

Seasonal variation in the nitrogen concentration and ^{15}N natural abundance of a pleurocarpous moss species in dependence on nitrogen deposition dynamics

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Abstract – The common weft-forming moss species *Pseudoscleropodium purum* (Hedw.) M.Fleisch. was periodically collected at two low mountain range monitoring stations differing in their rates and courses of atmospheric nitrogen deposition. The study explored possible associations between N deposition, its composition and its seasonal variation on one hand and changes in bryophyte tissue N and $\delta^{15}\text{N}$ on the other. Significant seasonal differences of tissue N concentration were clearly linked with the courses of bulk N deposition. Moreover, there were negative correlations between the ratio of $\text{NH}_4^+\text{-N} / \text{NO}_3^-\text{-N}$ in deposition and the bryophyte $\delta^{15}\text{N}$. The results suggest a strong dependence of *Pseudoscleropodium purum* on atmospheric nutrient supply. Furthermore, the isotopic composition of the moss tissue reflects seasonal variations in the composition of N deposition which are probably due to fluctuations of the different anthropogenic N emissions. It is concluded that seasonal variations in tissue nitrogen concentration and $\delta^{15}\text{N}$ must be taken into account when using mosses as bioindicators for atmospheric N deposition.

Bioindication / eutrophication / *Pseudoscleropodium purum* / *Scleropodium purum* / stable isotope ^{15}N / tissue N

INTRODUCTION

Bryophytes occur in most natural and anthropogenic landscapes and under various growing conditions. One of the reasons why they can colonize hard and almost impermeable surfaces like rocks, concrete or tree bark is their poikilohydric habit (Proctor, 2000). Another is that many of them (though not all) rely mainly on an atmospheric nutrient supply and to a much lesser extent on the underlying substratum (Brown and Bates, 1990). Since this holds particularly for the macro nutrient nitrogen, numerous studies investigating the relationship between N deposition and bryophyte tissue N concentration have been carried out (e.g. Malmer, 1988; Pitcairn *et al.*, 1995; Pitcairn *et al.*, 1998; Hicks *et al.*, 2000; Solga *et al.*, 2005). These studies demonstrated the suitability of certain bryophyte species for assessing spatial differences in N deposition and suggested their use as bioindicators. Moreover, it recently has been shown that measurements of the

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isotope ^{15}N enable the identification of different anthropogenic emission sources (Pearson *et al.*, 2000; Gerdol *et al.*, 2002; Bragazza *et al.*, 2005; Solga *et al.*, 2005; Solga *et al.*, 2006a). However, there have been very few studies of the temporal changes in bryophyte tissue N and ^{15}N in relation to seasonally varying rates of N deposition. Apart from the pure scientific interest of how fast bryophytes “react” to such variations, knowledge of this relationship is of practical importance, e.g. when sampling periods in a monitoring programme have to be defined (*cf.* Solga and Frahm, 2006).

Thus a study investigating seasonal changes of nitrogen concentration and ^{15}N natural abundance of the pleurocarpous moss *Pseudoscleropodium purum* was carried out. This common species typically grows in wefts and can hold considerable quantities of rainwater by capillary action (Mägdefrau, 1982). It is regarded as well adapted to obtaining both water and nutrients from precipitation (Rincon and Grime, 1989; Dierßen, 2001; Smith, 2004).

MATERIAL AND METHODS

Study sites

The study was conducted at two low mountain range sites in North Rhine-Westphalia, western Germany. The two sites were selected because of their proximity to Level II monitoring stations of the UN/ECE-ICP Forest programme. In North Rhine-Westphalia these stations are maintained by the North Rhine-Westphalian Agency for Ecology, Land Division and Forestry in Recklinghausen (LÖBF). From the two stations monthly data on bulk N deposition was available ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, N_{tot}). As discussed in an earlier paper (*cf.* Solga *et al.*, 2005), it can be assumed that bulk N data are suitable for comparison with nitrogen concentrations of ectohydric weft-forming mosses.

The monitoring station Velmerstot in the Weserbergland ($8^\circ 57' 04.0''\text{E}$, $51^\circ 49' 53.0''\text{N}$, 420m a.s.l.) is characterized by a relatively high bulk N deposition of $15.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (mean of 1996-2001). The mean annual temperature and precipitation at Velmerstot are 7°C and 1400 mm, respectively.

At the other monitoring station, Glindfeld in the Süderbergland ($8^\circ 41' 02.0''\text{E}$, $51^\circ 13' 20.0''\text{N}$, 470m a.s.l.), deposition rates are distinctly lower with a mean of $9.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (1996-2001). At Glindfeld mean annual temperature is 6.5°C and precipitation is about 1100 mm.

The differences in N deposition between the two sites are probably caused by their specific location to emission sources: while the Velmerstot station is influenced by livestock farming and excessive use of fertilizers in the westwards adjacent Westphalian Bay, the Glindfeld station is situated leeward to the Rothaargebirge which is characterized by large forested areas and agriculture of moderate intensity.

Sampling, analysis of plants and data

At both stations pure stands of *Pseudoscleropodium purum* were found within a radius of less than 1 km. Since the data of N deposition provided by the LÖBF were estimates of open area depositions, material was gathered exclusively in open habitats that were not influenced by throughfall precipitation. At Velmerstot, the sampling site was an extensive *Vaccinium myrtillus* heath, in Glindfeld sampling was carried out on a clearing.

The investigation period of 12 months was from September 2000 to August 2001. Initially, it was planned to take samples every quarter on the same day. However, because of heavy snow fall in the two areas in February 2001, the third sampling was postponed until March; the subsequent samplings were carried out in June and August 2001. At each sampling event, seven samples of *Pseudoscleropodium purum* were randomly taken at both sites on the same day. The distance between single samples was at least 50 cm. The plants were transported in PET bags and stored in a refrigerator before further processing.

In the laboratory, the material was cleaned to remove foreign material of other plants and soil but was not washed. All shoots of each sample were cut 2 cm below the apex. One part of the samples was deep-frozen for the later ^{15}N determination, the other was oven dried at 70°C for 48 h. For the determination of total nitrogen concentration which was carried out in seven replicates 10 shoot tips were randomly selected from each sample. The tips were ground with a vibratory ball mill (Retsch MM 200, Haan, Germany). Approximately 5 mg of each homogenized sample were used for further analysis. Measuring of total nitrogen concentration was conducted with a continuous flow elemental analyser (EuroVector EA 3000, Cascade Science, Gainesville, USA).

The plant material defrosted for the analysis of ^{15}N natural abundance after the end of the experiment was processed as described above. However, due to a shortfall in the material, determinations of ^{15}N were limited to six replicates. Determination was carried out with an isotope ratio mass spectrometer (20-20 IRMS, Europa Scientific, Crewe, UK). ^{15}N natural abundance was expressed as $\delta^{15}\text{N}$ values (e.g. Mariotti, 1984) with atmospheric N_2 as the standard for the $^{15}\text{N}/^{14}\text{N}$ ratio. All laboratory work was performed at the Institute of Plant Nutrition, University of Bonn.

The significance of seasonal differences in tissue N concentration and ^{15}N natural abundance of *Pseudoscleropodium purum* was examined by way of multiple comparison tests. Whenever the Levene test indicated homoscedasticity the Tukey HSD test was used. In the case of heterogeneous variances, the Games-Howell test was applied. Associations between the deposition data and the tissue N concentration/ ^{15}N natural abundance measurements were investigated by calculating Pearson product-moment correlation coefficients (c.c.).

It has been shown in earlier studies that ammonium and nitrate have different nitrogen isotope signatures (Freyer, 1978; Garten, 1992; Heaton *et al.*, 1997). Furthermore, both ammonium and nitrate seem to have an impact on bryophyte $\delta^{15}\text{N}$ (Bragazza *et al.*, 2005; Solga *et al.*, 2005). For this reason monthly $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$ quotients were computed in order to examine the relationship between the temporal variability of N deposition and changes of bryophyte $\delta^{15}\text{N}$. All statistical tests were performed with SPSS 12.0, graphs were created with SigmaPlot 9.0 (both SPSS Science, Chicago, USA).

RESULTS

Nitrogen concentration

At both stations a maximum of bulk N deposition was recorded in early spring of 2001 (Fig. 1). The second-highest values did not coincide and were reached at the Velmerstot in summer 2001 and in Glindfeld in the late summer of 2000. Considering both sites, the tissue N concentration of *Pseudoscleropodium*

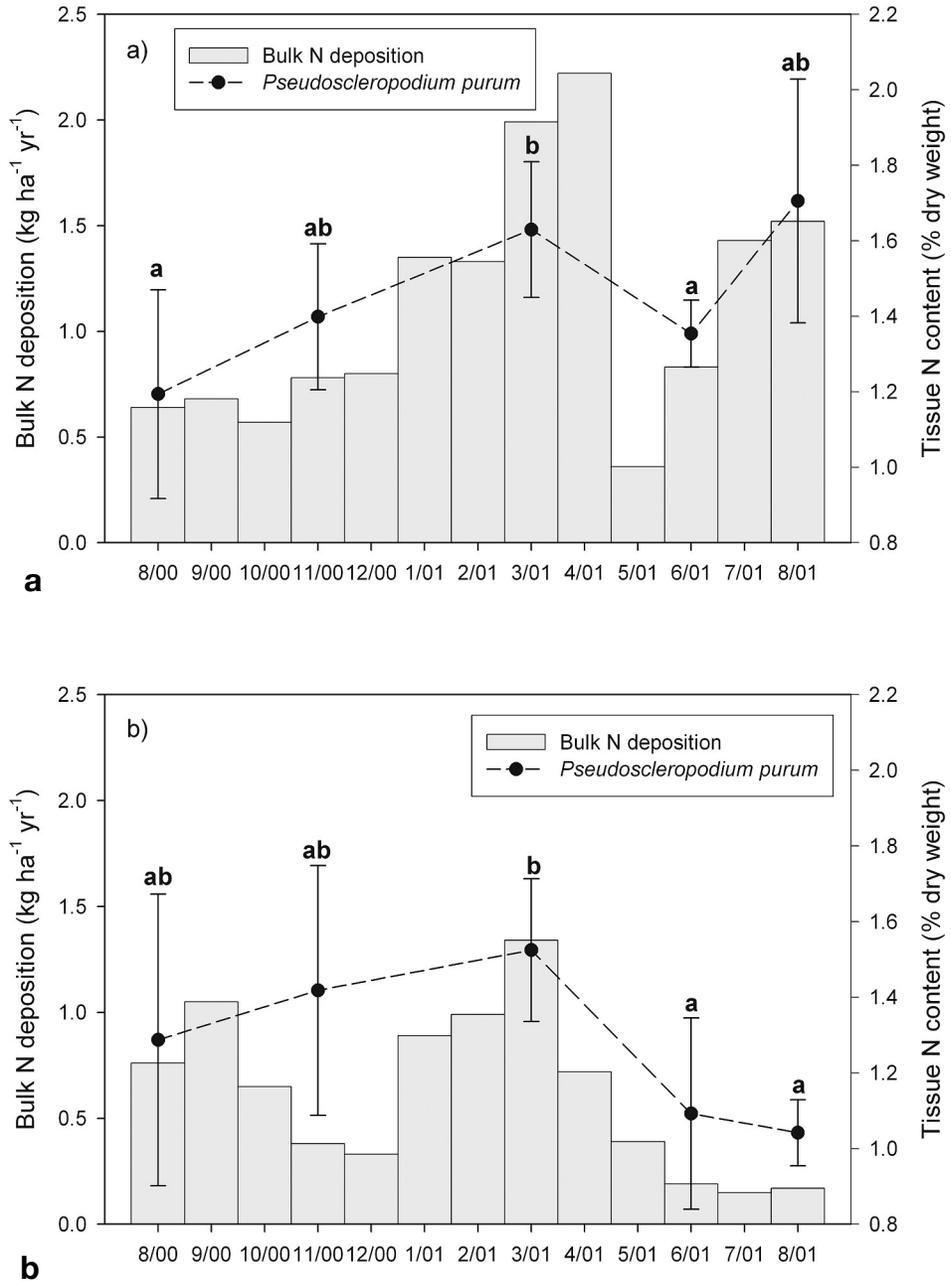


Fig. 1. Bulk N deposition and tissue N concentration of *Pseudoscleropodium purum* at the monitoring stations Velmerstot (a) and Glindfeld (b) between August 2000 and August 2001. Tissue N values are means of seven replicates, error bars are standard deviations. Data points sharing the same letter are not significantly different ($P > 0.05$).

purum ranged between 1.0 and 1.7%. Moreover, apparent seasonal changes of tissue N concentration were found which were partly significant and partly only a trend. At the Velmerstot in particular (Fig. 1 a) the tissue N concentration was clearly linked with the course of bulk N deposition (c.c. 0.88*, $P = 0.05$). At the Glindfeld station (Fig. 1 b) this relationship was somewhat less distinct (c.c. 0.81 n.s.). However, the decrease of N deposition in spring 2001 was reflected in a significant decline of tissue N concentration of the moss.

¹⁵N natural abundance

The highest quotients of $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ deposition exceeding 2.0 were calculated for both stations for the summer months, at Velmerstot in 2001 and at Glindfeld in 2000 (Fig. 2). Relatively low quotients were found especially during autumn and winter, with the exception of February, at Glindfeld. Taking into account both sites, $\delta^{15}\text{N}$ values of *Pseudoscleropodium purum* ranged between -4.1 and -4.9% . As with tissue N concentration, seasonal variation was also detected for ¹⁵N natural abundance. Both at Velmerstot and at Glindfeld relatively strong, even though not significant, negative correlations between the deposition quotients and the $\delta^{15}\text{N}$ values were found (c.c. -0.74 and -0.76 , respectively). At Velmerstot (Fig. 2 a) a significant decrease of $\delta^{15}\text{N}$ values was detected for the period of high $\text{NH}_4^+\text{-N} / \text{NO}_3^-\text{-N}$ quotients in spring and summer 2001. At Glindfeld (Fig. 2 b) the most negative $\delta^{15}\text{N}$ values were also found in the time of the highest quotient (i.e. August 2000). At both sites at least a trend to less negative $\delta^{15}\text{N}$ values during autumn and winter 2000 can be noticed.

DISCUSSION

Nitrogen concentration

There are various explanations for the maxima of bulk N deposition recorded at both stations in early spring. They may be due to increased spreading of fertilizers and/or to an increasing photochemical activity in the atmosphere. These processes lead to enhanced concentrations of NH_4^+ and NO_3^- in rainfall, the latter being linked with enhanced deposition rates if they coincide with high amounts of precipitation (Fricke *et al.*, 1997). At the two study sites the highest monthly amounts of precipitation were in fact recorded in April (Velmerstot) and March (Glindfeld), respectively (data not shown). Another explanation for the bulk deposition peaks could be the thaw after February (see “sampling analysis of plants and data”) as snow is regarded as a very efficient scavenger of atmospheric pollution (e.g. Woolgrove and Woodin, 1996).

The conspicuous and, in the case of the Velmerstot site, significant link between seasonally varying bulk N deposition and tissue N concentration supports the assumption that *Pseudoscleropodium purum* depends mainly on atmospheric nutrient supply (*cf.* Rincon and Grime, 1989; Bates, 1994; Solga *et al.*, 2006a). Furthermore, it became obvious that alterations of tissue N concentrations throughout the year can be considerable. Relationships between seasonally varying deposition rates and bryophyte tissue chemistry were also found by other researchers. Farmer *et al.* (1991) investigated the epiphytic moss *Isoetesium*

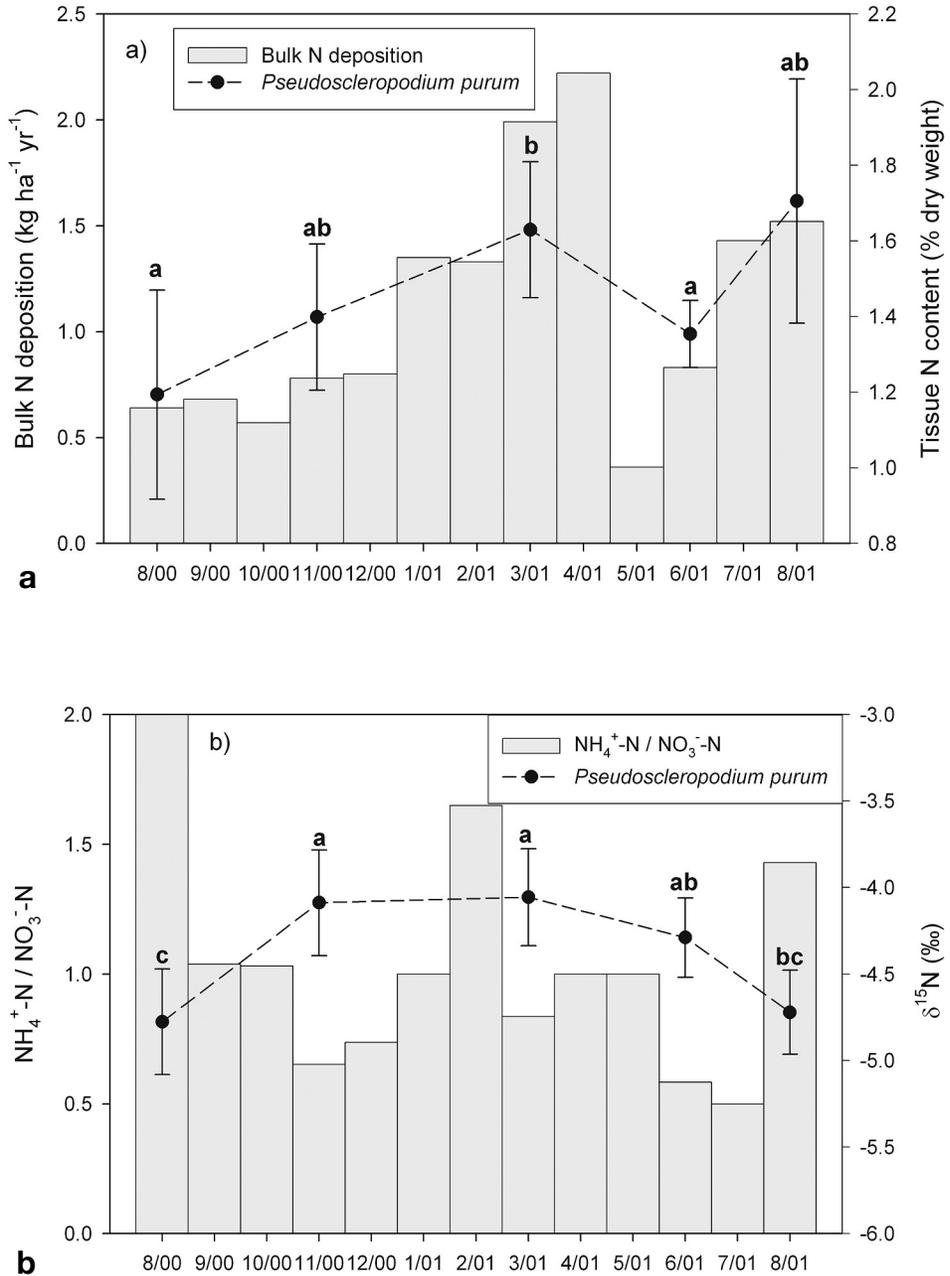


Fig. 2. NH₄⁺-N/NO₃⁻-N deposition quotient and nitrogen isotope signature of *Pseudoscleropodium purum* at the monitoring stations Velmerstot (a) and Glindfeld (b) between August 2000 and August 2001. δ¹⁵N values are means of six replicates, error bars are standard deviations. Data points sharing the same letter are not significantly different ($P > 0.05$).

myosuroides over a one year period. They determined the highest tissue N concentrations for the winter months when both the amounts of precipitation and the deposition of pollutants reached their maxima. In a five months experiment with the forest floor bryophyte *Dicranum majus* Bakken (1994) found increasing tissue N concentrations in the period of enhanced N deposition rates.

Apart from the varying atmospheric deposition some other factors which might have had an effect on tissue N concentration of *Pseudoscleropodium purum* must be taken into account. The lack of phanerogam vegetation during the winter season could have led to an increased availability of nitrogen for terricolous mosses resulting in maxima of tissue N in late winter and early spring. Furthermore, growth dilution (e.g. Brown, 1982) could partly be responsible for seasonal variation. However, as bryophyte growth is closely related to humidity (Pitkin, 1975) and maxima of precipitation at the two study sites coincided with maxima of tissue N concentration (see above), effects of growth dilution are of minor importance here (see also Farmer *et al.*, 1991; Bakken, 1994).

The tissue N concentrations of *Pseudoscleropodium purum* measured in the present investigation are closely in line with values determined in other studies (e.g. Bates, 1994; Solga and Frahm, 2006). The same is also true for the $\delta^{15}\text{N}$ values (Pearson *et al.*, 2000; Gerdol *et al.*, 2002; Solga *et al.*, 2006b).

^{15}N natural abundance

The seasonal variation of the $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ quotient can be explained by taking into account discussions in earlier studies on atmospheric chemistry. It is likely that fluctuations in anthropogenic N emissions have an impact on the ratio of the deposited N species: a higher share of ammonium in summer and spring may be due to agricultural activities, i.e. increased fertilizer application and ammonia volatilization from spreading of manure (Goulding *et al.*, 1986). On the other hand, a lower share of nitrate in precipitation during the summer months is probably the result of reduced domestic and industrial heating and reduced electrical power production (Söderlund *et al.*, 1985). However, contrary observations have also been made. Freyer (1978) found a lower $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ ratio in summer as compared to the winter season and explained this by an increased release of nitric oxides due to denitrification which is favoured by higher temperatures. It should be noticed that generally in most regions in Germany the deposition of NH_y distinctly exceeds that of NO_x (Gehrmann *et al.*, 2001).

Before examining in detail the correlation between the $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ quotient and the bryophyte $\delta^{15}\text{N}$ values the already mentioned different isotope signatures of ammonium and nitrate need further explanation. Oxidized (NO_x) and reduced (NH_y) nitrogen compounds have different $^{15}\text{N}/^{14}\text{N}$ ratios ($\delta^{15}\text{N}$ values). These ratios depend in particular on the sources of the compounds: The total area of Germany is commonly regarded as an artificial landscape (Kulturlandschaft), and consequently here NH_y originates mainly from agriculture, especially from livestock stables and the spreading of animal manure (Fangmeier *et al.*, 1994; Eichler and Schulz, 1998). This NH_y is strongly depleted in ^{15}N (Schulz *et al.*, 2001). NO_x , on the other hand, originates mostly from the combustion of fossil fuels (Lammel, 1993; Erisman *et al.*, 1998). NO_x is almost always enriched in ^{15}N (Heaton, 1990; Freyer, 1991).

Considering this, the negative correlations between the ratio of $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ and the $\delta^{15}\text{N}$ values of *Pseudoscleropodium purum* found for both sites

can be explained as follows: during the summer months the spreading of animal manure and the application of fertilizers cause a growing share of ammonium as nitrogen source. The moss takes up more ammonium and its $\delta^{15}\text{N}$ becomes more negative. On the other hand, in periods in which the share of ammonium in nitrogen deposition is lower (i.e. autumn and winter), the bryophyte $\delta^{15}\text{N}$ becomes less negative. Hence, the isotopic composition of the moss tissue and its alteration reflects reasonably well seasonal variations in the composition of N deposition.

In contrast to the results of this study, Jung *et al.* (1997) who investigated pine needles in eastern Germany found no significant seasonal variations of ^{15}N natural abundance. This is not really surprising: higher plants usually take up nitrogen from the soil and transport it to different parts. Therefore the $\delta^{15}\text{N}$ of a higher plant tissue is affected by many complex processes (isotope fractionations) which take place in the pedosphere, through nutrient uptake and in the plant itself (e.g. Dawson *et al.*, 2002). Ectohydric bryophytes like *Pseudoscleropodium purum*, on the other hand, predominantly take up nitrogen directly from the atmosphere. For this reason it can be expected that the connection between the $\delta^{15}\text{N}$ signature of N deposited from the atmosphere and the $\delta^{15}\text{N}$ of the plant tissue is much closer in an ectohydric bryophyte than in most higher plants.

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