## Global change and the functional diversity of cryptogams in northern biomes

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# Chapter 4

# Mapping nutrient resorption efficiencies of subarctic cryptogams and seed plants onto the Tree of Life

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## Summary

**1.** Nutrient resorption from senescing photosynthetic organs is a powerful mechanism for conserving nitrogen (N) and phosphorus (P) in infertile environments.

**2.** Evolution has resulted in enhanced differentiation of conducting tissue, which we hypothesized to have promoted nutrient resorption efficiency (RE, % of nutrient pool exported) as well.

**3.** Thereto, we compared RE among wide-ranging basal clades from the principally N-limited subarctic region, employing a novel method to correct for mass loss during senescence. Mosses, lichens and lycophytes generally showed low $RE_N$ (< 20%), liverworts and conifers intermediate (40%) and monilophytes, eudicots and monocots high (> 70%). $RE_P$ appeared higher in eudicots and liverworts than in mosses. Within mosses, taxa with more efficient conductance also showed higher $RE_N$.

**4.** *Synthesis*. This novel mapping of a physiological process onto the Tree of Life broadly supports the idea that the evolution of conducting tissues towards specialized phloem has aided land plants to optimize their internal nutrient recycling.

## Introduction

Plant adaptations to nutrient-poor environments include low nutrient requirements of plant tissues and high tissue longevity together with high resorption of nutrients from senescing parts (Chapin 1980; Reich *et al.* 1992; Aerts 1995; Killingbeck 1996). Although resorption of nutrients, especially nitrogen (N) and phosphorus (P), is a process well-known from higher plants (Aerts 1996; Killingbeck 1996), the controlling factors in nutrient resorption efficiency have remained elusive. While large differences in leaf nutrient resorption have been found among species, differences between plant growth

forms appear inconsistent (Aerts 1996; Killingbeck 1996; Yuan & Chen 2009). Some consistent variation in nutrient RE correlated with taxonomical position was reported by Killingbeck (1996), yet his study included a few seed plants only. Furthermore, environmental conditions have been suggested to influence intraspecific variation in RE (Killingbeck 1996) or may have led to adaptation of whole plant assemblages, as indirectly suggested by the large-scale increase of leaf N resorption efficiency ($RE_N$) and the decrease of P resorption efficiency ($RE_P$) of woody plants with latitude (Yuan & Chen 2009). This most likely reflects the predominant N deficiency of ecosystems at high latitudes, where soils are relatively young, as compared to P limitation in ancient soils, which predominate in the (sub-) tropics (Lambers *et al.* 2008). While much work has been done on seed plants, other terrestrial autotrophs have been largely neglected. Only few pteridophytes have been studied (e.g. Headley *et al.* 1985; Killingbeck *et al.* 2002), yet monilophytes other than ferns have been excluded. Moreover, research on variation in and controls on nutrient resorption in cryptogams is still in its infancy (Cornelissen *et al.* 2007), even though bryophytes and lichens are paramount contributors to biomass, especially at higher latitudes where they fulfill important controls on nutrient and carbon cycling (Longton 1997; Cornelissen *et al.* 2007).

Here we introduce a new concept to the debate about what controls nutrient resorption efficiency across taxa by proposing that species' resorption efficiencies are determined more by evolutionary changes in conducting tissues than by current environmental controls. Basic to this concept is that nutrients are translocated via the phloem during senescence (Gan 2007). Differences in conducting tissue should therefore importantly determine the extent of nutrient resorption. What do we know about tissue conductance of the main autotrophic, terrestrial clades of the Tree of Life? While non-vascular cryptogams contain no true sieve elements (SE) (Behnke & Sjolund 1990), conducting tissue as such, albeit simple, have evolved in both liverworts and mosses (Hébant 1977). Phloem emerged in early cryptogams (here: lycophytes or club mosses, monilophytes or ferns and horsetails) but was still relatively primitively built. In contrast, spermatophytes or seed plants (here: conifers, eudicots, monocots) feature a differentiated phloem with sieve cells or tubes accompanied by specialised parenchyma cells (Behnke & Sjolund 1990). Thus, the development of conducting tissue during land plant evolution, from non-vascular cryptogams to tracheophytes (vascular plants), did not only help to bring about increasingly complex plant structures (Behnke & Sjolund 1990) but also efficient transport of a variety of compounds such as photosynthates and amino acids from leaves to other plant parts (Van Bel 2003). We propose that this development also must have offered increasing possibilities of internal nutrient recycling, especially N and P, from

senescing photosynthetic tissues back to other plant parts, thereby helping the plants to gain relative independence from soil nutrient status. In this paper we ask the questions (i) whether the general lack or low degree of specialisation of conducting tissues in non-vascular cryptogams compared to that in vascular plants has left them less efficient at nutrient resorption from senescing parts; and (ii) whether interspecific variation within basal cryptogam clades corresponds with presence/absence or degree of differentiation of conducting tissues as related to their phylogenetic position.

Thus, we hypothesise that the appearance and specialisation of conducting tissues across the autotrophic branches of the Tree of Life has been accompanied by an evolution of increasing nutrient resorption efficiency. We test this new hypothesis across 16 lichen, 27 bryophyte and 25 vascular plant species together comprising the predominant components of the subarctic bogs, mires, tundras and forests of northern Europe, and covering the main basal clades of the Tree of Life present in a subarctic flora. We specifically chose to collect data from one climatic region, thus avoiding confounding effects of strong gradients in climate and nutrient availability and climate (see Yuan & Chen 2009), which in turn might affect nutrient resorption patterns, for instance through luxury consumption. We apply a new methodology to allow fair, calibrated comparisons of mass-loss-corrected nutrient resorption efficiencies among diverse taxa, by expressing nutrient pools of fresh and senesced tissues, respectively, relative to their contents of inert structural chemistry derived from infrared spectra (Fourier transform infrared attenuated total reflectance; FTIR-ATR). To our knowledge, this is the first paper to link a physiological process, nutrient resorption, explicitly to substantial branches of the Tree of Life.

## Materials and methods

### SAMPLING AND SPECIES CLASSIFICATION

Bryophytes and lichens were sampled in the summer of 2004 mainly around Abisko, Sweden (68º21’N, 18º49’E), but also on Andøya, Norway (69º07’N, 15º52’E) and in Kilpisjärvi, Finland (69°03'N, 20°50'E). The lichen *Cladonia stellaris* was sampled in the Altai Republic, S Siberia (51º04’N, 85º45’E) in 1999 and stored air-dry. We focused mainly on abundant species (see also Lang *et al.* 2009). For the vascular plants we used an existing database, for which common species were sampled from the predominant ecosystems within 10 km from Abisko in 1998 and 1999 (Quested *et al.* 2003). Since in this dataset no P was measured, we estimated $RE_P$, with an accuracy of 1%-point, for six vascular plants in the Abisko region from Van Heerwaarden *et al.* (2003b). Together these species were representative of the European subarctic region. For nomenclature see Lang *et al.* (2009).

Phylogeny followed Donoghue (2005). Species were allocated to basal clades, classes, orders and families according to Stevens (2001 onwards) for vascular plants, Goffinet & Shaw (2009) for bryophytes and Lumbsch & Hundorf (2007) for lichens (for the full list see Appendix S1 in Supporting Information). Not all cryptogam classes and orders could be represented by sufficient numbers of species, reflecting their low species richness in the European subarctic flora or the rarity of their occurrence. However, we feel that this imbalance, somewhat constraining detailed statistical analyses (see below) at the finer taxonomic levels, should still be acceptable compared to the disadvantages that would have been associated with adding species from other (climate) regions to artificially top up species numbers per group.

## PROCESSING THE CRYPTOGAM SPECIES

After return to the lab, samples were air-dried and kept in paper bags until further preparation. After careful remoistening without producing excess water to avoid leaching, cryptogams were thoroughly cleaned from dirt and other intermingled cryptogam species. Hereafter, liverworts and mosses were visually divided into the living green parts and the recently senesced (brown) parts (see Lang *et al.* 2009). Older, already visibly decomposed parts were not included. Similarly, lichens were divided into the living part and the recently senesced part, the latter with a seemingly softer structure, usually accompanied by a colour change, i.e. a dark brown, black or bleached appearance. For thallose lichens, senesced material was located in the centre of the lichen. In a second dataset, we furthermore distinguished between early and late RE, since the green tissue in mosses often consists of several years' growth. Mosses were visually divided into younger green tissue (bright green), older green tissue (darker green) and the recently senesced parts. Consequently, species that showed no differences in tissue colour were excluded from this dataset, including all sampled liverworts and a few mosses. Younger versus older 'green' tissue of all lichens was identified by its slightly green tinge (depending on thallus colour) versus its mature thallus colour, i.e. brown or yellow.

The influence of choice of material on the magnitude of RE is illustrated in Appendix S2. In general (except for $RE_P$ in lichens), RE was clearly higher in younger parts and lower in older tissue. Consequently, the measure of RE integrating all green tissue (Fig.1), was 10 - 20% lower compared to RE in the youngest tissue. Given also the fact that mosses are known to move photosynthates both upwards into the shoot and downwards into senesced tissue as an energy store (Hakala & Sewón 1992), RE in cryptogams is dependent on the choice of material. In this study, we chose to use RE integrating all green tissue, in accordance with the sampling procedure for evergreen vascular cryptogams (lycophytes).

## CALCULATION AND CALIBRATION OF RE

Absolute nutrient concentrations of green versus senesced tissues might give incorrect $RE_{\%}$ depending on the amount of translocation of carbon through plants or fungi (Van Heerwaarden *et al.* 2003a). Since for vascular plants, either area- (all except *Eriophorum vaginatum*) or leaf length-based $RE_P$ (solely *E. vaginatum*) were available as a stable reference (Van Heerwaarden *et al.* 2003b), we combined these measures in the later analysis. We aimed to express nutrient pools based on an immobile fraction, such as total acid-detergent fibers (ADF), lignin or cellulose. The latter occurs in vascular plants, bryophytes as well as in the algal part of lichens and can therefore be used as a stable reference for RE across clades. However, in most cryptogams, especially liverworts, the availability of material was too limited to perform the wet chemical laboratory analyses. Therefore, in a dataset where both wet chemical measurements and infrared measurements were available ($n$ = 14; one moss, 13 vascular plants from contrasting clades), we conducted partial least squares regression (PLS-R) to identify ADF-, lignin- or cellulose-characteristic wavelengths using The Unscrambler v9.2 (CAMO Software AS, Oslo, Norway). Based on significant variables only, which were determined with Jack-knifing (full cross validation), PLS-R was recalculated. In the final model, ADF and lignin were insufficiently described, while PLS-R for cellulose revealed an $R^2_{Calibration}$ of 0.98 and a small root mean square $error_{Calibration}$ of 0.95. In a second, independent dataset, we compared predicted cellulose values with conventional cellulose measurements. The linear relationship was significant ($P$ = 0.003, $R^2$ = 0.84). However, lichen cellulose content was not equally well expressed for all lichen species (details see Appendix S3). Calibration of lichen REs with Calcium (Ca) content (see Appendix S4), produced the same results for RE (and the interaction term method x lichen order was not significant). We are therefore confident that our results are representative despite the above-mentioned difficulties with cellulose calibration for some lichen species.

Nitrogen $RE_{\%}$ ($RE_{N\%}$) was calculated as $([N_{green}] - [N_{senesced}])/[N_{green}]$ x 100%, with $N_{green}$ and $N_{senesced}$ referring to N in green and senesced tissue, respectively. If calibrated with reference to immobile chemistry, e.g. cellulose, $RE_{Nsr}$ ($RE_N$ with stable reference) was expressed as ([Ngreen]/[cellulose] – [Nsenesced]/[cellulose])/[Ngreen]/[cellulose] x 100%. The corresponding parameters were calculated for P ($RE_{P\%}$ and $RE_{Psr}$, respectively). For vascular plants, a complete dataset was solely available for green tissue in 1998 and for litter in 1999. We therefore compared $[N_{senesced}]$, and $[N_{senesced}]$/[cellulose] of 1998 versus 1999, for species available in all datasets. Both linear regressions were highly significant, and, in the case of $[N_{senesced}]$/[cellulose], the intercept was close to zero and the slope close to 1 (see Appendix S5). Thus, we concluded that differences in

[$N_{senesced}$] between years were relatively small, allowing a direct comparison of RE across adjacent years.

## CHEMISTRY

Nitrogen concentrations of vascular plants were determined from ground samples, using a Tracermass mass spectrometer (Europa Scientific, Crewe, UK). For ADF, cellulose and lignin analyses see Quested *et al.* (2003). P in vascular plants was determined colorimetrically at 880 nm with molybdenum blue (details see Van Heerwaarden *et al.* 2003b). The cryptogam samples, for which the following analyses were carried out, were ground for approx. 2 min using a ball mill (MM 200, Retsch, Haan, Germany) before use in further chemical analysis. For concentrations of Ca and P, subsamples were acid-digested (teflon bomb under addition of 1 ml of the mixture $HNO_3$/HCl, ratio 4:1) for 7 hours at 140 ºC. After adding 4 ml distilled water, Ca was measured by atomic absorption spectrometry (1100B Spectrometer, PerkinElmer Inc., Waltham, Massachusetts, USA) under addition of 1% $LaNO_3$. For P analyses see above. N was determined by dry combustion with a Carlo Erba NA1500 (Rodana, Italy) elemental analyser. Since cryptogam samples were cleaned meticulously, LOI (mass loss of ignition, at 550 ºC for 4 hours) to correct for extraneous minerals, needed to be determined only for *Racomitrium fasciculare* and the lichen *Solorina crocea*. Both cryptogams originated from environments where contamination by minerals was possible. Molecular structure of the ground cryptogam and vascular plant samples was analysed spectroscopically by FTIR-ATR (Nexus$^{TM}$ 670, ATR cell DuraScope, Thermo Nicolet, Madison, WI, USA) with a resolution of 4 $cm^{-1}$ and 32 scans. Extinction was calculated from infrared spectra followed by ground correction to correct for multiple scattering of light inside the probe. Further details of this methodology are in Lang *et al.* (2009).

## DATA ANALYSIS

$RE_N$ of *Cetraria islandica* and $RE_P$ of *Nephroma arcticum* and *Tomenthypnum nitens* were unrealistically very negative and strongly suspected to represent sampling or measurement problems. These outliers were excluded from further analysis. Where necessary, data were ranked to improve normality. The influence of taxonomic level, across basal clades and cryptogam orders and classes, on $RE_{\%}$ and $RE_{sr}$ was tested in several one-way ANOVAs followed by Tukey post-hoc tests using SPSS 15.0 for Windows. The influence of method type on RE was tested in a two-way ANOVA with method type and taxonomical level as between-subject factors. Within lichens, the influence of $N_2$-fixing ability on RE was tested in a one-way ANOVA. Where Levene's test remained significant despite data transformation, we chose to reduce sample size

randomly down to five (or six) replicates (at basal clade level: $RE_P$; testing method type and clade: $RE_N$), since analysis of variance is robust to heterogeneity of variances as long as sample size is nearly equal (Zar 1999). Relating RE to [$N_{green}$] in linear regression (y = ax + b) would violate the assumptions of independence in statistical tests. Therefore, we compared [$N_{senesced}$] versus [$N_{green}$] across clades and outlined the isoclines of $RE_{N\%}$ (0, 10, …90), as a function of [$N_{green}$] and [$N_{senesced}$], in the same graphs. With a positive slope, RE increases if the intercept b > 0 and decreases if b < 0. If b = 0, RE is constant across clades. We also compared $N_{senesced}$/cellulose versus $N_{green}$/cellulose to evaluate whether results deviated depending on the type of $RE_N$ measure.

## Results

### $RE_N$ AND $RE_P$

At a broad taxonomic scale, clade identity influenced both $RE_{N\%}$ and $RE_{Nsr}$ significantly. Lichens (lichenized ascomycetes), mosses and lycophytes showed lower $RE_{Nsr}$ (and $RE_{N\%}$) (< 20%) compared to monilophytes, eudicots and monocots (> 70%) while liverworts held an intermediate position (40%). Conifer $RE_{Nsr}$ and $RE_{N\%}$ did not differ significantly from other basal clades (Fig.1, Table 1). Comparing the two methodologies, clade was a significant determinant of $RE_N$ ($F = 23.72$, $P < 0.001$; ranked) while method type ($F = 0.01$, $P = 0.93$) and the interaction of clade x method type ($F = 0.29$, $P = 0.95$) were not significant. There was a consistent trend for differences in $RE_P$ among clades. These differences are mainly due to the eudicots ($RE_{P\%}$ and $RE_{Psr}$: 54 and 61%; or angiosperms: 60 and 66%) resorbing more P than mosses (32 and 28%), while lichens (17 and 20%), encompassing a wide data range, were not clearly separated from the other clades. $RE_P$ in liverworts (42 and 50%) was almost as high as in eudicots.

**Table 1.** Statistical analysis of differences in $RE_N$ and $RE_P$ at clade, class and order level across the autotrophic sections of the Tree of Life (lichenised fungi and plants; $n$ = 2-20). Significant $P$-values are marked with bold letters. Note that the underlying species sets are more robust for $RE_N$ than for $RE_P$ since $RE_P$ of vascular plant clades encompassed solely eudicots (or angiosperms)

| Clade | Taxonomic level | Source | d.f. | $F$ | $P$ |
|---|---|---|---|---|---|
| All | Clade | $RE_{N\%}$ | 7 | 15.03 | **< 0.001** |
| | | $RE_{Nsr}$ | 7 | 19.94 | **< 0.001*** |
| | | $RE_{P\%}$ | 2 | 2.80† (2.62‡) | 0.073*† (0.079*‡) |
| | | $RE_{Psr}$ | 2 | 2.88† (3.26‡) | 0.068*† (**0.043***‡) |
| Moss | Class | $RE_{N\%}$ | 2 | 3.98 | **0.038** |
| | | $RE_{Nsr}$ | 2 | 2.79 | 0.089 |
| | | $RE_{P\%}$ | 2 | 0.78 | 0.47 |
| | | $RE_{Psr}$ | 2 | 1.11 | 0.36 |
| Lichen | Order | $RE_{N\%}$ | 1 | 0.10 | 0.76 |
| | | $RE_{Nsr}$ | 1 | 0.45 | 0.51 |
| | | $RE_{P\%}$ | 1 | 5.48 | **0.036*** |
| | | $RE_{Psr}$ | 1 | 3.37 | 0.089* |

* Ranked

† Eudicots

‡ Angiosperms (eudicots and monocots)

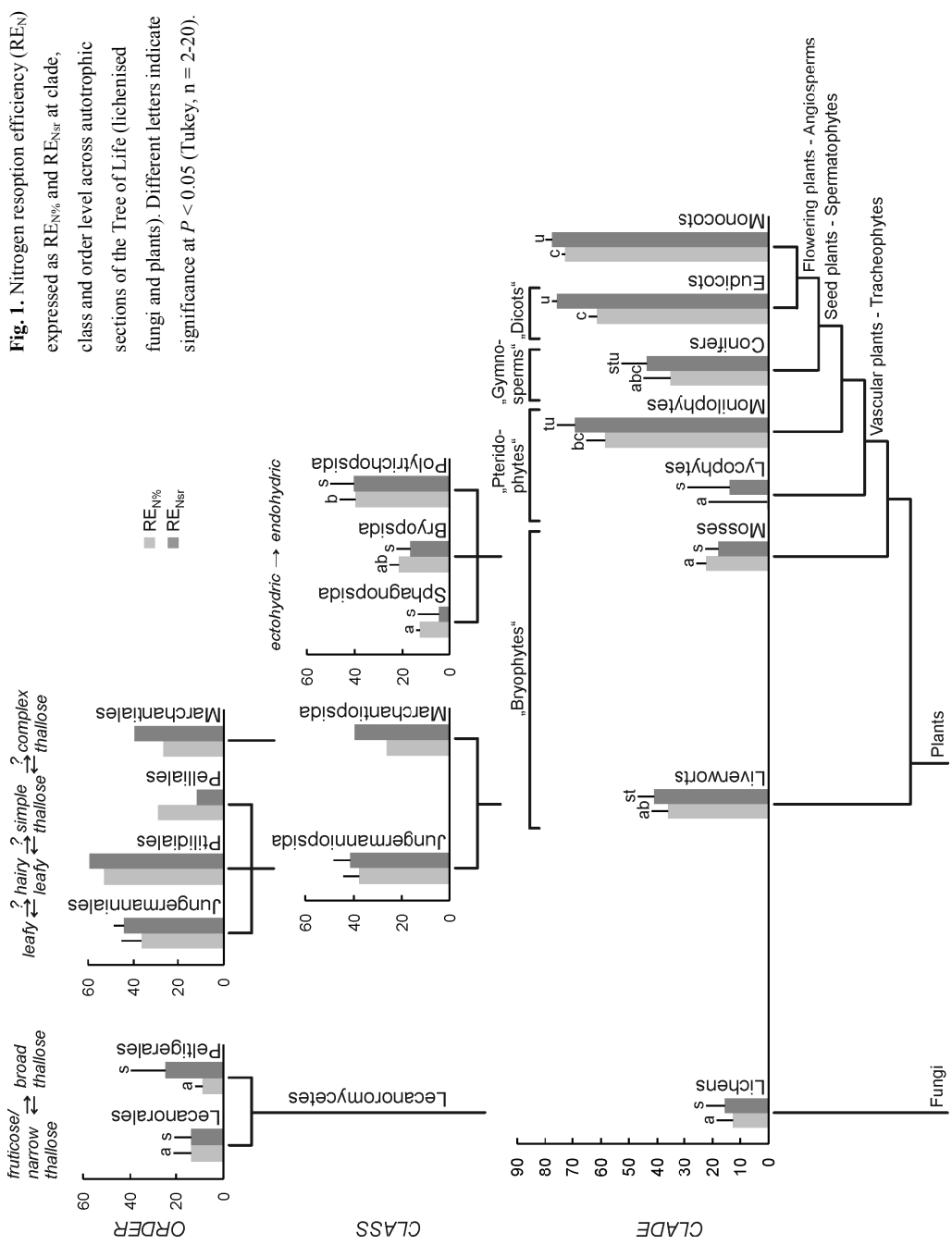


**Fig. 1.** Nitrogen resoption efficiency ($RE_N$) expressed as $RE_{N\%}$ and $RE_{Nsr}$ at clade, class and order level across autotrophic sections of the Tree of Life (lichenised fungi and plants). Different letters indicate significance at $P < 0.05$ (Tukey, n = 2-20).

At moss class level, the ectohydric Sphagnopsida showed lower $RE_{N\%}$ compared to the endohydric Polytrichopsida while Bryopsida were intermediate. $RE_{Nsr}$ showed a trend, showing the same pattern as $RE_{N\%}$ for the mean. When comparing the two methods, $RE_{N\%}$ versus $RE_{Nsr}$, class ($F$ = 6.12, $P$ = 0.005) was a significant determinant of $RE_N$ while method type ($F$ = 0.37, $P$ = 0.55) and their interaction effect ($F$ = 0.11, $P$ = 0.90) were not significant. Neither $RE_{P\%}$ nor $RE_{Psr}$ differed among moss classes.

At lichen order level, $RE_{N\%}$ or $RE_{Nsr}$ of the fruticose and narrow thallose Lecanorales were not significantly different from the broad thallose Peltigerales. $RE_{P\%}$ was significantly higher in the Lecanorales and $RE_{Psr}$ showed the same trend for these groups. $N_2$-fixing lichens, which were present in both the Lecanorales and Peltigerales, did not show significantly lower $RE_N$ ($RE_{N\%}$: $F$ = 0.96, $P$ = 0.35; $RE_{Nsr}$: $F$ = 0.19, $P$ = 0.67) whereas $RE_P$ was significantly higher in non-$N_2$-fixing lichens ($RE_{P\%}$: $F$ = 7.06, $P$ = 0.020; $RE_{Psr}$: $F$ = 5.89, $P$ = 0.031; ranked).

Whether liverwort taxa representing different growth forms, (hairy) leafy, simple or complex thallose, show differences in RE, remains unsubstantiated as most species in this study belonged to the leafy liverwort order Jungermanniales, while all other orders were represented by only one (Ptilidiales) or very few species in the study area.

## THE RELATION OF $N_{SENESCED}$ VERSUS $N_{GREEN}$

Across clades, linear regressions between $N_{senesced}$ and $N_{green}$, whether based on percentage or cellulose, were positive and significant (Fig. 2). With increasing [$N_{green}$] and $N_{green}$/cellulose, RE increased across clades (intercept of the regression line $b > 0$). Based on [$N_{green}$] and even more so when looking at N/cellulose, eudicots, monilophytes and monocots showed the highest $N_{green}$ content and highest $RE_N$ while liverworts, conifers, mosses, lichens and lycophytes were located at the lower end of the range.

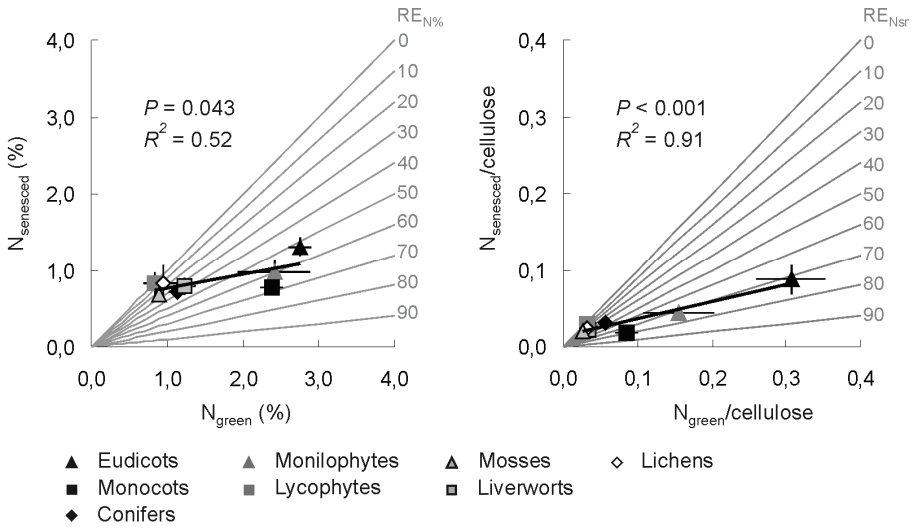


**Fig. 2.** $N_{senesced}$ (%) versus $N_{green}$ (%), and $N_{senesced}$/cellulose versus $N_{green}$/cellulose, across the basal clades of the Tree of Life (± SE; $n$ = 8). Isoclines for $RE_{N\%}$ and $RE_{Nsr}$ are outlined in grey.

## Discussion

To summarise our main findings, all clades across the Tree of Life resorbed nutrients during organ senescence, but the efficiency differed strongly among clades. Mosses, lichens and lycophytes generally showed low $RE_N$, liverworts and conifers intermediate and monilophytes, eudicots and monocots high. With reduced numbers of tracheophyte clades (only eudicots or angiosperms present), the pattern for $RE_P$ was similar to $RE_N$ but less clearly expressed. Within mosses, taxa with more efficient conductance also showed higher $RE_N$. Thus, the variation in nitrogen resorption efficiency in a subarctic flora broadly supports the hypothesis that the evolution of conducting tissues has aided plants to optimise their internal nutrient cycling in terrestrial environments. While we have most confidence for our results for RE as expressed on a stable secondary chemistry basis, derived with our novel application of FTIR-ATR, the general correspondence of the between-clade patterns for $RE_{sr}$ and $RE_{\%}$ show that the larger differences in RE are rather robust to methodological factors (interaction effect of clade x method type is not significant). Below we will discuss our findings in more detail with special focus on the types of conducting system that might support nutrient resorption of autotrophs across the Tree of Life.

## CONDUCTIVE SYSTEMS AS VEHICLES FOR NUTRIENT RESORPTION ACROSS AUTOTROPHIC CLADES

The fact that all non-tracheophyte cryptogam clades had distinctly low $RE_N$ compared to seed plants is consistent with the pattern of increasing differentiation of conducting tissue, from lichens, liverworts and moss clades with no or little conducting tissue to the high differentiation of phloem in seed plants, although the apparently lower $RE_N$ of conifers compared to angiosperms might be the results of other traits regulating RE (see below). Though based on fewer clades, the trend for $RE_P$ was similar, with angiosperms showing highest $RE_P$ in comparison to mosses, lichens and, to a lesser extent, liverworts. Our results for monilophytes and lycophytes were surprising, even though the numbers of species represented in this subarctic flora were too low for any firm statements. Still, the lycophytes showed low N resorption while monilophytes had particularly efficient N resorption, even higher than the $RE_{N\%}$ of 52% reported for a temperate fern (Killingbeck *et al.* 2002). The phloem of lycophytes (e.g. *Lycopodium*) differs from other vascular cryptogams in possessing plasmalemma-lined sieve area pores which are wide open, whereas the pores of certain monilophytes, e.g. *Equisetum* and leptosporangiate ferns, are traversed by membranes of endoplasmic reticulum (ER). In sieve tube members of eudicots, ER may play an important role in phloem loading processes (Behnke & Sjolund 1990). However, recent studies suggest ER facilitates the trafficking of proteins between sieve elements (SE) and companion cells (CC) (Van Bel 2003). P-proteins, known from eudicots and many monocots but absent in conifers and mosses, are replaced by refractive spherules in monilophytes but again are absent from lycophytes (Behnke & Sjolund 1990). The functions of P-proteins are subject to debate, but apart from plugging sieve plates upon injury (Behnke & Sjolund 1990), these may include sugar metabolism, transmembrane sugar transport, membrane water permeability and protein degradation (Van Bel 2003). Thus, we speculate that the absence of P-proteins or refractive spherules in conifers and lycophytes may partly explain their low RE. In addition, the evergreen habit of both conifers and lycophytes might compensate for insufficient RE, conserving nutrients by extending their mean residence time (Aerts 1995). Furthermore, the low N concentrations in green tissue found in these clades (Fig. 2), limit the extent of RE (Aerts 1996), given that there is always a pool of N that remains immobile during senescence (Killingbeck 1996). We expected that the emergence of specialized parenchyma cells, 'Strasburger cells' in conifers and CC in angiosperms (Behnke & Sjolund 1990), would lead to increased RE compared to vascular cryptogams. This hypothesis is based on the suggestion by Van Bel (2003) that evolutionary specialization led to increased longitudinal flow in the phloem due to increased porosity of the end walls. Also, most of the cytoplasmic structure is dismantled to decrease mass flow resistance while the

adjacent parenchyma cells gradually take over regulation of functions in the SE (Van Bel 2003). However, RE of monilophytes was equally high as RE in seed plants, possibly owed to the presence of single cytoplasmatic connections between SE and parenchyma cells in this clade (Behnke & Sjolund 1990). It is tempting to assign differences in RE to minor vein type structure and their related phloem loading types, from ancestral symplastic phloem loading (ferns, conifers) to apoplastic or mixed loading in evolutionary young angiosperms (Van Bel 2003). However, in the dicots all three minor vein types are found (Turgeon *et al.* 2001) and, moreover, symplastic phloem loading also occurred in evolutionarily young angiosperms such as *Salix* (Van Bel 2003). Gamalei (1991) suggested evolutionary specialisation of phloem loading in relation to climate. Typical in arctic-alpine tundra is the closed minor vein type, related to apoplastic phloem loading (except trees: symplastic) since symplastic plasmodesmal transport is sensitive to chilling. However, interactions between minor vein type and transported sugar identity on phloem loading type (Van Bel 2003), which is hypothesized to influence hydraulic gradient strength (Holbrook & Zwieniecki 2005), may further complicate the implications for resorption. Analogue to studies on xylem (Roth & Mosbrugger 1996), we can also speculate about possible implications of stelar morphology, from primitive protostele in the earliest land plants (e.g. *Cooksonia*, *Rhynia*) to eustele (e.g. eudicots), on phloem transport properties.

In conclusion, many open questions remain concerning evolution of SEs and their cell biology, pathways and modes of phloem loading, impact of environmental factors on phloem transport and possible adaptation of phloem loading modes to climatic conditions (Van Bel 2003). Yet answering these questions will provide the basis from which we can start to evaluate the factors influencing RE in tracheophytes.

## NOVEL SCREENING OF NUTRIENT RESORPTION IN HIGHER CRYPTOGAM TAXA

Our study is the first comprehensive study of nutrient resorption efficiency not only across basal cryptogam and seed plant clades, but also within several non-tracheophyte cryptogam taxa. While for some bryophyte and lichen taxa the subarctic flora did not support sufficient numbers of species for statistically sound comparison, we have quantified some consistent and logical patterns. Despite scattered evidence of N and P translocation, no study so far has investigated RE of a representative number of moss species in order to detect consistent taxonomic variation. Within mosses, translocation has been mainly linked to the endohydric Polytrichales (Collins & Oechel 1974; Reinhart & Thomas 1981) which feature leptoids (Hébant 1977), i.e. conducting tissue, somewhat

comparable to the sieve cells of higher plants. Also, this moss class alone features refractive spherules and callose, associated with plasmodesmata (Ligrone *et al.* 2000), which, in angiosperms, are associated with pores during sieve plate development (Behnke & Sjolund 1990). Indeed, $RE_{N\%}$ in the Polytrichaceae was higher than in other mosses, especially when compared to the ectohydric *Sphagnum*. However, though devoid of leptoids, transport of photoassimilates (Alpert 1989; Hakala & Sewón 1992) or N (Eckstein & Karlsson 1999) has also been reported for *Dicranum*, *Grimmia* and *Hylocomium*, respectively, most likely facilitated by an internal conducting strand of elongated parenchyma cells (Ligrone & Duckett 1994). Moreover, evidence has been found for conducting tissue in the Sphagnales differing from the Bryopsida only in lacking plastid - microtubules associations (Ligrone & Duckett 1998); this conducting tissue might explain P, C and N translocation found for the peat moss *Sphagnum* (Rydin & Clymo 1989; Aldous 2002). We have to be aware that some interspecific variation in RE may be due to environmental factors. Since not all mosses could be collected at the same time, species differences in seasonality of translocation (Skre *et al.* 1983), downwards for storage and upwards for growth, might have contributed to some of the observed variation. Furthermore, cyanobacterial $N_2$-fixation observed on feather mosses (Zackrisson *et al.* 2009), might have complicated the observed pattern. Indeed, $RE_N$ of these mosses was higher than in most other species of the Bryopsida (data not shown).

We are the first to study RE of N and P in senescing photosynthetic parts of liverworts. With the exception of the complex thallose liverwort *Marchantia* (Rota & Maravolo 1975), little is know about translocation in this clade. Ligrone *et al.* (2000) suggested a microtubule-based translocation system for the marchantialean liverwort *Asterella*. However, liverworts seemed to generally resorb nutrients at rather high rates, comparable to those of conifers and monilophytes. Analogue to mosses, cyanobacterial $N_2$-fixation is known for a few liverworts (Adams & Duggan 2008) and this might have affected RE. This might also hold for basidiomycetous infections found repeatedly in jungermannialean liverworts, leading to an increase or decrease of the host cytoplasm (Duckett *et al.* 2006). The latter phenomenon has been reported for *Lophozia*, *Barbilophozia* and *Nardia*, all represented in our study. A central strand of conducting tissue has so far only been found for species in the liverwort orders Calobryales, Pallaviciniales (Hébant 1977), Pelliales and Marchantiales (Ligrone *et al.* 2000), of which the latter two were represented in our subarctic flora. The hypothesized link between central strands and RE is in need of explicit comparison across liverwort orders.

This is also the first explicit study of nutrient resorption related to tissue senescence in lichens. Translocation of N and P between fresh tissues has been demonstrated for *Cladonia stellaris*, *Stereocaulon paschale* and *Cladonia portentosa*, respectively (Hyvärinen & Crittenden 2000; Kytöviita & Crittenden 2007), while for *Caloplaca trachyphylla*, transport of carbohydrates has been shown (Bench *et al.* 2002). Translocation in lichens, in which fungal hyphae provide the main structure, can most likely be compared to translocation in (ecto-) mycorrhizal mycelium. Out of three suggested transport mechanisms, diffusion, mass flow along turgor gradients or cytoplasmic streaming through pores of septa (Finlay 1992), the latter seems to be the most likely transport mechanism in lichens (Hyvärinen & Crittenden 2000). Assuming that a similar transport mechanism prevails in all fungal hyphae, we hypothesised that RE would not differ among lichens of different growth forms, when excluding $N_2$-fixing lichens. Indeed, $RE_N$ did not differ among lichen orders. However, $RE_{P\%}$ was higher in the Lecanorales and seemed to be negatively related to the $N_2$-fixing ability of lichens. The differences found in $RE_P$ may be due to P in cyanobacteria. Since cyanobacteria are located in cephalodia that are still present in dead lichen tissue, cyanobacterial P might not be accessible during resorption but is left behind in aging lichen tissue. Furthermore, algae in lichens are known to store P as polyphosphate in granules (Guschina *et al.* 2003), complicating the interpretation of the observed pattern. For $N_2$-fixing lichens, with a constant input of readily available N, one would expect N resorption to be less important than in non-$N_2$-fixing lichens, analogous to reduced $RE_N$ of $N_2$-fixing higher plants (Killingbeck 1996). However, $N_2$-fixing lichens did not differ in $RE_N$ from non-$N_2$-fixing lichens, which point to a similar transport mechanism across lichen species.

We conclude that the progressive evolution of tissues to facilitate internal transport across the major clades of land plants is, by and large, coupled with their nutrient resorption efficiency during organ senescence. As such, this has led to a lesser dependency of plants on external nutrient supply. While many organism characters have been mapped explicitly onto the Tree of Life before, nutrient resorption may, to the best of our knowledge, represent the first organismal *process* to have been given this treatment. Under the assumption that actual nutrient resorption, which involves several interacting physiological and chemical processes (Gan 2007), is influenced by a myriad of genes (Gan 2007), our approach will also be of great interest for phylogenetic analysis of many other complex organismal processes.

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**Appendix S1. List of vascular plant and cryptogam species assessed for nutrient resorption efficiency**

Species are sorted according to clades (class and order where appropriate). $RE_N$ was available for all species except *Eriophorum vaginatum*. Shaded species were not used in Figure 1 of the main manuscript. Marked with * are species for which $RE_P$ was available. Species nomenclature follows Hill *et al.* (2006) for mosses except *Sphagnum*; Daniels & Eddy (1985) for *Sphagna*; Damsholt (2002) for liverworts; Santesson *et al.* (2004) for lichens; and Mossberg *et al.* (1992) for vascular plants.

| Clade | Class | Order | Species |
|---|---|---|---|
| Lichens | Lecanoromycetes | Lecanorales | *Cladonia uncialis* |
| | | | *Cladonia amaurocraea* |
| | | | *Cladonia arbuscula* |
| | | | *Cladonia rangiferina* |
| | | | *Cladonia stygia* |
| | | | *Cladonia stellaris* |
| | | | *Cetraria islandica* |
| | | | *Cetrarialla delisei* |
| | | | *Flavocetraria cucullata* |
| | | | *Flavocetraria nivalis* |
| | | | *Alectoria ochroleuca* |
| | | | *Stereocaulon vesuvianum* |
| | | | *Stereocaulon* cf.*grande* |
| | | Peltigerales | *Nephroma arcticum* |
| | | | *Peltigera aphthosa* |
| | | | *Solorina crocea* |
| Liverworts | Jungermanniopsida | Jungermanniales | *Nardia scalaris* |
| | | | *Barbilophozia atlantica* |
| | | | *Barbilophozia floerkii* |
| | | | *Lophozia lycopodioides* |
| | | Ptilidiales | *Ptilidium ciliare* |
| | | Pelliales | *Pellia neesiana* |
| | Marchantiopsida | Marchantiales | *Marchantia alpestris* |
| Mosses | Bryopsida | | *Cinclidium stygium* |
| | | | *Dicranum montanum* |
| | | | *Dicranum fuscescens* |
| | | | *Racomitrium microcarpon* |
| | | | *Racomitrium fasciculare* |
| | | | *Racomitrium lanuginosum* |
| | | | *Hylocomium splendens* |
| | | | *Pleurozium schreberi* |
| | | | *Rhytidium rugosum* |
| | | | *Tomenthypnum nitens* |
| | | | *Aulacomnium palustre* |
| | | | *Aulacomnium turgidum* |

Continued

| Clade | Class | Order | Species |
|---|---|---|---|
| Mosses | Bryopsida | | *Paludella squarrosa* |
| | Polytrichopsida | | *Polytrichastrum sexangulare* |
| | | | *Polytrichum strictum* |
| | | | *Polytrichum commune* |
| | Sphagnopsida | | *Sphagnum balticum* |
| | | | *Sphagnum fuscum* |
| | | | *Sphagnum riparium* |
| | | | *Sphagnum teres* |
| Lycophytes | | | *Diphasiastrum complanatum* |
| | | | *Lycopodium annotinum* |
| Monilophytes | | | *Equisetum sylvaticum* |
| | | | *Gymnocarpium dryopteris* |
| | | | *Matteuccia struthiopteris* |
| Conifers | | | *Juniperus communis* |
| | | | *Picea* cf. *obovata* x *abies* |
| | | | *Pinus sylvestris* |
| Eudicots | | | *Achillea millefolium* |
| | | | *Astragalus frigidus* |
| | | | *Betula nana** |
| | | | *Betula pubescens* |
| | | | *Cornus suecica* |
| | | | *Empetrum nigrum** |
| | | | *Epilobium angustifolium* |
| | | | *Filipendula ulmaria* |
| | | | *Populus tremula* |
| | | | *Ribes spicatum* |
| | | | *Rumex obtusifolius* |
| | | | *Salix myrsinites* |
| | | | *Tanacetum vulgare* |
| | | | *Trollius europaeus* |
| | | | *Alnus incana* |
| | | | *Andromeda polifolia** |
| | | | *Angelica sylvestris* |
| | | | *Anthriscus sylvestris* |
| | | | *Arctostaphylos alpinus* |
| | | | *Astragalus alpinus* |
| | | | *Bartsia alpina* |
| | | | *Bistorta vivipara* |
| | | | *Caltha palustris* |
| | | | *Cassiope tetragona* |
| | | | *Dryas octopetala* |

Continued

| Clade | Class | Order | Species |
|---|---|---|---|
| Eudicots | | | *Geranium sylvaticum* |
| | | | *Lathyrus pratensis* |
| | | | *Orthilia secunda* |
| | | | *Pedicularis hirsuta* |
| | | | *Pedicularis lapponica* |
| | | | *Pedicularis sceptrum-carolinum* |
| | | | *Rhodiola rosea* |
| | | | *Rhododendron lapponicum* |
| | | | *Rubus chamaemorus** |
| | | | *Rubus saxatilis* |
| | | | *Salix herbacea* |
| | | | *Salix lapponum* |
| | | | *Salix reticulata* |
| | | | *Solidago virgaurea* |
| | | | *Sorbus aucuparia* |
| | | | *Trifolium pratense* |
| | | | *Vaccinium myrtillus* |
| | | | *Vaccinium uliginosum** |
| | | | *Vaccinium vitis-idaea* |
| | | | *Veronica alpina* |
| | | | *Vicia cracca* |
| Monocots | | | *Calamagrostis lapponica* |
| | | | *Carex rostrata* |
| | | | *Juncus arcticus* |
| | | | *Carex capitata* |
| | | | *Carex saxatilis* |
| | | | *Carex vaginata* |
| | | | *Deschampsia cespitosa* |
| | | | *Elytrigia repens* |
| | | | *Eriophorum angustifolium* |
| | | | *Juncus trifidus* |
| | | | *Luzula multiflora* |
| | | | *Phleum alpinum* |
| | | | *Eriophorum vaginatum* |

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### Appendix S2. Early versus late RE in lichens and mosses

Early RE of both N and P in mosses was always higher compared to late RE (Table 1), which even took on negative values (Fig. 1). These might indicate translocation of nutrients into brown moss tissue. Based on upward and downward movement of $^{14}C$ in the moss shoot, Hakala & Sewón (1992) suggested that senescent moss parts function as an energy store. Early RE in lichens was significantly higher for $RE_{N\%}$ while $RE_{Nsr}$ showed a trend for the same pattern. Early versus late $RE_P$ was not significantly different for lichens.

**Table 1.** Early versus late $RE_{N\%}$, $RE_{Nsr}$, $RE_{P\%}$ and $RE_{Psr}$ of mosses and lichens ($n$ = 15; the outliers late RE of *Cetraria islandica* and early RE of *Stereocaulon* cf. *grande* were excluded). Significant *P*-values are marked with bold letters

| Clade | Source | d.f. | *F* | *P* |
|---|---|---|---|---|
| Moss | $RE_{N\%}$ | 1 | 18.24 | **< 0.001** |
| | $RE_{Nsr}$ | 1 | 15.55 | **0.001** |
| | $RE_{P\%}$ | 1 | 6.83 | **0.014** |
| | $RE_{Psr}$ | 1 | 7.89 | **0.010*** |
| Lichen | $RE_{N\%}$ | 1 | 25.73 | **< 0.001** |
| | $RE_{Nsr}$ | 1 | 3.61 | 0.068 |
| | $RE_{P\%}$ | 1 | 0.16 | 0.69 |
| | $RE_{Psr}$ | 1 | 0.11 | 0.74 |

* Ranked

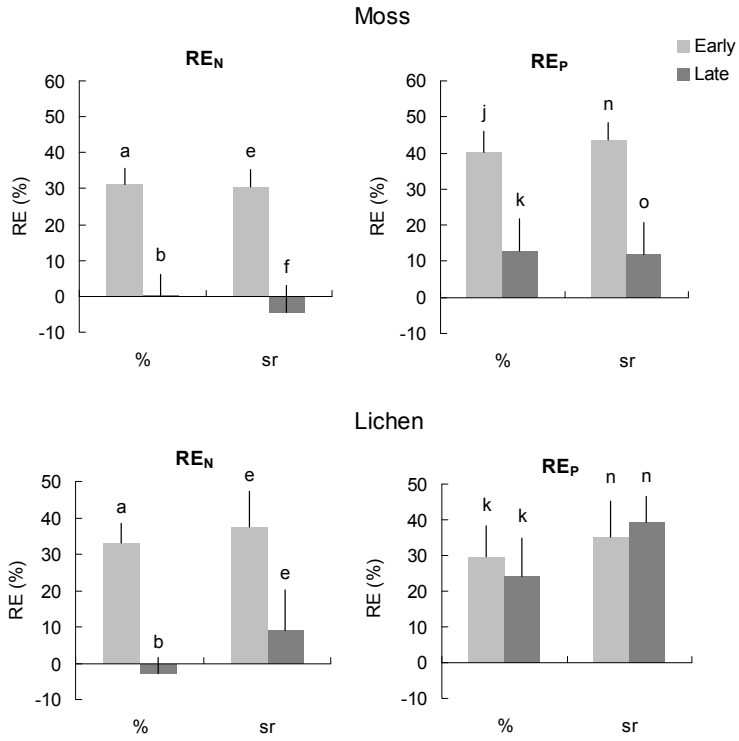


**Fig. 1.** Early versus late $RE_{N\%}$, $RE_{Nsr}$, $RE_{P\%}$ and $RE_{Psr}$ for mosses and lichens (Tukey, $n$ = 13-15).

Independent of time, non-$N_2$-fixing lichens showed significantly higher $RE_P$ (40-50%) compared to $N_2$-fixing lichens (3-12%) while the interaction of time x $N_2$-fixation was not significant (Table 2). Possible explanations for this finding are given in the Discussion (see main manuscript).

**Table 2.** Comparison of $N_2$-fixation and time of resorption on $RE_{P\%}$ and $RE_{Psr}$ of lichens ($n$ = 15; the outliers late RE of *Cetraria islandica* and early RE of *Stereocaulon* cf. *grande* were excluded). Significant $P$-values are marked with bold letters

| Variable | Source | d.f. | $F$ | $P$ |
|---|---|---|---|---|
| $RE_{P\%}$ | $N_2$-fixation | 1 | 5.78 | **0.024** |
| | Time | 1 | 0.23 | 0.63 |
| | $N_2$-fixation x time | 1 | 0.39 | 0.54 |
| $RE_{Psr}$ | $N_2$-fixation | 1 | 8.71 | **0.007** |
| | Time | 1 | 0.67 | 0.42 |
| | $N_2$-fixation x time | 1 | 0.51 | 0.48 |

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**Appendix S3. Prediction of cellulose content inferred from infrared spectra**

**Table 1.** Calibration and prediction of cellulose [%] from infrared spectra (PLS-R, $n$ = 14)

| | Cellulose |
|---|---|
| N | 14 |
| No. of PCs* | 6 |
| $R^2_{Cal.}$† | 0.98 |
| $R^2_{Pred.}$ | 0.91 |
| $RMSE_{Cal.}$‡ | 0.95 |
| $RMSE_{Pred.}$ | 2.02 |
| $Slope_{Cal.}$ | 0.98 |
| $Slope_{Pred.}$ | 0.99 |
| $Intercept_{Cal.}$ | 0.41 |
| $Intercept_{Pred.}$ | 0.08 |

* PC: Principal component

† Cal.or Pred.: Calibration or prediction

‡ RMSE: Root mean square error

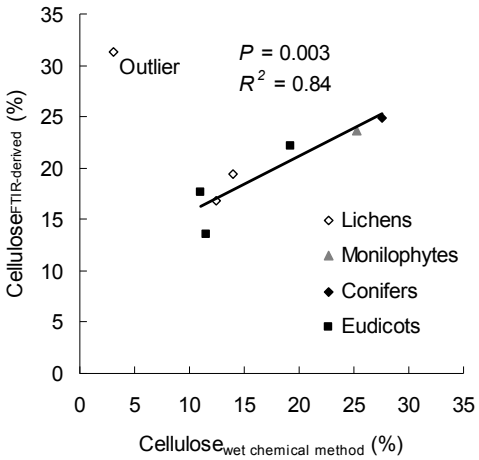


**Fig. 1.** FTIR-derived versus wet chemical measurements cellulose ($n$ = 7; the outlier was excluded).

**Appendix S4. Comparison of RE calibration approaches**

Although the pattern for most calibration methods seemed to be similar since none of the interaction terms was significant (Table 1), variation was especially high in $RE_{NCa}$ (Fig. 1). The use of $RE_{NCa}$ for mosses is problematic since Ca is known to either accumulate in old moss tissue (Vitt & Pakarinen 1987), move about mosses (Wells & Brown 1996) or even show no differences to slight decreases in young versus older tissue (Malmer 1993). It therefore provides an unreliable basis in contrast to this method used for vascular plants (Soudzilovskaia *et al.* 2007). Furthermore, in lichens, Ca may occur in trapped particles or as Ca oxalate but is also bound extracellularly (Brown 1987). As older material decomposes, trapped material or Ca oxalate might be lost from the tissue or decomposition of material might create additional exchange sites by increasing the tissue surface. Thus, it seems unsure whether Ca would provide a safe basis for RE.

**Table 1.** Comparison of measurement type of $RE_N$ at clade, class and order level (ranked; $n$ = 3-20)

| Taxonomical level | Source | d.f. | *F* | *P* |
|---|---|---|---|---|
| Clade | Clade | 2 | 8.94 | < 0.001 |
| | Method | 2 | 0.01 | 0.99 |
| | Clade x method | 4 | 0.39 | 0.82 |
| Class | Class | 2 | 6.84 | 0.002 |
| | Method | 2 | 0.30 | 0.74 |
| | Class x method | 4 | 0.81 | 0.53 |
| Order | Order | 1 | 1.22 | 0.28 |
| | Method | 2 | 0.64 | 0.54 |
| | Order x method | 2 | 1.77 | 0.19 |

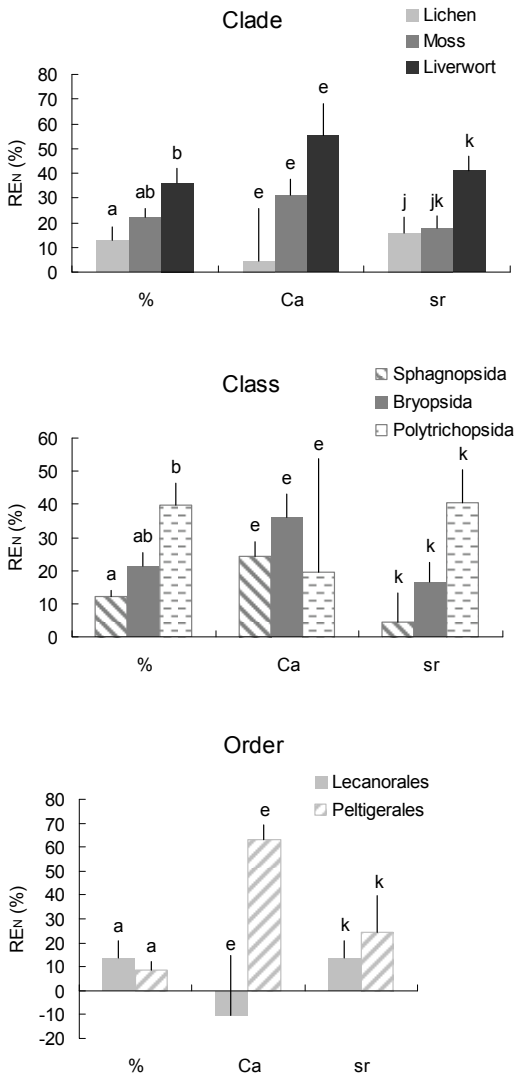


**Fig. 1.** $RE_{N\%}$, $RE_{NCa}$ and $RE_{Nsr}$ across and within clades. Different letters indicate significance at $P < 0.05$ (Tukey, $n = 3$-20).

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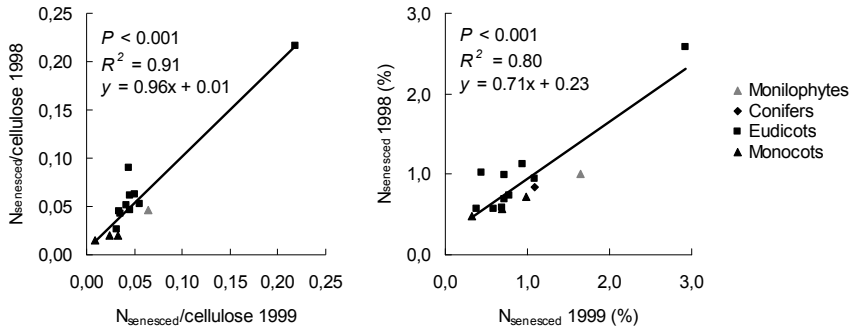
### Appendix S5. Vascular plant [$N_{senesced}$] and [$N_{senesced}$]/[cellulose] of adjacent years

Comparison of [$N_{senesced}$] and [$N_{senesced}$]/[cellulose] of adjacent years (1998 versus 1999, n=15), based on vascular plant data from Quested *et al.* (2003).

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