility conditions coincided with a N_2 fixation gradient in the feather moss. We found that *P. schreberi* takes up C and N directly from soil. However, the moss did not show a preference for inorganic or organic N sources and only 1.4% of the added amino acid appeared to be taken up from soil in an intact form. No differences in uptake of C or N from soil along the fertility gradients were detected. Nitrogen fixation rates in the moss were thus not correlated with C or N-uptake from soil. Nitrogen fixation as well as uptake of C and N from soil seem to be unaffected by C or N availability in the soil, suggesting that the moss can cover its nutrient demand by absorption of throughfall N and via associated N_2-fixing cyanobacteria without soil-N supplementation. We suggest further, that the moss can represent a (temporary) N-sink in the boreal forest, and that the moss' mechanism of uptake and release thereby will characterize the ecosystem N cycle.

1. Introduction

Boreal forests are considered to be nitrogen (N)-limited (Tamm, 1991) with N demand of plants and microbes satisfied by a combination of sources and pathways (Jones et al., 2005). Besides the uptake of mineralized, inorganic N (ammonium (NH_4^+) and nitrate (NO_3^-)), plants, as well as microbes, have the ability to take up organic N in form of amino acids, urea, polyamines and small polypeptides (Kielland, 1994; Schimel and Chapin, 1996; Schimel and Bennett, 2004; Persson and Näsholm, 2008; Hill et al., 2011). Furthermore, in N-limited systems like boreal forests, organic N can be found in higher concentrations than inorganic N in soil solution (Kielland, 1995; Nordin et al., 2001; Finzi and Berthrong, 2005).

The N-limited conditions in boreal forests result in strong competition for N resources between plants and soil microbes. This is particularly true in the rhizosphere where plant exudation of low molecular weight amino acids, sugars and organic acids creates a zone of high microbial activity (Curl and Truelove, 1986; Jones et al., 2004), resulting in rapid turnover rates of nutrients (Jones, 1999; Jones and Kielland, 2002; Rousk and Jones, 2010). In terrestrial ecosystems, microbes are usually carbon (C)-limited (Demoling et al., 2007), however, microbial N-limitation can also occur (Sistla and Schimel, 2012). Thus, organic N in the form of amino acids should be readily taken up by microbes and suggests that microorganisms are better competitors for organic N than plants (e.g. Jones et al., 1996; Owen and Jones, 2001). In addition to vascular plants, mosses, including feather mosses, have been found to take up amino acids from solution and directly from soil via

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rhizoids (Ayres et al., 2006; Krab et al., 2008). However, the lack of true roots in mosses primarily limits soil nutrient acquisition to passive transport from soil to moss shoots and possibly via fungal associations (Kauserud et al., 2008; Davey et al., 2009). Since it has been found that several moss species are able to use soil-N (Ayres et al., 2006; Hill et al., 2011), we will use the term "uptake" for mosses, without distinguishing between active or passive transport.

Feather mosses, however, are thought to satisfy most of their N demands via absorption of N originating from atmospheric deposition and throughfall (Li and Vitt, 1997; Kotanen, 2002) and via fixation of atmospheric N_2 through epiphytic cyanobacteria (DeLuca et al., 2002). Due to their association with cyanobacteria, which fix substantial amounts of atmospheric N_2 in pristine environments (DeLuca et al., 2002), feather moss turnover represents a major N input in nutrient poor systems, contributing approximately 2 kg N ha^{-1} yr^{-1} to the N pool in mature forest ecosystems (DeLuca et al., 2002; Zackrisson et al., 2004). However, N_2 fixation by cyanobacteria is highly sensitive to inorganic N inputs, and the more N is added and available, the less N_2 is fixed (DeLuca et al., 2008; Gundale et al., 2011; Ackermann et al., 2012). To date, it is unknown if there is a connection between N_2 fixation in feather mosses and their ability to take up N from soil and with that, their role as a net N source due to their cyanobacterial associates or as a N sink due to their ability to take up N from soil. Thus, the main aim of this study was to link N_2 fixation in the feather moss–cyanobacteria association with the moss' ability to acquire N from soil along natural fertility gradients providing varying nutrient conditions.

We investigated the N acquisition of pleurocarpous feather mosses in boreal forests. To do this, we tested the ability of the ubiquitous and dominant feather moss *Pleurozium schreberi* (Brid.) Mitt. to take up N directly from soil, assessing the competition for C and N with microbes by analysing moss and microbial ^{13}C and ^{15}N enrichment after injection of ^{13}C, ^{15}N-alanine and ^{15}N–NH_4Cl into soil. In addition, we analysed soil microbial community size and composition using PLFA-analyses and we tested mineralization rates of various amino acids in soil. To accomplish this, we collected intact cores of soil, humus and feather moss along natural fertility gradients in northern Sweden. The fertility gradients are the result of groundwater discharge (zones of high fertility) and recharge (zones of low fertility), enabling us to compare the uptake of N from soil at sites with contrasting fertility (Giesler et al., 2002). Further, and more importantly, we can relate uptake of N from soil to different rates of N_2 fixation in the moss–cyanobacteria association, which is expected to vary along the gradients. We hypothesize that (1) mosses are able to take up N from soil; (2) uptake of N from soil by the moss is negatively correlated with N_2 fixation since the moss presumably receives N from associated cyanobacteria (Adams and Duggan, 2008); (3) mosses are poor competitors for organic forms of N in contrast to inorganic N.

2. Materials and methods

2.1. Study sites

Feather moss and soil samples were collected in June 2010 along natural fertility gradients in Northern Sweden in three different forests (Varjisån, Kryddgrovan, Pite Älven). The sites are located between latitude 64–65° N, longitude 18–19° E and between 230 and 540 m above sea level. Each forest possesses three different fertility conditions (high, medium, low) due to respective inputs and losses from groundwater discharge and recharge. The low and medium fertility stands fall within a groundwater recharge zone while the high fertility stands fall on groundwater discharge zones thereby receiving subsurface nutrient inputs (Giesler et al., 2002). Mean annual temperature and precipitation are approximately 1 °C and 570 mm, respectively. The vegetation at the high fertility sites were dominated by *Paris quadrifolia*, *Gymnocarpium dryopteris*, *Sorbus aucuparia*, *Geranium sylvaticum*, *Actaea spicata*, *Pinus sylvestris*, *Picea abies*, *Betula pubescens*, *Solidago virgaurea*, *Rubus spec.* and *Maianthemum bifolium*. The medium fertility sites were dominated by *Vaccinium vitis-idaea*, *V. myrtillus*, *Hylocomium splendens*, *P. schreberi*, *Empetrum hermaphroditum* and at the low fertility sites by *V. vitis-idaea*, *V. myrtillus*, *P. sylvestris*, *P. abies*, *Cladonia spec.*, *Calluna vulgaris*, *H. splendens* and *P. schreberi*. Soils at the low and medium fertility sites were classified as Typic Haplocryods, the high fertility soils are Eutric Haplocryods (Soil Survey Staff, 2010). All sites represented stand successional ages older than 120 years (estimated by tree ring counts; O. Zackrisson, unpublished).

2.2. Sampling and soil nutrient analyses

For the uptake experiment, six moss–soil-cores (5 cm diameter, 15 cm depth) with shoots from *P. schreberi* were collected at each fertility stand in each forest site (a total of 54 moss–soil-cores). Additional soil samples for analyses of total C (TC), total N (TN), extractable NH_4^+–N, NO_3^-–N, free amino acids, amino acid mineralization and microbial community analyses (PLFA) were collected using a 2.5 cm diameter stainless steel soil core to a depth of ~10 cm, separating out only the O-layer, and were directly returned to the laboratory and stored at 5 °C until analysis within 5 days. Soil pH was determined in the laboratory on field-moist soil (1:1 w/w soil:distilled water). Soil moisture content was estimated gravimetrically by measuring the mass loss after drying for 24 h at 80 °C.

For analyses of net N mineralization and nitrification potential, we conducted 28-day aerobic incubations. In brief, a 5 g sample of fresh soil was extracted with 20 ml 0.5 M K_2SO_4, shaken for 30 min, centrifuged (15 min at 4000 rpm) and subsequently filtered through Whatman 42 filters. The extracts were analysed by microplate-colorimetric technique using the salicylate–nitroprusside method of Mulvaney (1996) for NH_4^+–N and the vanadium method for NO_3^-–N (Miranda et al., 2001). A second soil sample (5 g fresh weight) was incubated in 50 ml polycarbonate tubes at 20 °C for 28 days in a growth chamber with 16/8 h light/dark cycles; followed by extraction and analyses for NH_4^+–N and NO_3^-–N as described above. Net nitrification was calculated as NO_3^-–N at day 28 minus NO_3^-–N at time zero; net ammonification was calculated as NH_4^+–N at 28 days minus NH_4^+–N at time zero, and net mineralization was calculated as total inorganic N (NH_4^+–N plus NO_3^-–N) at day 28 minus total inorganic N at time zero.

Samples from the time zero extraction were used for analyses of dissolved organic N (DON) and dissolved organic carbon (DOC) using a Shimadzu TCV-TN analyser (Shimadzu Corp., Kyoto, Japan). Total C and TN in soil samples were analysed on dried soil by oxidative combustion using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (IRMS) (Sercon Ltd., Cheshire, UK).

To monitor throughfall N over one year we used an ion exchange system adapted from "resin lysimeters" described by Susfalk and Johnson (2002). Throughfall collectors were constructed by placing the resin capsule between 5 mm of clean, nutrient free mineral wool in open bottomed conical polycarbonate tubes, measuring 2.5 cm in diameter at the surface opening and 5 cm in depth and with a 0.8 cm bottom opening (DeLuca et al., 2008). The ionic resins in the collectors were 1.0 g of mixed bed, anion–cation exchange resins contained in a polyester mesh capsule (Unibest, Walla Walla, WA, USA). Throughfall collectors were placed at moss