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FROM LEAF TO LITTER

nutrient resorption in a changing environment

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FROM LEAF TO LITTER

nutrient resorption in a changing environment

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de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
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door

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geboren te Wormerveer

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voor en na

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General introduction

The ecological importance of nutrient resorption from senescing leaves

Ecosystems are complex structures where abiotic conditions and biota interact. The potential presence of a species is determined by the combination of abiotic conditions and the biota already present. However, biota also alter their environment, with the emergence of a high oxygen concentration in the atmosphere being one of the most important biotic driven changes of abiotic conditions in history.

Plants play an important role in ecosystems, because they are the primary producers and they strongly control nutrient cycles, especially those of N and P. A large part of the available N and P in the ecosystem is organically bound in plants, as organisms have a high demand of N and P to produce various components, like proteins, energy carriers, genetic material and phospholipids. These nutrients may be returned to the soil through exudation, leaching or turnover of dead material. A strategy to minimise nutrient losses through litter is to resorb these nutrients during tissue senescence, thus producing litter with low nutrient concentrations. Moreover, the slow turnover rate of litter with low nutritional value slows down nutrient cycling, and thus leads to a positive feedback between plant species dominance and nutrient availability (Chapin 1993, Aerts 1999).

Plant growth in natural terrestrial ecosystems is mostly N-limited, although P-limitation also occurs frequently (Chapin 1980). Therefore, resorption of N and P from senescing tissue is of great adaptive significance, because the resorbed nutrients are directly available for further use (e.g. seed filling, bud growth, storage), making a species less dependent on current nutrient uptake (Aerts and Chapin 2000). In spring, remobilisation of nutrients from storage organs can lead to (competitive) early regrowth of foliage, even before the start of nutrient uptake from the soil (Thornton and Millard 1993, Millard 1996, Bausenwein *et al.* 2001). As a considerable part of the total plant N pool is allocated to leaves, remobilisation of N from these plant organs contributes significantly to the annual N economy of plants (Aerts and Chapin 2000).

Plants adapted to sites with low nutrient availability seem to lose less nitrogen than species adapted to more fertile sites (Vázquez de Aldana *et al.* 1996). It has therefore often been suggested that species from low-nutrient habitats have higher nutrient resorption efficiencies (percentage of a nutrient withdrawn from mature leaves before leaf abscission). However, the evidence available so far does not support this contention: high nutrient resorption efficiency is characteristic of all perennial growth-forms and is not very responsive to changes in nutrient supply (Aerts 1996). This implies that although nutrient resorption is an important nutrient conservation mechanism, it does not explain the distribution of growth-forms over habitats differing in soil fertility.

Leaf litter is an important annual litter input in the soil and the process of N and P resorption from senescing leaves largely determines the actual N and P input through leaf litter. This emphasizes the importance of leaf nutrient resorption in the N and P cycles, because the vegetation retains a considerable part of N and P that otherwise would have been returned to the soil through leaf shed. A certain amount of N and P is not withdrawn from the litter, and the final concentration varies substantially among species, and is also determined by external environmental conditions (Pugnaire and Chapin 1993, Killingbeck 1996, Vitousek 1998, Aerts *et al.* 1999). However, there is still much debate on how leaf nutrient resorption is controlled by external environmental factors.

The aim of this thesis was therefore to study the response and variation in leaf nutrient resorption during senescence to the external (abiotic) factors N availability and CO₂, and intrinsic (biotic) factors that are related to species-specificity and growth form.

Biochemistry of resorption: the colourful phase of leaf senescence

Plant leaves enter the phase of senescence at the end of their life span, which involves many active processes that aid the retention of valuable nutrients like N and P. Efficient resorption of N from senescing leaves co-occurs with a marked de-greening (Thomas *et al.* 2002). This is due to the breakdown of photodestructive chlorophyll to colourless breakdown products, which unmasks the partially retained carotenoids and gives senescent leaves their typical yellow colour (Thomas 1997, Matile *et al.* 1999). The highly regulated chlorophyll breakdown and detoxification is necessary to gain access to the large pool of N-containing proteins associated with chlorophyll. Subsequently, proteins are hydrolysed and modified to N-rich amino acids before they are loaded onto the phloem (Feller and Fischer 1994). In the meanwhile, P containing DNA, RNA and phospholipids are also broken down (Matile 1992, Matile 1997). The energy required for these activities is supplied by the relatively unstable, but still active photosynthetic apparatus (Hoch *et al.* 2001). As excess light is particularly harmful at this stage, some species turn their leaves deeply red with anthocyanins as a photoprotective mechanism (Feild *et al.* 2001, Hoch *et al.* 2001). When finally the intercellular organisation and turgor collapses, phenol oxidases from the plastids can contact the phenolics that had been stored in the vacuole, leading to the formation of the brown melanin (Matile 2000). At this stage, hydrolases, which had been accumulating during the late stage of leaf senescence, are also released from the vacuole. Their function remains illusive and is not likely to be related to nutrient conservation (Matile 1997). To assure the ordered dehiscence of the old leaf, many species also form an abscission layer during senescence.

Despite the clearly beneficial aspects of nutrient resorption for the plant, the nutrient pool from leaves is never resorbed totally (Killingbeck 1996). The resorption machinery itself consists of proteins, and is logically not able to dismantle itself without direct loss of function. The same line of thinking applies to the phospholipids from membranes, as there is probably a minimal amount of phospholipids necessary for a cell to remain intact. In addition, the colourless N containing chlorophyll catabolites, which had been dumped in the vacuole, also contribute to the N in litter. As a result, average resorption efficiency of N and P is only about 50% \pm 20 (SD) (based mostly on shrubs and trees) during leaf senescence (Aerts 1996).

Parameters describing leaf nutrient resorption

A commonly used parameter to quantify nutrient resorption is resorption efficiency. This parameter describes the percentage of the nutrient pool withdrawn from the foliage before leaf abscission (or functional disconnection in leaves that remain attached to the plant), and is determined by measuring the nutrient pools of mature and abscised leaves. The nutrient pool is usually expressed on the basis of leaf mass or leaf area.

Various authors have recognised that using mass basis causes an underestimation of resorption efficiency because of mass resorption during senescence (*see references in both* Oland 1963 and Killingbeck 1984). This underestimation is intrinsic to measuring mass based resorption efficiency, because nutrients themselves contribute to mass, and in addition, also starch and other leaf components are resorbed. Nevertheless, mass based resorption efficiency data are still presented in research papers, with sometimes even negative values (Knops *et al.* 1997, Cordell *et al.* 2001). Woodwell (1974) concluded that due to seasonal variation in leaf mass 'the pattern of change in nutrient content through the season would also be distorted, of course', and suggested to use leaf area basis instead. This basis is now commonly used, but also in this case the assumption is often made that very small or no changes in leaf area occur during senescence (Shaver and Melillo 1984, Chapin and Van Cleve 1996). However, leaf area should not be considered stable either during senescence, as shrinkage can take place in several plant species (Trémolières *et al.* 1999, Lin and Wang 2001). These changes in the measurement basis have probably led to underestimation of nutrient resorption efficiency. This leads us to the first question of this thesis, which will be answered in Chapter 2:

How much is mass resorption or leaf shrinkage contributing to underestimation of nutrient resorption efficiency?

Another parameter to quantify leaf nutrient resorption is resorption proficiency: the level to which a plant has reduced an element in its senescing leaves (Killingbeck 1996). Species with high

nutrient resorption proficiency thus show low nutrient litter levels. The physiological constraints mentioned above are probably also determining the ultimate resorption proficiency: the minimum level to which a plant can potentially reduce its nutrients (Killingbeck 1996). This level is about 3 mg/g DW for N, and between 0.7 and 1 mg/g DW for P (Killingbeck 1996, Aerts and Chapin 2000, Côté *et al.* 2002). However, this level is not always attained. Killingbeck (1996) therefore also introduced for deciduous and evergreen woody perennials litter N and P levels that define complete, intermediate and incomplete resorption, to be able to distinguish between potential and realised resorption. Some external factors that can directly lead to incomplete leaf nutrient resorption are for instance frost, which prematurely arrests the resorption process (Norby *et al.* 2000), and strong wind, which can prematurely detach leaves from the plant (Oland 1963, Killingbeck 1988). These factors may contribute to the variation in nutrient resorption proficiency among different years and regions. Other external factors that correlate to the level of N and P resorption proficiency are latitude (Berg *et al.* 1995), N and P availability (Shaver and Melillo 1984, Pugnaire and Chapin 1993, Kemp *et al.* 1994, Vitousek 1998, Eckstein *et al.* 1999) and temperature (Berg *et al.* 1995, Nordell and Karlsson 1995). A certain part of the variation in N and P resorption proficiency might additionally be caused by intrinsic growth form differences. Plant species harbouring nitrogen-fixing symbionts are for instance a clearly distinct group, which show lower N resorption proficiency compared to other species (Killingbeck 1996). This might be related to the higher N availability within the symbiotic species.

Thus, a higher order classification of patterns in nutrient resorption that is based on differences among growth forms could simplify the description of nutrient cycles at the ecosystem level. If this would work, information at the ecosystem level can then be derived from the relative contribution of several growth forms to total plant mass. Of course, this would not be required, if gathering data of each species under all possible environmental conditions was not limited by practical constraints. However, a higher order classification can only be useful if a growth form ranking is consistent and robust against external variation. Testing the prerequisites for using growth-form based patterns in N and P resorption proficiency was the goal set out in Chapter 3, answering the next question:

Does growth form classification of N and P resorption proficiencies yield a consistent pattern that is robust against environmental and geographical variation?

Leaf nutrient resorption in a changing environment

Since the industrial revolution and the explosive increase of human population have such a large impact on the environment, the effects on natural communities have become a source of concern. Two undisputable and clear global changes are the increase of atmospheric CO₂ concentration and the increase in N availability for plants.

Burning fossil fuel to meet the large energy demand of the ever growing human population has caused a rapid and unprecedented increase of CO₂ concentration in the atmosphere, as can be observed from ice core records (Vitousek 1994). The pre-industrial CO₂ level has been reasonably steady for thousand years between 180 and 270 vpm (volume parts per million), and has rapidly increased up to 375 vpm since 1800, and is expected to reach a level of 700 vpm at the end of this century (IPCC 2001). This rise in atmospheric CO₂ concentration and other greenhouse gasses is predicted to raise mean global temperatures by 1.0-3.5 °C in the next 50-100 years with above-average increases at high-latitude and high-altitude sites (IPCC 2001).

Besides releasing huge amounts of C into the atmosphere, the burning of fossil fuels has also led to increased (bio) availability of N, although the production and use of industrial N fertiliser is the major contributor to the changes in the global N cycle. We have nowadays reached the point where more N is fixed annually by human-driven than by natural processes (Vitousek 1994). An additional indirect increase in N availability may result from the expected higher global temperatures, as this increases soil net N mineralisation rates (Rustad *et al.* 2001).

Plant growth can directly benefit from increases in N availability and CO₂ concentration, though responses are species specific, and responses to CO₂ are often dependent of N availability (Stitt and Krapp 1999, Poorter and Pérez-Soba 2001). In a changed environment, the competitive strength among plant species might change, leading to a shift in community composition, and therefore ecosystem properties.

Nutrient cycles are important ecosystem attributes, where leaf nutrient resorption from senescing leaves is a key process determining the fate of leaf nutrients: to recycle in the plant, or to return to the soil through litter. Therefore, influencing this balance with increased environmental N availability affects the N loading of ecosystems, but this also influences the P cycling (Aerts *et al.* 2001). First, the quality of litter changes when species composition shifts in response to the changed environment, as different species produce different litters. Second, direct responses of mature tissue might influence the quantity and/or quality of the shed litter. Third, as resorption is a physiological process, environmental factors can have a direct influence on the final quality during the production of the litter. The effect of increased N availability on N and P resorption is the topic of Chapter 4, where the following question will be answered:

How does increased N availability affect N and P resorption from leaves?

The remobilised leaf N contributes significantly to the N economy in plants (Aerts and Chapin 2000). The contribution of leaf N resorption may become smaller in an environment with increased N availability, due to a trade-off with other energy consuming processes. However, such a decrease in the contribution of leaf N resorption would probably not result from a decrease in N resorption efficiency (Aerts 1996).

Elevated CO₂ concentration on the other hand also affects plant N economy, though this is dependent of N availability and species (Stitt and Krapp 1999, Poorter and Pérez-Soba 2001). The contribution of leaf N resorption to the N economy of plants under elevated CO₂ and increased N availability is however not clear and hard to predict, as it is the result of effects on both N allocation to leaves and N resorption efficiency. Effects of increased N availability on N allocation (Aerts and De Caluwe 1989, Aerts *et al.* 1992a, Makino and Mae 1999), as well as N resorption efficiency (Aerts 1996) have been ambiguous and no clear patterns emerged. Enhanced CO₂ has occasionally been observed to decrease N allocation to leaves (Makino *et al.* 1997, Temperton *et al.* 2003), dependent on fertilization (Zak *et al.* 2000), or have no effect at all (Roumet *et al.* 1996, Harmens *et al.* 2001), whereas it probably increases N resorption efficiency (Norby *et al.* 2001). To gain insight in these matters, an experiment was performed in the greenhouse with the purple moor grass *Molinia caerulea*. The experiment is described in Chapter 5, and addresses the following question:

*How does the combination of high N availability and elevated CO₂ affect the late seasonal N economy in *Molinia caerulea*?*

Nutrient resorption in plants species form peatlands

The research described in this thesis was conducted on plant species from peatlands. Peatlands cover a large part of the northern hemisphere (especially at high-latitude sites), and plant growth in those ecosystems tends to be severely constrained by nutrient availability (Gorham 1991). Resorption of nutrients may therefore play an important role to minimise nutrient losses in the associated species.

The nutrient-limited growth conditions in these ecosystems make them particularly responsive to increasing N deposition. In addition, high latitude sites are expected to undergo above-average increases in global warming, which lead to an additional increase in N availability (Houghton 2001, Rustad *et al.* 2001). Understanding the plant-mediated processes in these ecosystems and the effects of environmental change may help to evaluate, and possibly predict some of the responses of ecosystems to global environmental change.

THESIS OUTLINE

- The fact that current measures of nutrient resorption efficiency lead to a substantial underestimation of real resorption efficiency is elaborated on in **Chapter 2**.
- In **Chapter 3** it is shown that Plant Functional Type based patterns in nutrient resorption proficiency are consistent along an environmental gradient.

- **Chapter 4** describes the effects of 4 years of increased N availability on the N and P resorption of six sub-arctic peatbog species, where methodology was also an aspect of consideration.
- Resorption from senescing leaves is placed in a whole plant perspective in **Chapter 5**, which describes the effects of increased N availability and elevated CO₂ concentration on the late seasonal N dynamics of the purple moor grass *Molinia caerulea*.
- Finally, in **Chapter 6** the results of the previous chapters are discussed in relation to the thesis aim and some new ideas are presented.

CHAPTER 2

Current measures of nutrient resorption efficiency lead to a substantial underestimation of real resorption efficiency: facts and solutions

with Sylvia Toet and Rien Aerts

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Nutrient resorption is an important process during leaf senescence, which helps plants to minimize nutrient losses. To quantify nutrient resorption, the parameter resorption efficiency is commonly used. This parameter describes the percentage of the nutrient pool withdrawn before leaf abscission. The nutrient pool is generally expressed on the basis of leaf mass or leaf area, assuming that these bases do not change during senescence. In this paper we firstly present a mathematical formula describing the effect of change in measurement basis on the difference between the real resorption efficiency (RRE) value and the measured resorption efficiency (MRE). This formula shows that even moderate senescence-related changes in a measurement basis can lead to considerable underestimation of RRE. Secondly, to estimate the general change in measurement basis we quantified leaf mass loss and leaf shrinkage during senescence from literature data. These data shows that mass loss percentages can be as high as 40%, and leaf shrinkage can be up to 20%. This level of change in basis seriously compromises the MRE when not corrected for. Using our formula and the reported average literature values of changes in leaf mass (21%) and leaf shrinkage (11%) during senescence, we calculated that the average RRE for nitrogen and phosphorous of terrestrial plants is 6% (leaf area) to 10% (leaf mass) higher than the 50%, respectively 52% as reported by Aerts (1996). This implies that nutrient resorption from senescing leaves is even more important for nutrient retention in terrestrial plants than thought so far. We advocate that preselecting leaves and monitoring the measurement basis throughout the duration of the experiment should minimize the difference between MRE and RRE.

Resorption efficiency, a troublesome parameter

Nutrient resorption during senescence is an important physiological process for nutrient retention in terrestrial plants (Aerts 1996). Especially nitrogen and phosphorus are largely withdrawn from senescing leaves before abscission, and used for new growth or stored in vegetative tissue until the next growing season. Therefore, plants are less dependent on current nutrient uptake from the soil, which can have important ecological consequences for e.g. competition, and plant fitness. A commonly used parameter to quantify nutrient resorption is resorption efficiency. This parameter describes the percentage of the nutrient pool withdrawn from the foliage before leaf abscission (or functional disconnection in leaves that remain attached to the plant), and is determined by measuring the nutrient pools of mature and abscised leaves. The nutrient pool is expressed on the basis of leaf mass or leaf area, but also unit leaf, leaf length, calcium concentration, and canopy area have been used. It is implicitly assumed that these bases do not change during senescence.

Various authors have recognised that using mass basis causes an underestimation of resorption efficiency because of mass resorption during senescence (*see references in both* Oland 1963, and Killingbeck 1984). This underestimation is intrinsic to measuring mass based resorption efficiency because nutrients themselves contribute to mass. Nevertheless, mass based resorption efficiency data are still presented in research papers, with sometimes even negative values (Knops *et al.* 1997, Cordell *et al.* 2001). Woodwell (1974) concluded that due to seasonal variation in leaf mass 'the pattern of change in nutrient content through the season would also be distorted, of course', and suggested to use leaf area basis instead. This basis is now commonly used, but also in this case the assumption is often made that very small or no changes in leaf area occur during senescence (Shaver and Melillo 1984, Chapin and Van Cleve 1996). However, leaf area should not be considered to be stable during senescence as shrinkage can take place in several plant species. The leaves of many herbs dry out and shrink, and many grasses curl before leaf abscission. Especially in grasses shrinkage can be observed on the transition from dead to green as they senesce from the tip down. Shrinkage is also an intrinsic physical problem to the measurement of abscised leaf area, because leaf cells cannot remain turgid when functionally disconnected or after collapse of intercellular organisation. As a consequence, the area of an abscised leaf is different from the leaf area when mature, although, compared to species with 'softer' leaves, this will be less apparent for sclerophyllous species due to the rigidity of their leaves. This will, analogous to leaf mass loss, lead to an underestimation of (area based) resorption efficiency.

In this paper we report leaf mass loss and leaf shrinkage data from literature, and quantify with a descriptive mathematical formula how these changes in the measurement basis (e.g. leaf shrinkage or mass loss) during senescence contribute to errors when measuring resorption efficiency.

Contribution of measurement basis to error in resorption efficiency

In this section we derive the formula describing the relationship between the **real** resorption efficiency (RRE) and the **measured** resorption efficiency (MRE) as influenced by the change in the measurement basis (expressed as the **fractional change** in the **measurement basis**; FCMB). The derivation of this formula is shown using nitrogen (N) as nutrient and leaf area as measurement basis, but the formula is applicable to any combination of nutrient and measurement basis.

The amount of N per unit leaf area can be described as:

$$[N] = \frac{N}{\text{area}} \quad (1)$$

After measuring the N contents of mature ($[N]_{\text{green}}$) and abscised ($[N]_{\text{abscised}}$) leaves the MRE can be calculated with the following formula:

$$\text{MRE} = 100\% * \left(1 - \frac{[N]_{\text{abscised}}}{[N]_{\text{green}}}\right) \quad (2)$$

MRE is an approximation of the RRE of a single leaf. The equation of RRE is therefore analogous to (2):

$$RRE = 100\% * \left(1 - \frac{N_{\text{abscised}}}{N_{\text{green}}}\right) \quad (3)$$

Here N_{green} and N_{abscised} describe the true N pool in a single green leaf before and after abscission. The RRE is thus the real value of resorption efficiency, and in contrast to MRE this parameter is bias free. Area based MRE is affected by leaf shrinkage during senescence, and to correct for this effect a new parameter is introduced: Fractional Change of Measurement Basis (for leaf shrinkage: FCMB_{area}). This parameter describes the fraction of change in leaf area during senescence, which theoretically can have values between 1 (meaning the leaf totally shrunk) and 0 (meaning no shrinkage occurred):

$$RRE = 100\% * \left(1 - \frac{N_{\text{abscised}}}{N_{\text{green}}}\right) \quad (4)$$

This parameter can be incorporated into the equation for MRE (2) by first rewriting equations (3) and (4) to express N content of an abscised leaf with parameters of a green leaf:

$$[N]_{\text{abscised}} = \frac{N_{\text{green}} * (1 - RRE/100)}{\text{area}_{\text{green}} * (1 - FCMB_{\text{area}})} \quad (5)$$

and substituting this into equation (2):

$$MRE = 100\% * \left(1 - \frac{N_{\text{green}} * (1 - RRE/100)}{\text{area}_{\text{green}} * (1 - FCMB_{\text{area}})} * \frac{\text{area}_{\text{green}}}{N_{\text{green}}}\right) \quad (6)$$

which equals:

$$MRE = 100\% * \left(1 - \frac{N_{\text{green}} * (1 - RRE/100)}{\text{area}_{\text{green}} * (1 - FCMB_{\text{area}})} * \frac{\text{area}_{\text{green}}}{N_{\text{green}}}\right) \quad (7)$$

which also equals:

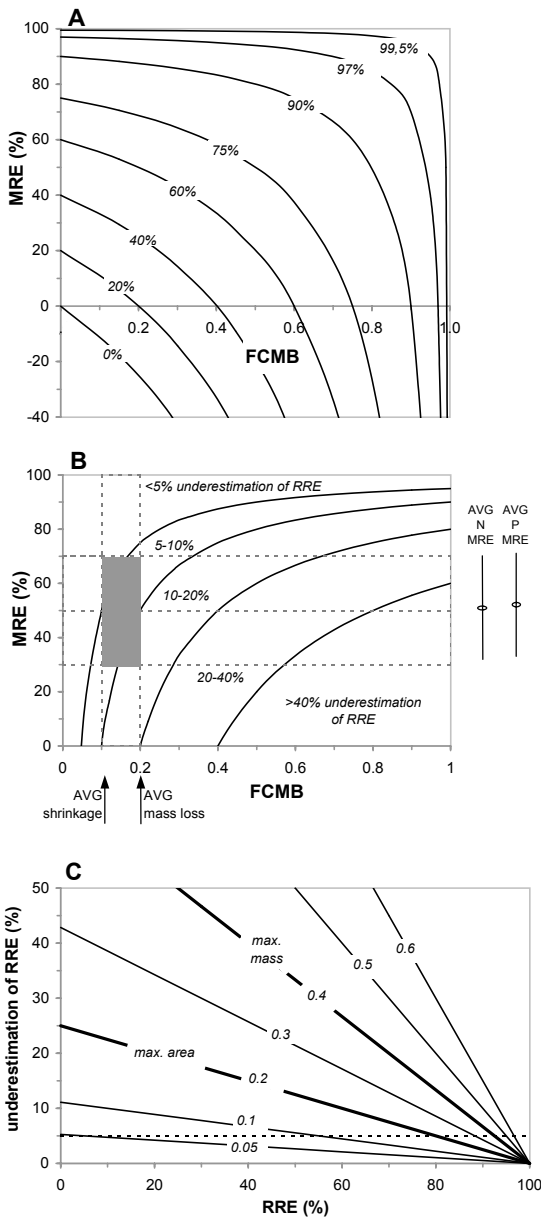
$$MRE = 100\% * \left(1 - \frac{N_{\text{green}} * (1 - RRE/100)}{\text{area}_{\text{green}} * (1 - FCMB_{\text{area}})} * \frac{\text{area}_{\text{green}}}{N_{\text{green}}}\right) \quad (8)$$

Formulas 7 and 8 show the effect of changes in the measurement basis on the measured resorption efficiency and the dependency on the value of real resorption efficiency. This relationship is visualised in Fig. 1A.

Quantification of error in resorption efficiency due to change in measurement basis

Due to the co-dependency of MRE on FCMB and RRE, a relative small FCMB can lead to substantial differences between MRE and RRE (Fig. 1A). Only when the FCMB is 0, the MRE is the same value as the RRE. In the case that FCMB is not 0, the value of MRE declines already at small FCMB and can even become negative. At high values of RRE, the decline in MRE is only large at high FCMB values. Low values of RRE are more susceptible to FCMB than high values of RRE. For example, if the RRE is 40%, and an FCMB of 0.20 was not accounted for, an MRE of only 25% will be measured (as calculated with Eq. 7). This would lead to an underestimation of 15% incorporated in the result (see also Fig. 1B and Fig. 1C).

Using published data on mass resorption and leaf shrinkage during senescence and on leaf N and P resorption, we calculated how large the underestimation of N and P resorption is. In a literature survey on terrestrial plant species, the average MRE appeared to be 50% ± 17% (n=287) for nitrogen, and 52% ± 22% (n=226) for phosphorous (Aerts 1996). Many data included in this review were measured on mass basis, so an underestimation is unquestionably incorporated in the average MRE. However, data on the mass resorption (or area shrinkage) were not available from this source. Therefore, the size of the FCMB incorporated in these MRE values can only be hypothesised on the basis of other studies. Table 1 presents a compilation of published data on mass loss and leaf shrinkage during senescence, and it illustrates that mass loss as well as leaf shrinkage can be considerable. The change during senescence ranged from 2 – 37% for mass, and from 4 – 15 % for leaf area between several species. A drawback in most of



the mass loss data is that they were compared to leaf area, while leaf area shrinkage will evidently have caused an underestimation of mass loss during senescence. Another drawback is that due to the small amount of available data on senescence related changes in leaf area, the following calculation of underestimation in area based RRE is an educated guess.

The data presented in Table 1 yields a weighted average value of 21% (n=126 species) for mass loss during senescence and 11% (n=9 species) for leaf shrinkage during senescence. In the hypothetical case that the average resorption efficiency value is based solely on area, then the average real nitrogen resorption efficiency is 56% instead of the reported 50%, and the average real phosphorous resorption efficiency 57% instead of the reported 52%. In the other hypothetical case that the average resorption efficiency value is based solely on mass, then the average real nitrogen resorption efficiency is 61%, and the average real phosphorous resorption efficiency is 62% (calculated with equation 8). Ignoring changes in the measurement basis during senescence could thus have led to an average underestimation between 6% (area basis) and 10% (mass basis) in these nitrogen and phosphorous MRE's (the real value is not clear due to uncertainty of the relative contribution of area or mass based data). This is also comprehensibly visualised in Fig. 1B, in which the grey area also includes the standard error bars of the average nitrogen and phosphorus MRE.

Another point of interest is that leaf area shrinkage during senescence is probably larger in 'soft-leaved' herbs, compared to species with sclerophyllous leaves, like evergreens. This implies that comparisons among species with differences in leaf toughness are subject to an additional systematic error in area based resorption efficiency measurements. From the few observations in Table 1 it also

Fig. 1. Visualisation of the relationship between fractional change of the measurement basis (FCMB), measured resorption efficiency (MRE), and real resorption efficiency (RRE). A: Effect of the FCMB on the MRE value for different values of RRE. In the curves, the value of corresponding RRE is plotted. The lines were calculated using equation 7 from the text. B: Underestimation of RRE for combinations of FCMB and MRE. The range in underestimation of RRE is plotted between the curves. The curves were calculated using equations 7 and 8 from the text. The average N and P MRE \pm s.e. (Aerts 1996) are depicted on the right side of the graph. Below the x-axis the average values of leaf shrinkage and mass loss during senescence from Table 1 are depicted with arrows. The grey area shows the range in underestimation of RRE for this combination of data. C: Relationship between RRE values and the underestimation of RRE (RRE-MRE) as influenced by different values of FCMB. In the lines the value of corresponding FCMB is plotted. The bold lines represent the relationship for FCMB values based on the maximum values of mass loss, respectively area shrinkage during senescence as reported in Table 1.

seems that leaves of evergreen species shrink less than leaves of deciduous species. For example, calculation of the RRE with data from Table 1 (average evergreen FCMB_{area}: 0.05; average deciduous FCMB_{area}: 0.15) and the average MRE for deciduous (54% N, 50% P), and evergreen species (47%N, 51%P) as reported by Aerts (1996) yields values with an underestimation in both N and P RRE of deciduous and evergreen species of approximately 7%, and 3% respectively. Of course, this is speculative, but it is a potential systematic error that could lead to underestimation of the difference between MRE of deciduous and evergreen species.

In conclusion, resorption is even more important for nutrient retention than thought so far. Not half, but on average almost two third of the nitrogen and phosphorus seems to be resorbed from green leaves of terrestrial plants during senescence, which also has the ecological implication that plants have a lower need for nutrient uptake from the soil than thought so far.

Table 1. Leaf mass loss (%) and leaf area shrinkage (%) during senescence of different species as reported in several studies. In the species list of shrinkage data the leaf type of each species is shown (deciduous: D; evergreen: E)

Species	Compared to	Study
Mass loss (%)		
17 ± 4 (s.e.)	leaf area	Pugnaire and Chapin (1993)
5 ± 4 (s.e.)	leaf area	Pugnaire and Chapin (1993)
37	leaf area	Jonasson (1995)
35	leaf area	Jonasson (1989)
30 - 36	leaf area	Karlsson (1994)
25	leaf area	Gessner (2001)
9	leaf area	Knops and Koenig (1997)
13	leaf area	Knops and Koenig (1997)
3 - 5	dried leaf area	Lajtha (1987)
> 17 **	-	Knops and Koenig (1997)
> 29 **	-	Knops and Koenig (1997)
> 30 **	-	Knops and Koenig (1997)
24 ***	punched leaf area	Lal et al. (2001)
30 ***	punched leaf area	Lal et al. (2001)
14 ***	punched leaf area	Lal et al. (2001)
~35 *	leaf units	Birk and Vitousek (1986)
16	preselected leaf area	Oland (1963)
31	preselected leaf units	Trémolières et al. (1999)
30	preselected leaf units	Trémolières et al. (1999)
25	preselected leaf units	Trémolières et al. (1999)
Average: 21% (n=126)		
Leaf area shrinkage (%)		
15	preselected leaf units	Trémolières et al. (1999)
13	preselected leaf units	Trémolières et al. (1999)
11	preselected leaf units	Trémolières et al. (1999)
> 16 **	-	Knops and Koenig (1997)
> 22 **	-	Knops and Koenig (1997)
> 1	-	Knops and Koenig (1997)
8(-15to 20)	leaf units	Lin and Wang (2001)
4(-4to 13)	leaf units	Lin and Wang (2001)
6(-6to 18)	leaf units	Lin and Wang (2001)
Average: 11% (n=9)		

* Determined from the difference between resorption efficiency based on fascicle basis and mass basis, using Fig. 1C.
 ** Minimum percentage that explains the negative recorded value of phosphorus resorption efficiency, assuming this is not lower than 0% for phosphorus.
 *** Calculated as the percentage decrease in specific leaf mass from mature and senescent punched leaf discs of fixed, known area.

Good practice for measuring resorption efficiency

Using values of literature data on mass resorption and area shrinkage to estimate average FCMB, we showed that not incorporating these values could lead to a serious underestimation of RRE. However, as Table 1 shows, certain species have much higher values. So, for these species the problem is even worse. Therefore, the maximum reported values of mass resorption and leaf area shrinkage during senescence from Table 1 are depicted in Fig. 1C to show their influence on the MRE. Since mass loss during senescence can have such large values (up to 37%), ignoring changes in leaf mass basis may introduce a large error in the determination of resorption efficiency. This is also valid for leaf area shrinkage: leaf area can shrink considerably during senescence (up to 20%) and thus, ignoring changes in leaf area basis may also introduce a substantial error in the measurement of resorption efficiency. This illustrates that monitoring the FCMB during the experiment is important. Fig. 1C also shows that if an (arbitrary) underestimation of 5% is allowed, the maximum FCMB can only be as small as 0.05 (conservative indication at MRE = 0%). As leaf shrinkage can be larger than 5%, leaf area should not be used as measurement basis without monitoring the shrinkage factor (and correct for this). Therefore, the experimental set-up needs to be designed in such a way that these errors are minimised.

How should an ideal experimental set-up for measuring resorption efficiency look like? In the easiest and most ideal situation the FCMB is 0 when measuring resorption efficiency of a leaf. The measurement before and after senescence is ideally also non-destructive within the same leaf. As there is no method to do so, the available methods are intrinsically compromised in one way or another. Choosing a measurement basis with a small FCMB (and measuring it accurately) is important in this respect. The choice of the measurement basis should be dependent of the morphology of the species. Leaf area can only function as a measurement basis if the leaf morphology allows this. Needle-formed leaves require an alternative measurement basis. For species with these kind of leaves and grasses with small leaves, leaf length is a better measurement basis than leaf area. However, before using leaf length as basis, the relation between leaf length and another parameter of leaf morphology has to be looked at as variation between leaves of the same length could compromise the results. Most species exhibit a clear relationship (Cornelissen 1992), but this has to be checked for each species. Calcium has also been used as an alternative basis because it is a nutrient that is not resorbed during senescence, but as large portions of this nutrient leach out of leaves (Tukey Jr. 1970, Lambers *et al.* 1998), it is not suitable to use with field grown species. Lignin has recently been used as a basis (Quested *et al.* 2003), but the analysis is laborious and would not be useful with lignin-poor species.

Another important aspect is monitoring of the measurement basis. This can be done by preselecting mature leaves, which are to be collected when they have senesced. Preselection also minimises the chance of comparing green and abscised leaves of different cohorts. The mature leaves and leaves to be collected when dead should be comparable according to their position in the plant, leaf life history, light stratification, leaf age, leaf size, nutrient status, etc. Only then can the measured difference between the two leaf classes be attributed solely to resorption, apart from some leaching (Chapin and Moilanen 1991). This is not common practice, as measuring the nutrient concentration in random selected senesced leaves (leaves 'about to fall' or which detach 'when slightly shaking a branch') and leaf litter (collected from the ground) are often used to obtain resorption efficiency data. In these cases the comparability of the two leaf classes (mature and dead) is left to coincidence.

After preselection, the leaf area of these leaves can be monitored (e.g. with digital camera) during the experiment. An example of such an experimental set-up is the study of Trémolières *et al.* (1999). Other important guidelines and considerations on the design of resorption studies are given by (Chapin and Van Cleve 1996).

In conclusion, data on nutrient resorption efficiency are intrinsically biased. This bias can be minimised by preselecting leaves and monitoring the measurement basis of these leaves during the process of senescence.

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Plant functional types are good predictors of nitrogen resorption proficiency along environmental gradients

with Ellen Dorrepaal, Sylvia Toet and Rien Aerts

Resorption proficiency is a measure of the process of leaf nutrient resorption during senescence, and is expressed as the level to which nutrient concentrations have been reduced in litter. As nutrient resorption proficiency (NRP) varies considerably among species, we investigated (1) whether NRP of different species segregates into a predictable pattern when using the concept of Plant Functional Type (PFT), and (2) whether such pattern is consistent, and robust against large-scale environmental and geographical variation. Hereto, NRP was measured along a large scale environmental north-south gradient in European peatlands, spanning three climatically distinct regions (warm temperate, cool temperate, and sub-arctic), taking care that the species identity within PFTs differed among regions as much as possible. A consistent PFT-ranking of N and P resorption proficiency along this gradient emerged at each region: *Sphagnum* mosses had the highest N resorption proficiency, followed by graminoids, evergreens, deciduous species, and forbs. For P, the only difference was that *Sphagnum* mosses were the second most resorption proficient group. Consequently, the PFT concept is an adequate tool to predict patterns in NRP along large-scale environmental and geographical gradients. Differences in absolute values of NRP among regions are largely determined by regional climatic and management factors.

INTRODUCTION

Nitrogen and phosphorus cycles are important features of ecosystem functioning, where living plants play a key role by annually producing large amounts of leaf litter. Apart from quantity, also litter quality, in terms of N and P concentrations, is important for N and P inputs into the system.

Green leaves contain a significant amount of N and P, but not all of it enters the decomposition pathways in the soil through leaf litter production. Before leaves detach from the plant, about 50% of the N and P in green leaves is resorbed and redirected towards growing parts or storage tissues (Aerts 1996). This percentage indicates the ability of a plant to resorb nutrients, and is termed resorption efficiency. Due to resorption of nutrients from senescing leaves, soil nutrient input through leaf litter is considerably smaller than potentially available in green leaves.

The level to which a plant has reduced the nutrient level in its senescing leaves is quantified with the parameter nutrient resorption proficiency (NRP) and is measured as the nutrient level in litter (Killingbeck 1996). The level of NRP can be directly influenced by external factors like wind (Oland 1963), frost (Norby *et al.* 2000) and nutrient availability (Aerts and Chapin 2000), but NRP is also a feature that intrinsically varies among species. Some intrinsic patterns are that species harbouring N-fixing bacteria have a clearly lower N resorption proficiency (higher N levels in litter) than non-symbiotic species, and that woody evergreens have a higher P resorption proficiency (lower P levels in litter) than woody deciduous species (Killingbeck 1996).

NRP values are directly related to ecosystem functioning as they express the amount of N and P that becomes available for decomposition after leaf shed. Therefore, the NRP of the vegetation is an important parameter to incorporate in the description of nutrient cycles at the ecosystem level. However, as with many other important parameters, practical constraints limit the amount of N and P resorption proficiency data of each species under all possible environmental conditions. Identifying patterns in a higher order classification scheme may therefore be convenient. This was for instance also done for plant traits such as litter decomposability (Enríquez *et al.* 1993, Cornelissen 1996, Pérez-Harguindeguy *et al.* 2000) and nutrient use efficiency (Aerts *et al.* 1999). In this way information at the ecosystem level can be derived from the relative contribution of several functional groups of the classification scheme to total plant mass.

The concept of Plant Functional Type (PFT) is commonly used as a higher order classification. This concept is generally used to group species according to their specific traits, response to environmental variation, or their effect on ecosystem functions (Lavorel *et al.* 1997, Semanova and Van der Maarel 2000, Dormann and Woodin 2002). A frequently used PFT classification, which in fact separates species into growth forms, is based on a cluster analysis of traits like leaf longevity, specific leaf area, photosynthetic rate and fire survival, and distinguishes trees, deciduous shrubs, evergreen shrubs, sedges, grasses, forbs, *Sphagnum* mosses, non-*Sphagnum* mosses and lichens (Chapin *et al.* 1996).

Due to small but possibly ecologically relevant differences in the N and P resorption efficiency among different growth forms, the pattern in NRP may not follow the growth-form specific pattern as has been found for green leaves (Aerts 1996, Aerts *et al.* 1999). Therefore, we investigated whether NRP values cluster in a useful and predictable pattern in the PFT scheme of Chapin *et al.* (1996). We hypothesized that (1) a PFT-based ranking of both N and P resorption proficiency would yield a consistent pattern that is robust against environmental and geographical variation; and (2) *Sphagnum* mosses would have the highest resorption proficiency, followed by graminoids, woody evergreens, woody deciduous species, and forbs. To test these hypotheses, the N and P resorption proficiency was measured in litter from the five dominant PFTs (deciduous and evergreen species, grasses, forbs, and *Sphagnum* mosses) that occur in European peatlands. Samples were collected at warm temperate, cool temperate and sub-arctic sites (Köppen 1931) that differed largely in latitude, N deposition, and management practice.

MATERIALS AND METHODS

Study sites

Senesced leaf material was collected during the period of maximum leaf senescence at a number of *Sphagnum*-dominated peatlands in three geographically distinct regions. These were sub-arctic (sampled in September 2000), cool temperate (sampled in October 2000), and warm temperate regions (sampled in November 2000), with the climatic classification based on Köppen (1931). The regions are positioned in a large-scale environmental gradient in northwest Europe, with the sub-arctic region having the lowest mean annual temperature, thus the shortest growing season, and the lowest atmospheric N deposition (Table 1). Within each region the sampling locations were chosen within a radius of about 150 km to ensure similar climatic conditions and nitrogen deposition, and they included both fens and bogs.

Species selection and leaf sampling

For each distinguished PFT, four to six abundant species were selected. We avoided selecting a species that was already selected in one of the other regions, or selecting two species of the same genus in the same region, except when no other species were available. Also the range of included families was chosen as wide as possible. In this way, we ensured that we made our comparison at the PFT-level without confounding effects of species similarity. A list of all species that were sampled in the three different regions is given in Table 2.

At each site, freshly died leaves were collected following the criteria as described by Cornelissen (1996). Laminae and rhachis of compound leaves were both included when they fell off simultaneously and attached to each other. This was not the case for *Sorbus aucuparia* and only the laminae were used. For the collection of *Sphagnum* litter, *Sphagnum* sods were cut in the field and stored at 4°C until further use. Only the middle part of the *Sphagnum* shoots, which was usually light brown and with a fresh and coherent structure, was taken as litter (Aerts *et al.* 2001). The green upper and dark brown lower parts were discarded.

The dead leaves were cleaned and oven-dried at 70°C for 48h, and ground. Phosphorus concentration was measured colorimetrically (Murphy and Riley 1962) after digesting ground material in a 1:4 mixture of 37% (v/v) HCl and 65% (v/v) HNO₃ (as in Sneller *et al.* (1999)). Total N concentration was measured using a Perkin Elmer 2400 series II CHNOS/O analyzer.

Table 1. Some characteristics of the study sites

Climatic region ^a	Cfb warm temperate	Df/Cfb cool temperate	EF/Df sub-arctic
Geographical location	51° N, 5° E The Netherlands and Belgium	57°0' N, 14°3' E Southern Sweden (Småland)	68°2' N, 18°5' E Northern Sweden (Lapland)
Annual precipitation (mm)	793 ^b	618 ^d	299 ^e
Mean annual temperature (°C)	9.8 ^b	6.4 ^d	-0.5 ^f
N deposition (kg.ha ⁻¹ .yr ⁻¹)	20-40 ^c	10-20 ^c	<5 ^c
Sites	Het Guisveld De Amstelveense Poel De Grote Peel Fagne Wallone	Store Mosse Dala Mosse Kopparås mire Åkhult mire Björnekulla	Stordalen Abisko region

a Köppen (1931)

b KNMI, Climate normals Station De Bilt, 1971-2000

c Bobbink *et al.* (1998)

d SMHI, Climate normals Station Växjö, 1961-1990

e Aerts *et al.* (2001)

f Abisko Scientific Research Station, Climate normals, 1971-2000

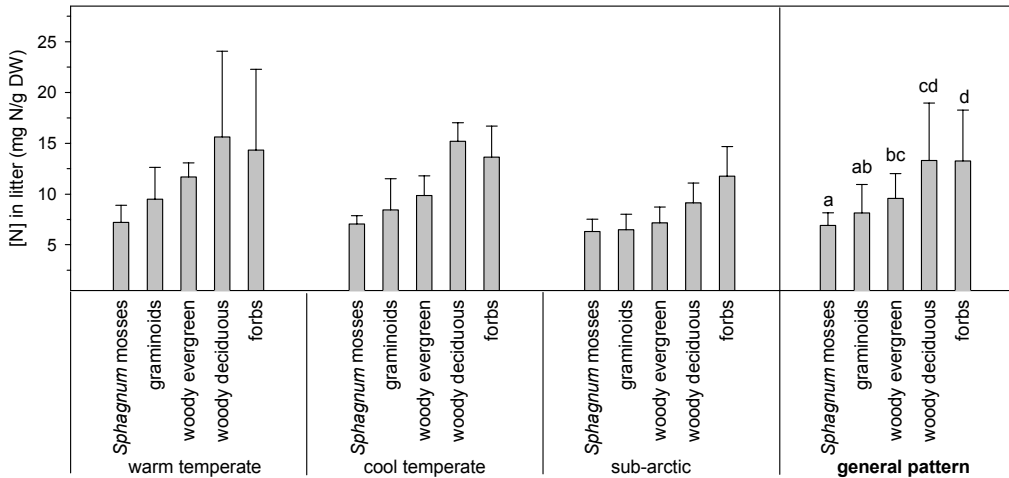


Fig. 1. Nitrogen resorption proficiency (expressed as N concentration in leaf litter) of five Plant Functional Types (PFT) sampled in peatlands from distinct regions. The value of each PFT is the average of various species (\pm SD, $n=4-6$). The 'general pattern' shows the results of the multiple comparisons on the factor PFT after the performance of a two-way Anova (region \times PFT) that did not result in an interaction term. PFTs sharing the same letter are not significantly different from each other (Tukey; $P>0.05$).

Statistical analysis

Both for N and P resorption proficiency, we conducted a two-way ANOVA with PFT and region as the main factors. Within each combination of region and PFT, the different species were treated as replicates ($n=4-6$). The litter N and P concentrations of each species were $\log(x)$ transformed before statistical testing, to make replicates normally distributed. Levene's test was used to examine the homoscedasticity of the transformed datasets, and showed that variance was homogeneous for N ($P = 0.37$), but not for P ($P = 0.001$). Nevertheless we present all statistics for P (and N) based on $\log(x)$ transformed data, because when testing the P data non-parametrically (ranked), this did not lead to a better Levene statistics ($P < 0.001$), or alter overall statistics. As we did not find interactions between the main factors in our ANOVA's we performed Post Hoc multiple comparison (Tukey) tests on the data set as a whole to unravel patterns in nutrient resorption proficiency at the Plant Functional Type and region level. Data were analysed using SPSS for Windows 10.1.0.

RESULTS

Nitrogen resorption proficiency ranged between 4.8 and 30.2 mg N/g DW, with the lowest proficiencies (highest litter N concentrations) occurring in the warm temperate region (Table 2). Phosphorus resorption proficiency ranged from 0.01 to 1.33 mg P/g DW, and the lowest proficiency also occurred in the warm temperate region.

Nitrogen resorption proficiency differed significantly among PFTs ($P < 0.001$) and among regions ($P = 0.001$) without a significant interaction ($P = 0.73$) (Fig. 1). The lack of interaction indicates that the pattern in N resorption proficiency among PFTs was comparable among the three regions like we hypothesized. The general ranking of PFTs according to their N resorption proficiency was also as hypothesized: *Sphagnum* mosses > graminoids > woody evergreens > woody deciduous species > forbs (Fig. 1). Differences among PFTs were significant with the exception of adjoining PFT groups. The highest levels of N resorption proficiency were found at the sub-arctic sites, followed by cool temperate and warm temperate sites, with differences being significant only between the sub-arctic and the temperate sites ($P < 0.007$).

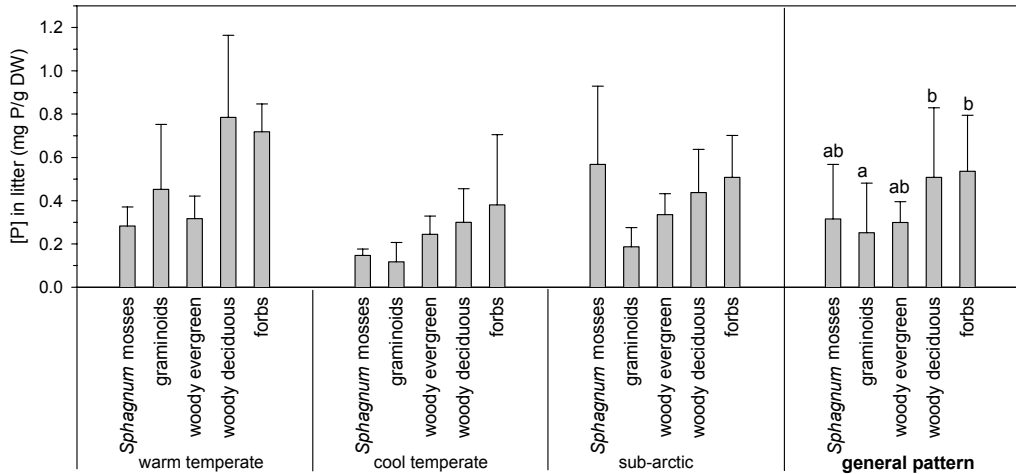


Fig. 2. Phosphorus resorption proficiency (expressed as P concentration in leaf litter) of five Plant Functional Types (PFT) sampled in peatlands from distinct regions. The value of each PFT is the average of various species (\pm SD, $n=4-6$). The 'general pattern' shows the results of the multiple comparisons on the factor PFT after the performance of a two-way Anova (region \times PFT) that did not result in an interaction term. PFTs sharing the same letter are not significantly different from each other (Tukey; $P>0.05$).

Phosphorus resorption proficiency also differed significantly among PFTs ($P < 0.001$) and regions ($P < 0.001$) without a significant interaction ($P = 0.31$). Compared to N, the patterns were less consistent among regions (Fig. 2). The general ranking of PFTs according to their P resorption proficiency was slightly different compared to the hypothesis: graminoids > *Sphagnum* mosses > woody evergreens > woody deciduous species > forbs (Fig. 2). Differences were significant only when comparing graminoids to both forbs and woody deciduous species. In contrast to that of N, the highest levels of P resorption proficiency were found at the cool temperate sites, followed by sub-arctic and warm temperate sites, with differences being significant only between the cool temperate and other sites ($P < 0.001$).

DISCUSSION

Consistent PFT-based patterns in N and P resorption proficiency

To our knowledge, this is the first study in which patterns in nutrient resorption proficiency are studied for five PFTs as well as three climatically distinct locations. Due to this large-scale design, we were not able to include more than six species for each PFT per region. Our dataset is limited to species from peatland ecosystems, which might result in higher N and P resorption proficiencies compared to other types of terrestrial ecosystems (Aerts *et al.* 1999). Moreover, we thereby probably also constrained some of the interspecific variation in nutrient resorption proficiency among species. Despite these limitations, our data support the hypothesis that consistent PFT-based patterns in nutrient resorption proficiency occur irrespective of large environmental and geographical variation. The observed patterns were generally in accordance with those reported in the literature (Enrriquez *et al.* 1993, DeLucia and Schlesinger 1995, Killingbeck 1996, Knops and Koenig 1997, Aerts *et al.* 1999, Gerdol *et al.* 2000, Pérez-Harguindeguy *et al.* 2000, Lal *et al.* 2001, Shaw and Harte 2001). However, most of these studies only dealt with two or three PFTs (mostly evergreen versus deciduous species, and occasionally also graminoids), which makes the comparison with our data limited. This also shows the need for data like ours. Nevertheless, insofar as the comparison could be made it showed that: (1) woody evergreens have a higher N and P resorption proficiency compared to woody deciduous species, (2) graminoids have a higher N resorption proficiency compared to forbs, and (3) that *Sphagnum*

mosses clearly have the highest N resorption proficiency from the five PFTs we studied. Thus, the PFT concept is an adequate tool to predict NRP patterns at the ecosystem level. Yet, what causes the observed pattern and its consistency?

The fact that environmental factors only influenced the absolute level of the whole PFT-grouped NRP pattern, but not the ranking, indicates that the ranking is intrinsic to these plant groups. This suggests that inheritable factors that influence NRP play a role. These might include phylogenetic relationships, source-sink relations, timing of abscission, activity of the resorption machinery and initial green leaf nutrient concentration (Killingbeck 2004). It may be the combination of these factors that leads to the observed PFT-ranking in NRP.

The pre-senescent nutrient concentration of green leaves is important, as this is the 'starting-point' for the resorption process. The pattern that emerges from PFT grouped green leaf nutrient concentrations is more or less comparable to the pattern observed for NRP in this study (Aerts *et al.* 1999). Evergreens have lower mature leaf N and P concentrations compared to deciduous species (Aerts and Chapin 2000), whereas forbs appear at the high end of the spectrum (Aerts 1996). Also is the segregation within the green leaf and litter PFT patterns comparable: PFT grouped P data show more overlap (less clear differences) compared to N data. This implies that for N the PFT-based classification has more distinguishing power compared to that of P.

Resorption efficiency of nutrients is another important factor that influences the final nutrient concentration in litter. Growth form differences in nutrient resorption efficiency are small (Aerts 1996), but can sometimes lead to a shift in the PFT ranking when comparing nutrient concentrations in litter and green leaves. Graminoids, with a high P concentration in their green leaves, have low litter P concentration due to their high P resorption efficiency. In contrast, *Sphagnum* mosses have the lowest nutrient concentration in litter, because, although not really efficient in conserving nutrients (Aldous 2002), they already have very low nutrient content in their living tissue.

As growth-form differences in mean nutrient resorption efficiency are small (Aerts 1996), we conclude that nutrient content of green leaves is the main determinant of NRP patterns as reported in this paper. However, there are some cases, like with graminoids, where nutrient resorption efficiency could be the main determinant of its nutrient content after senescence.

Consistent patterns, but regional differences in N and P resorption proficiency

We did not only find differences in NRP among PFTs, but also found that the level of NRP differed among regions. The highest region-averaged N resorption proficiency was found at the sub-arctic sites whereas it was considerably lower at the cool temperate and the warm temperate sites (Fig. 1). This pattern is clearly associated with the regional characteristics of the sites (cf. Table 1), as earlier studies showed that N resorption proficiency correlated positively with latitude (Berg *et al.* 1995), and responded negatively to increasing N availability (Shaver and Melillo 1984, Pugnaire and Chapin 1993, Kemp *et al.* 1994, Vitousek 1998, Eckstein *et al.* 1999) and higher temperatures (Berg *et al.* 1995, Nordell and Karlsson 1995). Compared to this north-south gradient in N resorption proficiency, a different pattern emerged for P: the cool-temperate region in the middle of the gradient showed the highest region-averaged P resorption proficiency. As P resorption proficiency is related to P availability (Shaver and Melillo 1984, Vitousek 1998, Anderson and Eickmeier 2000), it seems that there is no north-south gradient in P availability.

An explanation for the observed regional pattern for P may be that this is caused by the combined effects of the north-south gradient in atmospheric N deposition, and the fact that many peatlands in the warm temperate region are man-managed for biodiversity conservation purposes. The sub-arctic sites have N-limited plant growth, whereas high amounts of atmospheric N deposition at the cool temperate sites lead to P-limitation (Aerts *et al.* 1992b). At those cool temperate sites (which included some of the sites from this study), plant tissues had lower P concentrations (due to growth dilution), and this may explain the difference in P resorption proficiency that we found between the sub-arctic and the cool temperate region. In this line of thinking the warm temperate region, with even higher N deposition, is expected to have the lowest litter P concentrations. However, this is clearly not the case (Fig. 2). Management practices can additionally explain this anomaly: many of the peatlands in the warm temperate region are annually mown and hay is removed, because they are under severe threat of

biodiversity loss due to the high atmospheric N deposition (Bobbink *et al.* 1998). Due to the removal of hay not only N, but also other nutrients like P and K are removed, which may prevent plant growth to become P limited like at the cool temperate region (Verhoeven *et al.* 1996). Other nutrients, like K, could become limiting instead (Van Duren and Pegtel 2000), and this may be the reason that P resorption proficiency is similar to that of the sub-arctic sites, in which plant growth is neither P-limited.

The regional pattern of P resorption proficiency could also be related to limitations in the completeness of resorption at certain regions. We think, however, that this is not the case. The completeness of resorption can be qualified as complete, intermediate or incomplete with established litter nutrient levels from woody perennial species (Killingbeck 1996). Resorption of P is qualified as 'complete' when litter concentrations are less than 0.5 mg P/g DW in deciduous species, or 0.4 mg P/g DW in evergreen species. Litter concentrations higher than 1.2 mg P/g DW in deciduous species, or 0.8 mg P/g DW in evergreen species characterize 'incomplete' resorption. According to this classification, most of the woody species (Table 2) completely resorbed their P irrespective of regional differences. Assuming that this qualification can be extrapolated to the other PFTs, regional patterns as were found for P resorption proficiency cannot be explained by differences in completeness of resorption.

In conclusion, our data, which consists of measurements on 73 different species, support the validity of the proposed PFT based ranking, especially for resorption proficiency of N. We also showed that large regional differences (of factors like climate, N deposition and ecosystem management) lead to a shift in the absolute level of the PFT ranked pattern in N and P resorption proficiency. The ranking of NRP based on Plant Functional Types is, however, unaffected and might thus be of predictive importance.

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Table 2. Species included in each PFT along the climatic gradient from warm temperate to sub-arctic peatlands, and the N and P concentrations (mg/g) in the leaf litter from these species.

warm-temperate		cool-temperate		sub-arctic	
[N] _{litter}	[P] _{litter}	[N] _{litter}	[P] _{litter}	[N] _{litter}	[P] _{litter}
Sphagnum mosses*					
<i>S. contortum</i> Schultz	6.2	0.20	<i>S. angustifolium</i> (Warnst.)	7.1	0.17
<i>S. fallax</i> (H. Klinggr.) H. Klinggr.	5.5	0.34	C.E.O. Jensen	5.7	0.14
<i>S. palustre</i> L.	6.5	0.21	<i>S. cuspidatum</i> Ehrh. ex Hoffm.	7.7	0.13
<i>S. squarrosum</i> Crome	9.5	0.40	<i>S. magellanicum</i> Brid.	7.0	0.11
<i>S. subnitens</i> Russow & Warnst.	8.4	0.26	<i>S. papillosum</i> Lindb.	7.8	0.18
			<i>S. rubellum</i> Wilson		
graminoids					
<i>Calamagrostis canescens</i>	7.9	0.51	graminoids	6.7	0.06
<i>Carex acutiformis</i>	12.2	0.64	<i>Carex lasiocarpa</i>	5.5	0.01
<i>Carex rostrata</i>	12.5	0.82	<i>Eriophorum angustifolium</i>	8.3	0.16
<i>Juncus subnodulosus</i>	10.0	0.10	<i>Molinia caerulea</i>	13.5	0.25
<i>Scirpus lacustris</i>	5.0	0.20	<i>Phragmites australis</i>	8.1	0.11
			<i>Rhynchospora alba</i>		
woody evergreen					
<i>Calluna vulgaris</i>	12.1	0.19	woody evergreen	6.7	0.16
<i>Erica tetralix</i>	12.4	0.26	<i>Andromeda polifolia</i>	10.7	0.27
<i>Ledum groenlandicum</i>	10.7	0.37	<i>Calluna vulgaris</i>	10.1	0.36
<i>Pinus sylvestris</i>	13.4	0.30	<i>Ledum palustre</i>	9.9	0.27
<i>Vaccinium vitis-idaea</i>	10.0	0.47	<i>Picea abies</i>	11.9	0.17
			<i>Pinus sylvestris</i>		
woody deciduous					
<i>Alnus glutinosus</i>	28.9	0.44	woody deciduous	15.9	0.04
<i>Aronia x prunifolia</i>	5.8	0.56	<i>Myrica gale</i>	13.9	0.35
<i>Betula pubescens</i>	16.7	1.03	<i>Salix cinerea</i>	16.2	0.29
<i>Lonicera periclymenum</i>	13.5	1.33	<i>Salix pentandra</i>	17.3	0.46
<i>Salix cinerea</i>	13.2	0.56	<i>Sorbus aucuparia</i>	12.8	0.35
			<i>Vaccinium myrtillus</i>		
forbs					
<i>Angelica sylvestris</i>	14.3	0.70	forbs	13.4	0.61
<i>Calpha palustris</i>	30.2	0.92	<i>Epilobium palustre</i>	17.4	0.40
<i>Chamerion angustifolium</i>	10.9	0.74	<i>Filipendula ulmaria</i>	8.4	0.17
<i>Cirium palustre</i>	10.0	0.79	<i>Iris pseudacorus</i>	12.6	0.05
<i>Hydrocotyle vulgaris</i>	10.3	0.57	<i>Narthecium ossifragum</i>	15.1	0.17
<i>Rubus fruticosus</i>	10.4	0.60	<i>Rubus chamaemorus</i>	15.1	0.90
			<i>Stachys palustris</i>		
			<i>Bartsia alpina</i>	13.1	0.83
			<i>Polygonum viviparum</i>	13.4	0.37
			<i>Potentilla palustris</i>	9.6	0.51
			<i>Rubus chamaemorus</i>	13.2	0.63
			<i>Saussurea alpina</i>	14.4	0.35
			<i>Saxifraga aizoides</i>	6.8	0.35

* *Sphagnum* nomenclature follows Bouman and Van der Pluijm (2002). Vouchers are kept in L

Nitrogen and phosphorus resorption efficiency and proficiency in six sub-arctic bog species after 4 years of nitrogen fertilisation

with Sylvia Toet and Rien Aerts

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- Plant growth at high-latitude sites is usually strongly nutrient-limited. The increased nutrient availability predicted in response to global warming may affect internal plant nutrient cycling, including nutrient resorption from senescing leaves.
 - The effect of increased N supply ($10 \text{ g N m}^{-2} \text{ yr}^{-1}$) on nitrogen and phosphorus resorption efficiency and proficiency in six sub-arctic bog species, belonging to four different growth-forms, was studied in northern Sweden.
 - We hypothesised that while increased N supply would not affect N nor P resorption efficiency, it would lead to lower N resorption proficiency (higher N concentrations in leaf litter) and higher P resorption proficiency (lower P concentrations in leaf litter). We also investigated whether the basis on which resorption was expressed (leaf mass, leaf area or unit leaf) influenced the patterns observed.
 - Contrasting to our hypothesis, a general trend of decreased N resorption efficiency occurred in response to increased N supply, but the expected decrease in N resorption proficiency was seen in all species except *Betula nana*.
 - P resorption efficiency did not change in four species (*B. nana*, *Empetrum hermaphroditum*, *Eriophorum vaginatum*, *Rubus chamaemorus*) but it decreased in *Andromeda polifolia*, and increased in *Vaccinium uliginosum*. P resorption proficiency showed the expected increase in only two species (*B. nana* and *V. uliginosum*).
 - Apart from P resorption efficiency, the different calculation methods generally produced similar responses of resorption efficiency and proficiency to N supply.
 - Increased N supply at high-latitude sites clearly leads to more N being returned to the soil through leaf litter production. However, decomposition of such litter will probably become P-limited.
 - Considerable interspecific differences in nutrient resorption proficiency were found, indicating that long-term changes in vegetation composition need to be considered when evaluating plant-mediated effects on ecosystem nutrient cycling in response to increased nutrient supply.
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INTRODUCTION

Resorption of nutrients from senescing leaves is of great adaptive significance, because such nutrients are directly available for further use, making a species less dependent on current nutrient uptake (Aerts and Chapin 2000). It has often been suggested that species from low-nutrient habitats have higher nutrient resorption efficiencies (percentage of a nutrient withdrawn from mature leaves before leaf abscission). However, the evidence available so far does not support this contention: high nutrient resorption efficiency is characteristic of all perennial growth-forms and appears not to be very responsive to changes in nutrient supply (Aerts and Chapin 2000). This implies that although nutrient resorption is an important nutrient conservation mechanism, it does not explain the distribution of growth-forms over habitats differing in soil fertility.

Nutrient resorption can also be quantified by resorption proficiency, a parameter describing the level to which a nutrient is reduced during senescence (Killingbeck 1996), with higher proficiencies corresponding to lower final nutrient concentrations. Resorption proficiency seems to be more responsive than resorption efficiency to nutrient availability. Several studies reported that nitrogen (N) fertilisation resulted in higher N concentrations in the litter of most species (Shaver and Mellilo 1984; Kemp *et al.* 1994; Vitousek 1998) suggesting that N fertilisation leads to lower N resorption proficiencies. This relationship between N resorption proficiency and N availability is also found along natural fertility gradients (Pugnaire and Chapin 1993; Eckstein *et al.* 1999). In contrast with resorption efficiency, there are clear differences in resorption proficiency among growth-forms (Aerts 1996). On average, evergreen species have higher N proficiencies than woody deciduous species, which in turn have higher proficiencies than herbaceous species. This has profound consequences for soil nutrient cycling as the nutrient content of herbaceous and deciduous leaf litter that is returned to the soil is higher than that of evergreens. This is an important aspect of the positive feedback between growth-form dominance and nutrient availability in ecosystems (Chapin 1993; Aerts 1999), which, although plant-driven, may be affected by external nutrient inputs.

In many ecosystems N availability has increased during the past decades because many industrialised countries are subject to enhanced atmospheric N deposition (Bobbink *et al.* 1998) and also due to climate change. Greenhouse gas emissions are predicted to raise mean global temperatures by 1.0-3.5 °C in the next 50-100 years with above-average increases at high-latitude and high-altitude sites (Houghton 2001). This temperature increase is expected to have a significant impact not only on the abundance and phenology of organisms (Wookey *et al.* 1993; Wookey *et al.* 1994; Chapin *et al.* 1995; Harte and Shaw 1995), but also on element cycling in those regions, where plant growth tends to be severely constrained by nutrient availability (Gorham 1991). A global meta-analysis of 32 warming experiments, including several at high-latitude and high-altitude sites, has shown that, across all sites and years, 2-9 years of experimental warming in the range 0.3-6.0 °C significantly increased soil net N mineralisation rates by 46% (with a 95% confidence interval of 30-64%) (Rustad *et al.* 2001). There are no clear effects of warming on soil P availability, because the breakdown of detrital N is a biological process, whereas breakdown of detrital P is a chemical process with a much lower Q_{10} value (Aerts and Chapin 2000). These observations raise the question how increased inorganic N availability affects N and phosphorus (P) resorption from senescing leaves of dominant species and thereby the amount of N and P returned to the soil through leaf litter production. A secondary effect of increased N availability in N-limited ecosystems is a shift toward P-limitation (Bobbink *et al.* 1998), which seems to lead to lower P concentrations in litter, due to growth dilution of the P in the leaves (Shaver and Mellilo 1984; Vitousek 1984; Kemp *et al.* 1994).

We hypothesised that, in N-limited ecosystems, increased inorganic N supply would not affect leaf resorption efficiency for either N or P, but would lead to lower leaf resorption proficiency for N and higher values for P. Leaf litter concentrations are thus expected to increase for N, and to decrease for P. We investigated these hypotheses by studying the effects of 4 years of N fertilisation on leaf nutrient resorption of six dominant plant species in a sub-arctic bog in northern Sweden. We also investigated, as a secondary aim, whether the observed patterns in resorption efficiency and proficiency were dependent on the basis on which resorption was expressed (per unit of leaf mass, leaf area or per unit leaf).

MATERIALS AND METHODS

Field site and experimental set-up

Leaves were collected from a long-term N fertilisation experiment that started in 1998 in a peat bog at Stordalen, near the Abisko Scientific Research Station, northern Sweden (68 °21' N, 18°49' E). This bog, which is a former tundra site of the International Biosphere Programme (IBP), has been described in detail by Sonesson (1980). Annual precipitation amounts to 320 mm per year, and mean summer and winter temperatures are 7 °C and -6 °C respectively. The length of the growing season is 130 days. The vegetation of the bog is characterised by great heterogeneity both at small and large scales, reflecting the topographical heterogeneity of the site. We conducted our study in the elevated ombrotrophic part of the bog in the *Empetrum hermaphroditum*-*Vaccinium microcarpum* association.

In May 1998, 16 plots measuring 1.5 by 1.5 m were selected on drier hummocks. The plots were separated by buffer zones of at least 0.5 m. Since 1998, eight replicate plots have been N fertilised each year (10 g N m⁻²yr⁻¹ as NH₄Cl). Nitrogen was added in solution in three equal portions in mid-June, mid-July and mid-August. The other eight replicate plots served as controls (C) and received only an equal amount of water to that used to dissolve the NH₄Cl.

Six dominant perennial plant species that occurred within each plot were selected, namely: *Empetrum hermaphroditum* Hagerup, and *Andromeda polifolia* L., both woody evergreen; *Vaccinium uliginosum* L., and *Betula nana* L., both woody deciduous; *Eriophorum vaginatum* L., graminoid; and *Rubus chamaemorus* L., herb. From their mycorrhizal infection and low leaf ¹⁵N values (cf. the data of Michelsen *et al.* 1996, 1998 for this area), the four woody species are most probably capable of taking up organic N sources. No such data are available for *E. vaginatum* and *R. chamaemorus*. However, given the widespread capability of northern non-mycorrhizal species to take up organic N (Näsholm *et al.* 1998), these species are probably also capable of such assimilation. It must be emphasized, however, that the present study focuses on the effects of increased inorganic N supply on nutrient resorption from senescing leaves.

Leaf collection

In July 2001, preceding the N fertilisation that month, mature green leaves of the selected species were collected at random from each plot. In autumn (late September-early October) recently senesced, brown, but still attached leaves were collected in the same plots. For each plot and each species, sufficient leaves were collected to enable chemical analyses. For *E. hermaphroditum* this meant randomly sampling 200 leaves from whole branches, as different cohorts were not clearly distinguishable. Approximately 50, 60 and 35 leaves were collected from *B. nana*, *V. uliginosum* and *A. polifolia* respectively and in autumn, recently senesced leaves were collected from the plants as well as from the ground. For *R. chamaemorus* about 12 leaves were collected in summer, but 25 were collected in autumn. About 30 leaves were collected from *E. vaginatum*, but as they had not yet totally senesced down to the base in the control plots only the senesced parts (>75%) were collected in autumn. The leaves were transported to the laboratory, oven dried at 30°C to a constant weight and stored until further analysis.

Laboratory methods

For both mature and senesced leaves, the number of collected leaves of each species in a plot was counted, and their dry mass measured. Total projected leaf area per sample was determined for each species using a scanner and Photoshop 5.0. However, this was not possible for the evergreen species *A. polifolia*, because the tough and bent leaves of this species did not allow accurate measurement of leaf area. For *E. vaginatum*, which has long, narrow and partly folded leaves, a similar problem occurred, and for this species the total leaf length per plot was determined as an alternative. Therefore, only four species provided data on all three different measurement bases (mass, area, unit leaf) for comparison. Leaf material of each species was bulked per plot and ground. Leaf P concentration was determined after digesting ground leaf material in 37% HCl:65% HNO₃ (1:4, v/v) (as in Sneller *et al.* 1999). Phosphorus was measured

colorimetrically at 880 nm after reaction with molybdenum blue (Murphy and Riley 1962). Total N concentration was measured using a Perkin Elmer 2400 series II CHNOS/O analyser.

Nitrogen and phosphorus resorption

Resorption of N and P was calculated for each species and plot individually. Resorption efficiency (RE) was calculated as:

$$RE = 100\% \times \left(1 - \frac{[\text{nutrient}]_{\text{dead}}}{[\text{nutrient}]_{\text{green}}} \right)$$

where [nutrient] is the N or P concentration of the dead or the green leaves expressed as the nutrient mass per leaf dry mass. Because of the sampling of the leaves in summer and autumn, alterations in leaf structure during senescence (due to mass resorption or area shrinkage) can bias the parameter resorption efficiency to a large extent (Van Heerwaarden *et al.* 2003a). Therefore, resorption efficiency was also calculated on a leaf unit basis (RE_{unit}), or calculated per unit leaf area (RE_{area}). Different ways to express nutrient levels (mass based (concentration), leaf area based (content), and leaf unit based) were used as measurement of resorption proficiency (Killingbeck 1996) for the same reason.

Statistics

Due to unequal variances, data of N and P concentrations were $\log(x)$ transformed, and resorption efficiencies were $\log(1/1-x)$ transformed prior to statistical testing (SPSS for Windows 10.1.0, SPSS Inc., Chicago). Data were tested for homogeneity of variance with Levene's test. When transformed data showed unequal variances, data were tested non-parametrically (ranked), but only if this increased Levene's P-value.

In a two-way ANOVA, with species and N treatment as factors, we tested whether treatment effects were general or species specific (shown by a significant treatment \times species interaction term). The results of the tests on the differently based data (mass, area or unit leaf) were compared to see whether the measurement basis influences the outcome of the test. As we were only able to collect mass-, and area-based, as well as leaf unit-based, data for four out of six species, we repeated the statistical analysis including only data from these four species. The statistics of the two-way ANOVAs are therefore presented both for the complete set of species, and for only the four species for which we had complete data. To test whether the N treatment influenced resorption of N or P for each species, the resorption efficiencies and proficiencies were also separately tested in a one-way ANOVA with N treatment as a fixed factor with two levels.

RESULTS

Nitrogen fertilisation effects on green leaf N and P concentrations

In the control plots, the N concentration of mature leaves clearly differed among species (Fig. 1a-c, Table 1). The leaves of the evergreen shrubs *E. hermaphroditum* and *A. polifolia* had the lowest N concentrations, and the herb *R. chamaemorus* the highest, while the other species exhibited intermediate values (Fig. 1a, b). For P the patterns were different, with the highest P concentrations occurring in the two deciduous species *B. nana* and *V. uliginosum* (Fig. 1d, e, Table 1), although the large leaves of *R. chamaemorus* logically had the highest nutrient contents when expressed on a unit basis (Fig. 1f).

In general, N treatment led to higher N concentrations in green leaves, but the size of this effect was species-specific, as shown by the significant species \times N treatment interaction (Table 1, Fig. 1a-c). The N fertilisation effect was not significant in *B. nana* and *R. chamaemorus*. In *E. hermaphroditum* a more than 3-fold increase in N concentration occurred, whereas the other species increased their green leaf N concentration by up to 75%. Only in *R. chamaemorus* did the measurement basis (dry mass, area or leaf unit) influence the significance of these responses.

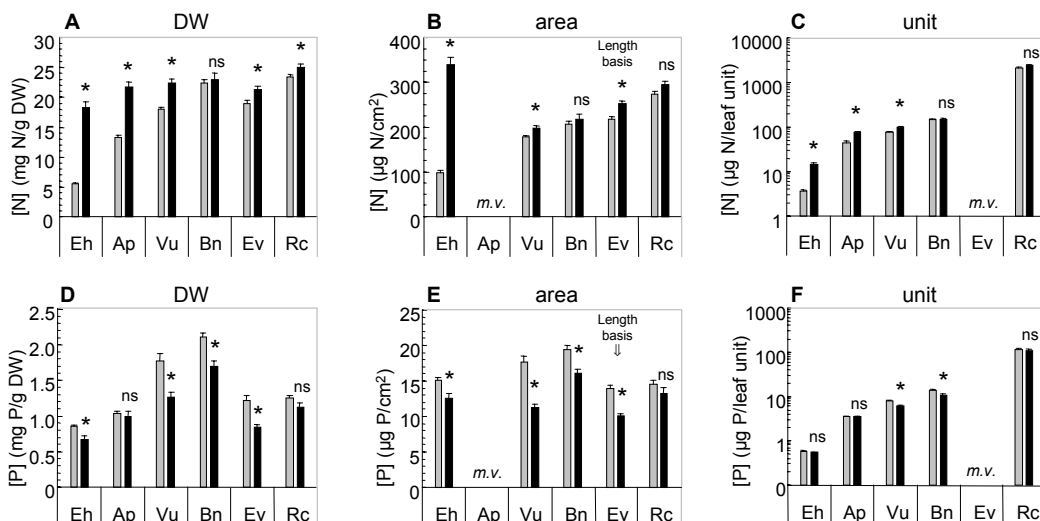


Figure 1. Effects of 4 years of N fertilisation ($10 \text{ g N m}^{-2} \text{ yr}^{-1}$) on green leaf N and P concentrations (mean \pm SE, $n=8$) of six sub-arctic bog species expressed on dry mass basis (a, d), area basis (b, e), and unit leaf basis (c, f, note log-scale). White bars: unfertilised controls; grey bars: +N. For *Eriophorum vaginatum*, N and P are expressed on leaf length ($\mu\text{g N}$ or P mm^{-1} leaf) rather than area basis. Species: *Empetrum hermaphroditum* (Eh), *Andromeda polifolia* (Ap), *Vaccinium uliginosum* (Vu), *Betula nana* (Bn), *Eriophorum vaginatum* (Ev), and *Rubus chamaemorus* (Rc). m.v.: missing value; * indicates significant fertilisation effect ($P < 0.05$); ns: non significant.

Table 1. Results of two-way ANOVA on the effects of species and N treatment and their interaction on N and P concentrations in green leaves of six sub-arctic bog species, growing on 8 replicate plots. The left hand set of results included all species available for the measurement basis concerned: dry mass (DW) based data: 6 species; unit based data: 5; area based data: 4. The right hand set analyses only the four species that had all three sets of data: these were used to compare results between measurement bases.

	Basis	Source	4-6 species			4 species		
			df	F	P	df	F	P
Nitrogen	DW	Species	5	193	.001	3	279	.001
		N	1	357	.001	1	240	.001
		Species * N	5	92	.001	3	126	.001
	unit	Species	4	3183	.001	3	4339	.001
		N	1	203	.001	1	152	.001
		Species * N	4	56	.001	3	78	.001
	area	Species	3	70	.001	3	70	.001
		N	1	225	.001	1	225	.001
		Species * N	3	140	.001	3	140	.001
Phosphorus	DW	Species	5	86	.001	3	133	.001
		N	1	61	.001	1	48	.001
		Species * N	5	3.2	.011	3	1.9	.136
	unit	Species	4	2085	.001	3	2741	.001
		N	1	9.5	.003	1	13	.001
		Species * N	4	2.4	.059	3	1.8	.151
	area	Species	3	14	.001	3	14	.001
		N	1	54	.001	1	54	.001
		Species * N	3	5.6	.002	3	5.6	.002

Phosphorus responded differently, with N fertilisation generally resulting in a decreased P concentration in green leaves, although not in all species as indicated by the significant species x N interaction (Table 1). Green leaf P concentration decreased by almost 30% in some species (*V. uliginosum* and *E. vaginatum*), while in others (*A. polifolia*, *R. chamaemorus*, and unit based *E. hermaphroditum*) differences were not significant (Fig. 1d-f). The different measurement bases

generally yielded the same patterns of significance in all the statistical tests, except for *E. hermaphroditum* individually and the interaction term of the overall test. This irregularity in the overall test could be due to the different number of species used. However, when only the four species for which dry mass, unit, and area based data were available were analysed, rather than all species, only the interaction term of dry mass based data changed significance (Table 1).

N fertilisation changed the N:P-ratios, clearly reflecting the contrasting effects of N fertilisation on N and P concentrations in green leaves. In the control plots, N:P-ratios of green leaves were lower than 14 in most species, which points to N-limited plant growth (Fig. 2). In the N-treated plots, N:P-ratios were significantly higher in all species (except in *B. nana*) and exceeded the critical value of 16 that may indicate P-limited plant growth.

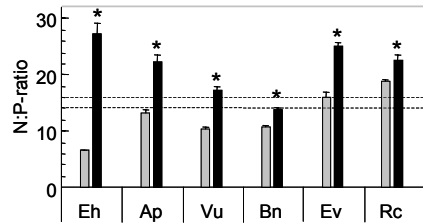


Figure 2. Effects of 4 years of N fertilisation ($10 \text{ g N m}^{-2} \text{ yr}^{-1}$) on green leaf N:P ratios (mass basis) ($\bar{x} \pm \text{SE}$, $n=8$) of six sub-arctic bog species. White bars: unfertilised controls; grey bars: +N. * indicates significant fertilisation effect ($P < 0.05$). Horizontal lines in the graph represent the boundary N:P values correlating with N limitation ($\text{N:P} < 14$) and P limitation ($\text{N:P} > 16$) as proposed by Koerselman and Meuleman (1996). See Fig. 1 for abbreviation of species

Nitrogen fertilisation effects on N and P resorption efficiency

In the unfertilised controls, N resorption efficiency also clearly differed between species, with values between 40 and 80% (Fig. 3a). No clear patterns emerged in relation to growth forms. In general, N treatment led to lower N resorption efficiencies but, as indicated by the significant species \times N interaction, the response was species-specific (Fig. 3a, Table 2). At the individual species level, N resorption efficiency significantly decreased only in *A. polifolia*, *R. chamaemorus* (only on DW basis) and *V. uliginosum*. The largest decrease in N resorption efficiency after N treatment occurred in *A. polifolia*, down to a value that was even not significantly different from zero ($p=0.513$, DW based data). The type of measurement basis did not influence the significance of these responses, except for *R. chamaemorus* and *V. uliginosum*.

Resorption efficiency of P also differed significantly among species in the control plots (values between 30 and 90%), but again no clear pattern emerged when considering growth-form differences (Fig. 3b). Nitrogen fertilisation did not have a clear general effect on P resorption efficiency, as responses were species-specific, dependent on the measurement basis (Fig. 3b, Table 2) and, for most species insignificant. P resorption efficiency only changed significantly in *A. polifolia*, decreasing (DW basis) from 71% to 20%, a value not significantly different from zero ($P=0.115$ of the effect on N resorption efficiency). It was also less clear whether P resorption efficiency of *V. uliginosum* and *R. chamaemorus* responded to N fertilisation, as differences were only significant when expressed on unit or DW basis respectively. Moreover, in the overall statistics, only the DW based data yielded a significant interaction term. An explanation for this dependency on measurement basis is that a different number of species had been included in each test (Table 2), although differences and responses in mass resorption may also have played a role. In contrast with N, the measurement basis significantly influenced the overall statistical significance pattern for P when either all species or the four with complete datasets were compared (Table 2 and Fig. 3b).

Nitrogen fertilisation effects on N and P resorption proficiency

The patterns in N and P concentrations in senescent leaves in the unfertilised control plots were noticeably similar to the N and P concentration patterns in green leaves (compare Fig. 4 with Fig. 1). Thus, there is high N proficiency (low litter N concentration) in both evergreen shrubs and low N proficiency in the herb *R. chamaemorus* (Fig. 4a-c, Table 3). The patterns were also comparable in fertilised plots, although N and P resorption proficiencies clearly responded differently to N fertilisation in *A. polifolia*. In this species, differences between N concentrations in the control and fertilised plots were considerably larger in litter compared to green leaves; for P, there was no significant N fertilisation effect in green leaves, but litter P concentration increased 3-fold.

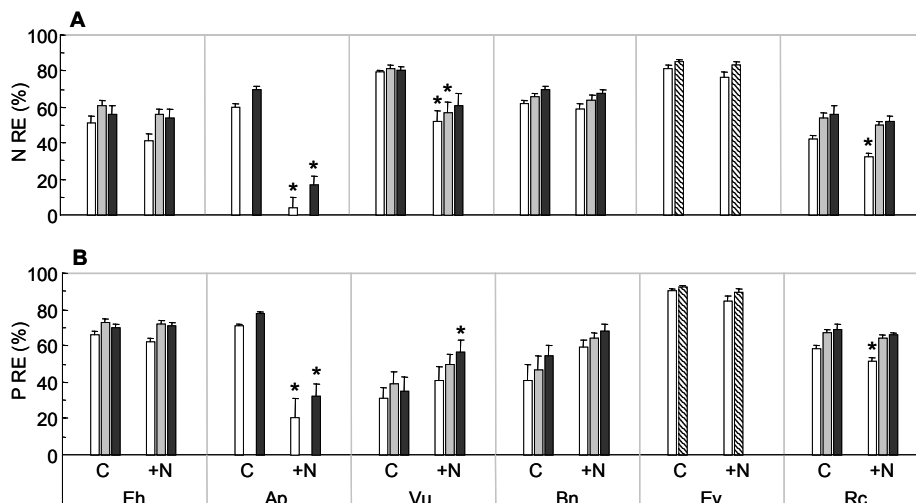


Figure 3. Effects of 4 years of N fertilisation (10 g N m⁻² yr⁻¹) on resorption efficiency (x ± SE, n=8) of nitrogen (a) and phosphorus (b) of six sub-arctic bog species expressed on dry mass basis (white bars), area basis (light grey bars), unit leaf basis (dark bars), and leaf length basis (striped bar, only for *Eriophorum vaginatum*). C: unfertilised control; +N: N fertilised. * indicates significant fertilisation effect (P < 0.05) within each measurement basis. See Fig. 1 for abbreviation of species.

Table 2. Results of two-way ANOVA on the effects of species and N treatment and their interaction on N and P resorption efficiency of six sub-arctic bog species. See Table 1 for further explanation.

	basis	Source	df	F	P	df		F	P
						4-6 species	4 species		
Nitrogen	DW	Species	5	64	.001	3	39	.001	
		N	1	79	.001	1	38	.001	
		Species * N	5	11	.001	3	11	.001	
	unit	Species	4	21	.001	3	17	.001	
		N	1	48	.001	1	11	.001	
		Species * N	4	13	.001	3	4.9	.004	
	area	Species	3	20	.001	3	20	.001	
		N	1	26	.001	1	26	.001	
		Species * N	3	12	.001	3	11	.001	
Phosphorus	DW	Species	5	40	.001	3	14	.001	
		N	1	11	.002	1	.15	.704	
		Species * N	5	14	.001	3	3.5	.021	
	unit	Species	4	6	.001	3	7.2	.001	
		N	1	2.25	.138	1	3.0	.087	
		Species * N	4	15.0	.001	3	2.5	.073	
	area	Species	3	23	.001	3	23	.001	
		N	1	.90	.347	1	.90	.347	
		Species * N	3	1.9	.143	3	1.9	.143	

In general, N treatment led to lower N resorption proficiencies, but the effect sizes were species-specific as indicated by the significant species x N treatment interaction (Table 3). The N treatment effect was significant except in *B. nana* (Fig. 4a-c), and the largest response occurred in *A. polifolia*, which had a 4-fold higher N litter concentration when expressed on DW basis, and almost 5-fold higher when expressed on unit basis. *Empetrum hermaphroditum* and *V. uliginosum* responded with more than doubling of litter N concentration whereas other species had smaller increases.

Nitrogen fertilisation also affected P litter concentrations, but responses were species-specific (right hand side of Table 3, Fig. 4d-f). When more than the 4 'complete' species were tested the overall N fertilisation effect was not significant (left hand side of Table 3).

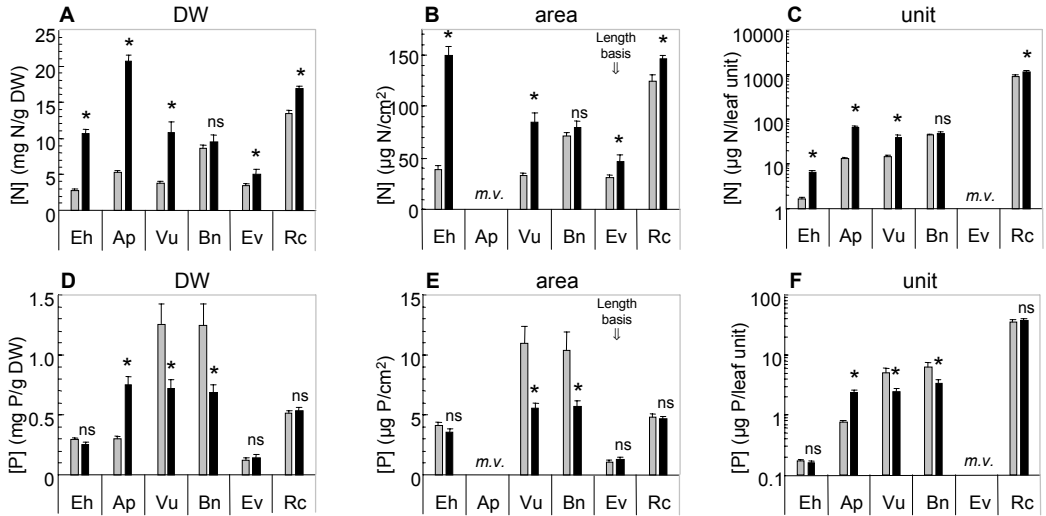


Figure 4. Effects of 4 years of N fertilisation ($10 \text{ g N m}^{-2} \text{ yr}^{-1}$) on dead leaf N and P concentrations ($x \pm \text{SE}$, $n=8$) of six sub-arctic bog species expressed on various bases. For *Eriophorum vaginatum* (Ev), N and P are expressed on leaf length ($\mu\text{g N}$ or P mm^{-1} leaf) rather than area basis. White bars: unfertilised controls; grey bars: +N. * indicates significant fertilisation effect ($P < 0.05$). See Fig. 1 for further explanation.

Table 3. Results of two-way ANOVA on the effects of species and N treatment and their interaction on N and P resorption proficiency of six sub-arctic bog species. See Table 1 for further explanation.

	basis	Source	df	F	P	df	F	P
			4-6 species			4 species		
Nitrogen	DW	Species	5	84	.001	3	75	.001
		N	1	289	.001	1	160	.001
		Species * N	5	30	.001	3	33	.001
	unit	Species	4	204	.001	3	1430	.001
		N	1	44	.001	1	106	.001
		Species * N	4	3	.001	3	25	.001
	area	Species	3	60	.001	3	60	.001
		N	1	147	.001	1	147	.001
		Species * N	3	34	.001	3	34	.001
Phosphorus	DW	Species	5	100	.001	3	68	.001
		N	1	.173	.679	1	18	.001
		Species * N	5	12	.001	3	4.5	.007
	unit	Species	4	41	.001	3	757	.001
		N	1	340	.562	1	16	.001
		Species * N	4	2	.001	3	4.9	.004
	area	Species	3	24	.001	3	24	.001
		N	1	25	.001	1	26	.001
		Species * N	3	5.1	.004	3	5.1	.004

Considering individual species, responses of P resorption proficiency to N fertilisation were more variable than green leaf P responses (which decreased in all species, Fig. 1). Both *B. nana* and *V. uliginosum* had almost 50% lower P concentrations in their litter in response to the N treatment, meaning that they had achieved higher P resorption proficiency, whereas no change occurred in other species, apart from *A. polifolia* where it decreased dramatically. This clearly confirms that the response of P resorption proficiency to N fertilisation is species-specific. Different measurement bases gave similar patterns for both N and P resorption proficiency in overall and species level statistics.

DISCUSSION

Methodological considerations

It is well known that values of resorption efficiency can be biased by the measurement basis that is used. In a theoretical paper we have shown that considerable underestimations of resorption efficiency can occur when mass based data are used instead of area based data (Van Heerwaarden *et al.* 2003a). This is due to the fact that, during leaf senescence, substantial resorption of non-structural compounds can occur, although leaf shrinkage can also lead to bias when using area based data. In this study we also found considerable mass loss (up to 25%) during senescence in some species (data not shown). In comparative studies such as this one, the species-specific biases have to be taken into account. Moreover, when applying a treatment there is also the possibility of treatment-induced change in mass loss or leaf area during senescence that can lead to bias in the treatment effect. This can partly be overcome by expressing the data on various measurement bases, and checking if the significances of the treatment effect are similar among measurement bases. Our data showed that the treatment and species patterns were hardly influenced by the different measurement bases except for P resorption efficiency. This could be caused by the inhomogeneous group-variances in the P resorption efficiency data, in combination with a species-specific treatment-induced change in mass loss and leaf area shrinkage during senescence, as was the case in *R. chamaemorus* (area based mass loss in control plots: $20\% \pm 3.6$ (sd); in fertilised plots: $26\% \pm 2.1$ (sd); $p=.002$) and *V. uliginosum* (unit based area loss in control plots: $11\% \pm 13$ (sd); in fertilised plots: $17\% \pm 8$ (sd); $p=.001$).

Another general cause for bias in results is that nutrient resorption efficiency has to be determined from two different cohorts of green and dead leaves, as nutrient concentration is measured destructively. This is a problem inherent to all resorption studies. These problems can partly be minimised if the leaves are pre-selected and followed (e.g. photographically) throughout the growing season. This might be feasible for studies of species with large leaves that can easily be tagged and followed. However, most species in this sub-arctic bog habitat are extremely small (height less than 5 cm; i.e. considerably smaller than similar or related species in more fertile and southern habitats) and their leaves are tiny (millimetres, for some of the species). Thus, for a species as *E. hermaphroditum* it is not possible to differentiate between leaf samples with an average leaf length of 4 mm or of 5 mm (i.e. a 20% difference) when the leaves are collected in the field. In addition, it is of course possible to choose which mature leaves are sampled, but for the dead leaves of the evergreen species one has to sample 'what is there'. Moreover, tagging of leaves of these species is practically impossible due to their small size. Thus, in the present study this problem cannot be solved.

There is a risk that strongly different leaf cohorts are compared, especially when investigating evergreen species with long leaf lifespan. Theoretically, evergreen dead leaves could have been subjected to pre-experimental conditions, whereas green leaves developed during the treatment. This could be the case in *E. hermaphroditum*, as this species keeps some leaves about 4 years (Jonasson 1989). Given that this is just as long as the duration of the experiment this type of error is probably small.

For some species we found that leaf areas of green and dead leaves were significantly different. In the control plots, the leaf area of dead Empetrum leaves was 11% larger than that of the green leaves (14% for *B. nana*). This might be caused by sampling young, not yet maximally expanded green leaves, or by a change in leaf shape during senescence. In contrast, *V. uliginosum* leaf cohort collected in autumn from N-treated plots had 17% smaller leaves than the cohort collected in summer, whereas in the control plots no significant differences were observed. These findings could be explained by accelerated senescence due to N treatment since, if larger leaves had already dropped due to accelerated senescence and their larger weight, this would have led to selectively finding the smaller leaves still attached to the plants in the N-treated plots. Such size related leaf shedding was also found in another study, where the oldest leaf cohort of the evergreen *E. hermaphroditum* consisted of few and relatively smaller leaves (Jonasson 1989). Accelerated senescence was also observed in this study in the graminoid

E. vaginatum during autumn collection, as leaves from N-treated plots had totally senesced all the way to the leaf base, whereas they had not in the control plots. However, other studies mainly reported that N fertilisation delayed senescence (Larcher 1995). Thus, it seems that the response of senescence timing to N fertilisation is species-specific.

Leaf N and P resorption efficiency and proficiency in sub-arctic peat bog species

The plant species in our study site showed high N and P resorption efficiencies. The four investigated sub-arctic bog species with area based data available withdrew between 54 and 82 % of the N from their senescing leaves. The graminoid *E. vaginatum* even withdrew 86% (length basis) of its N, and the evergreen *A. polifolia* 69% (unit basis). These resorption efficiency values are rather high compared to the average N resorption efficiency (50%) found in a large literature survey (Aerts 1996), but are comparable to those found in another study in the same region (Qustedt *et al.* 2003). This was also the case for P resorption efficiency: it varied between 39 and 72% in the four species with area based data, but was even 92% (length basis) in the grass *E. vaginatum*, and also very high (78%, unit basis) in the evergreen *A. polifolia*. The lowest P resorption efficiency values were those of the two deciduous species. Our values are very similar to the values reported by (Berendse and Jonasson 1992) who made a compilation of resorption data for arctic and sub-arctic plant species, including four species that were also studied by us (*B. nana*, *V. uliginosum*, *E. hermaphroditum*, and *E. vaginatum*). These data suggest that nutrient resorption efficiency in high-latitude species is at the high end of the values as compiled by Aerts (1996) and Killingbeck (1996) for a wide range of ecosystems.

The average litter N concentrations in the control plots varied between 3.7 and 13 mg N g⁻¹ DW among the different species and, for the species with area based data available, between 33 and 125 µg N cm⁻². Phosphorus concentrations in dead leaves grown in the control plots varied between 0.12 and 1.25 mg P g⁻¹ DW among the different species, or (for the species with area based data available) between 4.1 and 10.9 µg P cm⁻². When comparing these resorption proficiency values with the values of Killingbeck (1996) that are indicative for the completeness of resorption, the data for the woody perennial species suggest that resorption of N is complete in some species and intermediate in others, whereas P resorption is classified as less complete (intermediate to incomplete). This pattern correlates well with the observation that plant growth in sub-arctic peat bogs is N-limited, as was found in an earlier study (Aerts *et al.* 1992b), because plants under N limitation are more likely to reach complete resorption of this nutrient compared to the unlimiting nutrients.

Effects of increased N supply on N and P resorption efficiency and proficiency

We hypothesised that N resorption efficiency would not be affected by increased N supply, but we found a general decrease in N resorption efficiency, although decreases were significant in only two species individually. In accordance with our hypothesis, N resorption proficiency decreased in all species except *B. nana* upon N treatment. This implies that the litter N content was higher, and that the amount of N returned to the soil by leaf litter production probably increased.

Our hypothesis that P resorption efficiency would not change after N fertilisation was supported by four out of six species. The other two species showed a large decrease (*A. polifolia*) and a moderate increase (*V. uliginosum*) in P resorption efficiency after N fertilisation. In contrast to our hypothesis, P resorption proficiency only increased in two species (*B. nana* and *V. uliginosum*), showing no response to N fertilisation in three species, and a decrease in *A. polifolia*. This pattern might also be explained with the concept of 'complete resorption'. Using the reference values of Killingbeck (1996), the evergreens *A. polifolia* and *E. hermaphroditum* had complete resorption of P in the control plots, whereas *B. nana* and *V. uliginosum* had incomplete resorption. As the demand for P probably increased after N fertilisation, the species with incomplete resorption could hypothetically increase the resorption proficiency to use the potentially available P, leading to lower litter P concentrations after N fertilisation. The species that already had complete resorption under normal conditions were unable to acquire extra P from their senescing leaves owing to physiological constraints and did not show a lower P concentration in their senesced leaves. The herbaceous species did not show significantly changed P resorption proficiency, which could mean that these species also had complete

resorption proficiency. In this case, P proficiency could not increase even though the need for P became higher, as indicated by the increased green leaf N:P ratio in all species.

In this study *A. polifolia* showed anomalous behaviour compared to the other species, with the results suggesting prematurely arrested resorption of N and P after N fertilisation. In the fertilised plots the mature leaves appeared to be red instead of green, probably due to anthocyanins (personal observation), and indicating a plant stress response (Chalker-Scott 1999). Such a response is not a general observation as, in other studies, *A. polifolia* showed a significant increase in net primary production (Thormann and Bayley 1997) and increased density (Redbo-Torstensson 1994) after N fertilisation. We suspect that under the specific conditions of our experiment this species suffered from strong P limitation, since anthocyanin accumulation is often a sign of severely P-limited plant growth (Marschner 1995).

Consequences for nutrient cycling

Given the expected increase in nutrient availability at high-latitude sites due to global warming, our fertilisation study provides insight into possible changes in the amount of nutrients returned to the soil through leaf shedding. Mass based nutrient resorption proficiency is directly related to decomposition characteristics (in the form of litter nutrient concentration) (cf. Quedsted *et al.* 2003) and, together with litter quantity, determines the amount of nutrients returned to the soil. Our data show that in all the studied species (except *B. nana*) the concentration of N in the shed leaves increased strongly (e.g. in *A. polifolia* four-fold!) upon increased N availability. It is most likely that litter quantity also increased upon fertilisation, thus adding to the amount of nutrients returned to the soil. The secondary effect of increased N availability on P resorption proficiency is also important: it increased in *B. nana* and *V. uliginosum* upon fertilisation. Combining the patterns in N and P proficiency suggests that, in many species, the N:P ratio of leaf litter will strongly increase in response to increased N availability, thereby possibly leading to a strong P control over the decomposition process. Such a change from N-controlled to P-controlled litter decay has already been observed in southern Sweden and north-west Europe as a result of increased atmospheric N deposition during the past few decades (Aerts and De Caluwe 1997; Bobbink *et al.* 1998). It should be noticed, however, that we added high amounts of N (10 g N m⁻² yr⁻¹) to our plots, almost certainly overestimating future effects. Nevertheless, it is obvious from our data that the potential changes are substantial.

Earlier work (Killingbeck 1996; Aerts and Chapin 2000) and also this study have shown that there are considerable species-specific and growth-form differences in nutrient resorption proficiency, and that growth-form dominance is thus an important component of the plant-mediated feedback on soil nutrient cycling (Chapin 1993; Aerts 1999). It has also been shown that increased N availability can have direct effects on decomposition rates, but also indirect effects through shifts in the species composition of the vegetation (Wookey *et al.* 1993; Wookey *et al.* 1994; Chapin *et al.* 1995) and subsequent effects on the chemical composition of the bulk litter (Shaver and Chapin 1991; Cornelissen 1996; Hobbie 1996; Shaw and Harte 2001). We did not determine quantitative changes in species composition of our plots, but cover estimates carried out after four years of N fertilisation suggested that the cover of both *A. polifolia* and *R. chamaemorus* had decreased, whereas the cover of *E. vaginatum* had increased (data not shown). Increased dominance of a graminoid species following long-term N fertilisation was also found in a dwarf shrub heath community close to our site (Press *et al.* 1998). This emphasises that, for long-term assessment of plant-mediated effects on soil nutrient cycling, including the partitioning of nutrients between the resorption and the decomposition pathway, we need to know not only how given species respond to global changes, but also how the species composition of the vegetation changes.

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Effects of increased N availability and CO₂ concentration on late seasonal N dynamics in the graminoid *Molinia caerulea*

with Sylvia Toet, Richard S.P van Logtestijn and Rien Aerts

Global environmental change very likely affects the internal N economy of plant species. We investigated the effect of increased N availability and CO₂ concentration on the contribution of leaf N resorption to the late seasonal N economy of the perennial deciduous graminoid *Molinia caerulea*.

Plants were grown in a factorial combination of two levels of N and CO₂ in the greenhouse. After measuring N pools before and after leaf senescence, the leaf N allocation, N resorption efficiency and contribution of leaf N resorption to late seasonal N economy were calculated.

Increased N availability reduced N allocation to leaves, but did not affect N resorption efficiency, which led to decreased contribution of leaf N resorption to late seasonal N economy. The CO₂ treatment did not affect N allocation to leaves or N resorption and there was no CO₂xN interaction.

Late seasonal N economy probably shifted from recycling strategy towards reserve formation during growth in response to N fertilization. We conclude that internal N cycling in *Molinia caerulea* is not affected in a CO₂ richer world, but only in an N richer world.

INTRODUCTION

The annual N demand of natural vegetation is supplied by root uptake of N from the soil, but also by N pools stored within perennial tissues (Aerts and Chapin 2000). In spring, the stored N pool can be used for (competitive) early regrowth of foliage, even before the start of N uptake from the soil (Thornton and Millard 1993, Millard 1996, Bausenwein *et al.* 2001). At the end of the growing season, important N sinks are seed production, and in perennial species also storage formation for next seasons' regrowth. To meet these late seasonal demands, not only may the soil act as a source of N (Millard 1996), but also the senescing vegetative tissue. As a considerable part of the total plant N pool is allocated to leaves (Aerts and De Caluwe 1989, Zak *et al.* 2000), resorption of the N before leaf fall is crucial to reduce N losses and it contributes significantly to the annual N economy of plants (Aerts and Chapin 2000).

The potential contribution of leaf N resorption to the late seasonal N economy of plants can be substantial. Two factors play an important role herein: the relative size of the leaf N pool relative to the total plant N pool (leaf N allocation), and N resorption efficiency. As already mentioned, the N allocation to leaves can be substantial: e.g. in the grass *Molinia caerulea* it was about half of the total aboveground N pool (Aerts and De Caluwe 1989). Only part of this pool can be resorbed: the N resorption efficiency of naturally occurring plant species is about 50% on average (Aerts 1996, Van Heerwaarden *et al.* 2003a). Defoliation experiments showed the importance of the contribution of N from senescing leaves to N storage. In *M. caerulea* defoliation resulted in halving the stored N pool at the end of the season (Thornton and Millard 1993).

Variation in environmental factors can cause substantial interspecific and intraspecific variation in the factors that control late seasonal N economy. Therefore, the rapidly increased availability of nitrogen and CO₂ in natural ecosystems as a result of the industrial revolution and explosive growth of the human population (Vitousek 1994) will most likely affect some of the late seasonal processes in plants and also their N economy. This may significantly affect the fitness of plant populations in many (semi-) natural ecosystems (Aerts 1999). The observed effects of increased N availability on N allocation (Aerts and De Caluwe 1989, Aerts *et al.* 1992a, Makino and Mae 1999), as well as N resorption efficiency (Aerts 1996) have been ambiguous and no clear patterns emerge. Enhanced CO₂ has occasionally been observed to decrease N allocation to leaves (Makino *et al.* 1997, Temperton *et al.* 2003), dependent on fertilization (Zak *et al.* 2000), or have no effect at all (Roumet *et al.* 1996, Harmens *et al.* 2001), whereas it probably increases N resorption efficiency (Norby *et al.* 2001).

The lack of consistent effects of increased nutrient and CO₂ availability on leaf N allocation and N resorption efficiency makes it difficult to predict their effects on the late seasonal internal N cycling of plant species. So far, most of the studies on N economy of plants have either assessed CO₂ or nutrient availability effects separately. The aim of this study was therefore to investigate role of N allocation to leaves and N resorption from senescing leaves to the late seasonal N demands and the combined effects herein of increased N and CO₂ availability during growth. The experiments were performed with the perennial deciduous graminoid *M. caerulea*, which stores a substantial amount of N in basal internodes and cord roots at the end of the season (Taylor *et al.* 2001). Thereto, we studied the changes in N pools during leaf senescence in response to factorial combinations of two levels of N availability and CO₂ concentrations. Such a design enables to study both the separate effects and their possible interaction. Only by such a design can a proper evaluation of the combined effects be made.

MATERIALS AND METHODS

Plant material and growth conditions

Sods with *Molinia caerulea* (L.) Moench were collected in the first week of May 1999 from grassland on peat soil in the Gagelpolder, The Netherlands. Individual shoots with a root system were separated from the sod and washed. Per experimental unit (a 1750 ml polyethylene pot, diameter 13 cm) eight shoots were planted in nutrient poor dune sand. An inner lining of lace in

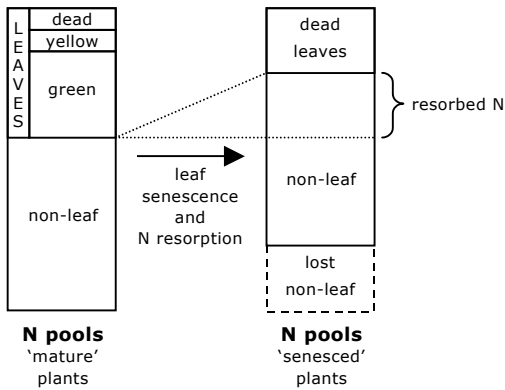


Fig. 1. Schematic description of the different N pools in mature and senesced plants.

the pots prevented loss of sand, but allowed percolation of excess water. The experimental units were placed in the greenhouse to acclimatize for two weeks before the start of the experiment.

A full factorial design with two levels of CO₂ concentration (ambient: A; elevated: E) and two levels of N addition (low-nitrogen: LN; high-nitrogen: HN) with six pots per treatment were established in the following way. Pots were placed in two greenhouse compartments with similar conditions. During the day, the light period was 14 h, temperature 22 °C and the relative humidity (RH) 70%. Photosynthetic Photon Flux Density (PPFD) was 450 μmol m⁻² s⁻¹ at plant height, which was supplied by 400 W HPI-T lamps (Philips, Eindhoven, The Netherlands). These lamps were turned off in

the second half of September, in order to induce leaf senescence under autumn daylight conditions (cf. Aerts and De Caluwe 1989). During the night period, the temperature was 15 °C and the RH 80%. Compartments were kept at either ambient CO₂ conditions (380 vpm) or automatically maintained at elevated CO₂ conditions (700 vpm) with CO₂ from cylinders (Hoekloos, type K 50H). The N applications were 65 kg N ha⁻¹ yr⁻¹ in the LN treatment (comparable to the level of atmospheric N deposition in the Netherlands), and 265 kg N ha⁻¹ yr⁻¹ in the HN treatment (comparable to the combined level of atmospheric N deposition and soil N mineralization in old *Molinia*-dominated heathlands; Berendse 1990). The annual amount of N was applied during the four months growing season in the form of NH₄, as this is the dominant form of N in the natural environment of *M. caerulea*. Other nutrients were given in non-limiting amounts. Nitrogen treatments were applied by fertilizing plants twice a week with 50 ml nutrient solution. In the low-nitrogen (LN) treatment it contained 3.9 mM NH₄Cl, 0.13 mM KH₂PO₄, 0.75 mM K₂SO₄, 0.18 mM MgSO₄ and 0.75 mM CaCl₂, whereas in the high-nitrogen (HN) treatment it contained 15.9 mM NH₄Cl and the other nutrients in a five times higher concentration compared to the LN solution. The solutions of both treatments also contained 3.75 μM H₃BO₃, 0.3 μM MnSO₄, 0.3 μM ZnSO₄, 0.015 μM CuSO₄ and 3 μM Fe(Na)EDTA. The day before each fertilizer treatment, pots were flushed with demineralised water to avoid accumulation of ions (especially chloride), and once a week pots were rotated within each compartment to avoid site effects.

The N treatment was terminated after 15 weeks (end of August), when pots in the LN treatment had received 82 mg N in total and pots in the HN treatment 334 mg. At this stage plants were fully mature with green leaves and unmaturing flowers. By stopping the N application, the increases in N pools of reserve forming organs ('reserve formation' *sensu* Chapin *et al.* 1990) could be fully accounted to remobilisation of N and not to N uptake. Plants were kept well watered with demineralised water after the termination of the N treatment. A control group of plants that grew in outdoor conditions showed clear signs of senescence after 22 weeks (end of October). This was the sign to also stop the CO₂ treatment and transfer the plants to the experimental garden and make the outdoor conditions induce senescence. The lack of CO₂ treatment during the rest of the experiment was justified by the fact that the cold outdoor conditions most certainly minimised photosynthetic activity. Moreover, we wanted to study the effects of CO₂ through alterations in the initial N pools, and not the direct effects of CO₂ on the process of resorption. Moreover, there is no mechanistic hypothesis to expect direct effects of CO₂ on resorption of N. To prevent wind injury, pots were placed in open top chambers, which are described in the study of Visser *et al.* (1997).

Analysis

Per treatment six randomly selected replicate pots were harvested 17 weeks after the start of the experiment (beginning of September). At this time plants were fully mature, bearing panicles, and 90% of the leaf mass was still green. The plants were counted and separated in leaves,

stems and roots. The roots were rinsed, and leaves were cut into green, yellow and dead parts. The different plant parts were dried for 48 h at 70 °C and weighed, except for the green and yellow leaf parts, which were frozen in liquid nitrogen and lyophilised before weighing. These plants will be referred to as 'mature'. A similar second harvest was conducted in the first week of January 2000, when leaves (and stems) had all senesced. These plants will be referred to as 'senesced'. As leaves senesced sequentially between the two harvest dates, dead leaves were regularly collected, dried (48 h at 70°C) and stored for measurements. For each replicate pot pooled yellow and green leaves, dead leaves, and pooled non-leaf plant tissues (roots and stems with panicles) were ground to powder with a ball mill and analysed for total nitrogen content with a Perkin Elmer 2400 series II CHNOS/O analyser.

Calculations and statistics

Calculations are based on values obtained per experimental unit (all the plant material that grew in one pot). See also Fig. 1. Assumptions in the calculations were that (1) the 'mature' total N pool will not increase any further during senescence, (2) the total 'senesced' N pool is not reduced by leaching from the leaf N pool, and (3) resorption takes place from green (g) and yellow (y) parts of the leaves.

Leaf N allocation in 'mature' plants was calculated as the percentage of the total N pool in green and yellow leaves. This part of the pool is potentially available for leaf resorption and redistribution. Leaf N resorption efficiency was calculated as the percentage of the yellow and green leaf N pool of mature plants that was resorbed during leaf senescence. The contribution of leaf N resorption to the late seasonal N requirement (for reserve formation and seed production) was calculated as the percentage of total non-leaf tissue of 'senesced' plants that was provided by the resorbed N from senesced leaves.

Due to unequal variances between different treatments, percentage N allocation to leaves was arcsin(\sqrt{x}) transformed, and dry weights, N pools and N concentrations log(x) transformed prior to statistical testing. This yielded homogeneity of variances in most cases (Levene's test). In a two-way ANOVA, with CO₂ and N treatment as factors, we tested treatment and interaction effects (SPSS for Windows 10.1.0, SPSS Inc., Chicago). The proportion of N lost through litter, N resorption efficiency and contribution of leaf N to late seasonal N requirement were calculated from the untransformed N pool averages and their SD. As these parameters are calculated from other parameters, we manually performed two way ANOVAs with the same set-up as for the other parameters.

Table 1. Results of two-way ANOVAs on various biomass and internal N cycling parameters of *Molinia caerulea* as dependent on N supply and CO₂ level and their interaction. Indicated are P-values.

	Mature plants			Senesced plants		
	N	CO ₂	NxCO ₂	N	CO ₂	NxCO ₂
Biomass						
whole plants	.000	.015	ns	.000	.042	ns
non-leaf tissue	.000	.020	ns	.000	.048	ns
leaves	.000	.007	ns	.000	ns	ns
Nitrogen						
whole plant pool	.000	.008	ns	.000	ns	ns
non-leaf tissue pool	.000	.007	ns	.000	ns	ns
leaf pool	.000	ns	ns	.000	ns	ns
allocation to leaves	.000	ns	ns			
concentration in leaves	.000	.000	.022	ns	ns	ns
resorption efficiency				ns	ns	ns
proportion lost though litter				.019	.013	ns
Contribution of leaf N resorption to reserve formation and seeds				.002	ns	ns

RESULTS

Both N and CO₂ addition increased the total biomass of mature *M. caerulea* plants considerably, without a statistical interaction (Fig. 2, Table 1). The N treatment increased total biomass by 92% under ambient CO₂, and 120% under elevated CO₂. This was associated with an increase of almost 80% in the number of tillers per experimental unit (data not shown). Elevated CO₂ caused an increase of the total biomass by 26% in LN conditions, and 44% in HN conditions (Fig. 2) without an increase in the number of tillers.

The total N pools in the mature plants also increased strongly in response to increased N and CO₂ availability (Fig. 3, Table 1), and increases were even larger than for total biomass. There was no CO₂ × N interaction. The N treatment increased the total N pool by 300% under ambient CO₂, and by 250% under elevated CO₂ (Fig. 3, Table 1). Elevated CO₂ increased the N pool by 38% in LN conditions, and by 21% in HN conditions (Fig. 3).

These effects of the treatments on biomass and N pools were in most cases also significant for the mature leaf and non-leaf tissue mass and N pools separately (Table 1). Total mass showed a trend to increase during the period of leaf senescence, though this was not significant ($P = 0.051$). The treatment effects on mass were also present in senesced plants (Fig. 2, Table 1). These parameters neither showed significant interaction effects of N and CO₂ in the senesced plants.

The allocation of N to active leaves within mature plants was about 30% of the total N pool under LN conditions, and significantly decreased in response to the N treatment (Fig. 4, Table 1). This decrease was mainly due to a larger part of the total N pool being directed to the rest of the plant (Fig. 3), and not because of lower N concentrations in the leaves (Fig. 5,

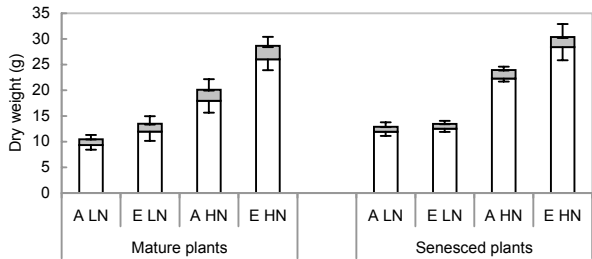


Fig. 2. Total dry weight (+ SE) of leaf tissue (grey bars; - SE) and non-leaf tissue (white bars; - SE) of mature and senesced *Molinia caerulea* plants. Plants were grown in experimental units ($n=6$) under ambient (A; 350 vpm) or elevated (E; 700 vpm) concentrations of CO₂, and low N availability (LN; 65 kg N ha⁻¹ yr⁻¹) or high N availability (HN; 265 kg N ha⁻¹ yr⁻¹).

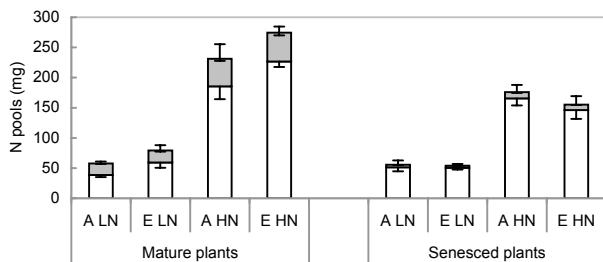


Fig. 3. Total N pool (+ SE) in leaf tissue (grey bars; - SE) and non-leaf tissue N (white bars; - SE) of mature and senesced *Molinia caerulea* plants. Plants were grown in experimental units ($n=6$) under ambient (A; 350 vpm) or elevated (E; 700 vpm) concentrations of CO₂, and low N availability (LN; 65 kg N ha⁻¹ yr⁻¹) or high N availability (HN; 265 kg N ha⁻¹ yr⁻¹).

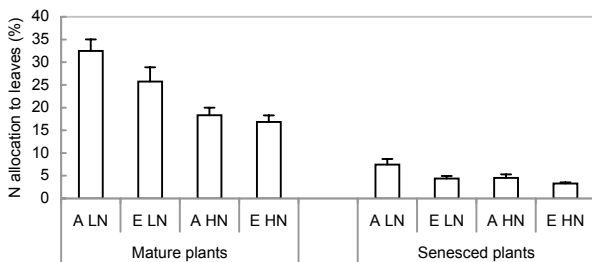


Fig. 4. Percentage (+SE) of total N pool allocated to leaves of mature *Molinia caerulea* plants, and lost through litter after leaf senescence. Plants were grown in experimental units ($n=6$) under ambient (A; 350 vpm) or elevated (E; 700 vpm) concentrations of CO₂, and low N availability (LN; 65 kg N ha⁻¹ yr⁻¹) or high N availability (HN; 265 kg N ha⁻¹ yr⁻¹).

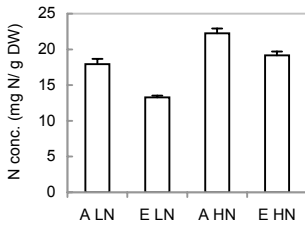


Fig. 5. Nitrogen concentration in green leaves (+ SE) of mature *Molinia caerulea*. Plants were grown in experimental units (n=6) under ambient (A; 350 vpm) or elevated (E; 700 vpm) concentrations of CO₂, and low N availability (LN; 65 kg N ha⁻¹ yr⁻¹) or high N availability (HN; 265 kg N ha⁻¹ yr⁻¹).

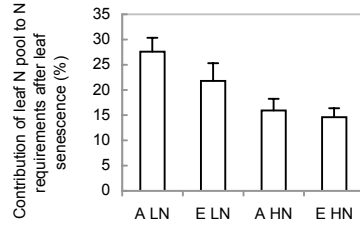


Fig. 6. Percentage (+SE) of total non-leaf N pool that originates from green leaves after leaves have senesced in *M. caerulea* plants. Plants were grown in experimental units (n=6) under ambient (A; 350 vpm) or elevated (E; 700 vpm) concentrations of CO₂, and low N availability (LN; 65 kg N ha⁻¹ yr⁻¹) or high N availability (HN; 265 kg N ha⁻¹ yr⁻¹).

Table 1). The CO₂ treatment showed a trend ($P = 0.09$) of decreasing N allocation towards leaves, which was presumably caused by a significantly lower leaf N concentration in plants grown under elevated CO₂ (Fig. 5, Table 1). There were no significant CO₂ × N interaction effects on N allocation. The changes in leaf N allocation did not coincide with a change in biomass allocation to leaves in response to the treatments: when averaged over all treatments leaves made up $12.4\% \pm 0.6$ (SE; n=24) of the total biomass. The N pool potentially available for leaf N resorption during senescence thus varied only as a result of the N treatment and not of the CO₂ treatment.

Before leaf abscission, a considerable part of the leaf N pool was withdrawn from the senescing leaves. Nitrogen resorption efficiency was neither affected by the N nor by the CO₂ treatments (Table 1). When averaged over the treatments, the N resorption efficiency was $84.9\% \pm 1.0$ (SE; n=24). The final N concentration in the litter (indicating the N resorption proficiency - *sensu* Killingbeck (1996)) was neither altered by the treatment (Table 1). Averaged over the treatments, N resorption proficiency was 3.6 ± 0.25 mg N g⁻¹ DW (SE; n=24). The fraction of N that was not resorbed from the leaves during senescence and lost through litter decreased about one third due to the N and CO₂ treatments, and was only about 5% of the total N pool (Table 1, Fig. 4; senesced plants).

The contribution of resorbed N from leaves to the late seasonal N requirements of *M. caerulea* plants was only significantly affected by the N treatment, and again no interaction effects were found (Fig. 6, Table 1). A marked decrease in response to N occurred, as under LN conditions still $25.0\% \pm 1.6$ (SE; n=12) of the non-leaf N pool, which originated from the mature leaf N pool, was attributable to the process of leaf N resorption, whereas this percentage was only $15.0\% \pm 1.0$ (SE; n=12) under HN conditions. This decrease was mainly due to the previously described change of leaf N allocation in mature plants, and the absence of changes in N resorption efficiency.

DISCUSSION

The experimental design

We measured the contribution of leaf N resorption to the late seasonal N requirement of *Molinia caerulea*, under the assumptions that (1) the N pool of the 'mature' stage was at its maximum, (2) observed late seasonal N losses only occurred from the non-leaf N pool, and (3) the LN treatment represented an N limiting condition. First, the assumption that the N pool was at maximum at the 'mature' stage is supported by the fact that the N treatment was terminated two weeks before the first harvest. Besides, most of the applied N had been taken up before the harvest of the 'mature' stage (particularly under E LN conditions). Second, the discrepancy between the N pools of the mature and senesced stage was most probably not due to leaching

from the leaf N pool, as it is a minor N loss process in *M. caerulea* (Morton 1977). However, it might become significant when plants are grown under very high N availabilities (Chapin and Van Cleve 1996). Nevertheless, the senescing plants have been relatively shielded from rain in OTC's. Leaching from other non-leaf N pools cannot be ruled out, but the largest part of the N loss is probably due to seed dispersal. Third, despite the fact that our LN treatment represented a rather high N dosage compared to other N fertilisation experiments, the biomass production of *M. caerulea* was N limited, as can be concluded from the biomass increase under HN, and the observation that in the LN treatments the applied N dosage had almost entirely been assimilated. So the aforementioned assumptions are correct to our best knowledge.

No effects of CO₂ or NxCO₂ interaction on late seasonal N economy

In this study, interaction effects between N and elevated CO₂ were not observed in the late seasonal N economy of *M. caerulea*, despite the fact that such effects have been reported for photosynthesis, N metabolism, and growth in various other species (Stitt and Krapp 1999). Effects of CO₂ on the contribution of remobilized leaf N to the late seasonal N requirement were also non-significant in our experiment. This resulted from CO₂ neither having an effect on N allocation to leaves, nor on N resorption efficiency, though there seemed to be a trend for decreased leaf N allocation with elevated CO₂ concentration. A comparable picture emerges from the literature, as a lack of response of leaf N allocation to elevated CO₂ was observed in the forage grass *Dactylis glomerata* (Harmens *et al.* 2001) and 11 Mediterranean grass species (Roumet *et al.* 1996), though *Alnus glutinosa*, *Pinus sylvestris* (Temperton *et al.* 2003), and rice (Makino *et al.* 1997) appeared to decrease the allocation of N to leaves in response to elevated CO₂. The meta-analysis of Norby *et al.* (2001) that investigated the effects of elevated CO₂ on mature and senesced leaf nutrient concentrations suggested that N resorption efficiency is somewhat decreased. This would make the contribution of leaf N resorption to late seasonal N economy smaller. However, this did not happen in our experiment. Therefore, we will further confine the discussion to the effects of N only.

Nitrogen supply effects on late seasonal N economy

Although we applied fairly high N amounts in (in the LN treatment), we found that increased N availability clearly reduced the contribution of resorbed leaf N to late seasonal N requirements. This was not the result of a changed N resorption, as both N resorption efficiency and proficiency showed no response to fertilization. For N resorption efficiency, this lack of response was anticipated, as a literature review showed that there are no clear nutritional controls on N resorption efficiency (Aerts 1996). The unresponsiveness of N resorption proficiency was, however, not expected. In many cases, N resorption proficiency decreases notably with increasing nutrient availability (Shaver and Melillo 1984, Kemp *et al.* 1994, Vitousek 1998, Van Heerwaarden *et al.* 2003b). It appears that the N resorption proficiency of *M. caerulea* is unresponsive to fertilization. Moreover, the low litter N concentration suggests that the plants exhibited complete N resorption, as can also be derived from the high N resorption efficiency. High N resorption efficiency of about 75% seems to be typical for *M. caerulea* (Pfadenhauer and Lütke Twenhöven 1986, Aerts and De Caluwe 1989). The unresponsiveness of N resorption proficiency is also in contradiction with the idea that N resorption from senescing leaves is related to the N status of green leaves.

The clear effect of increased N availability on the contribution of resorbed leaf N to late seasonal N requirements was mainly the result of a reduced N allocation to leaves. Such a response of leaf N allocation to fertilization has been observed before in *M. caerulea* (Aerts and De Caluwe 1989). However, this may be species-specific, as N allocation to leaves increased upon enhanced N supply in four *Carex* species (Aerts *et al.* 1992a). The reduced allocation of N to leaves observed in our experiment implies that resorption from stems and/or direct reserve and seed formation during growth became relatively more important for late seasonal N economy than resorption from leaves. Resorption from the stem N pool presumably contributed little to late seasonal N requirement, because compared to leaves, a considerable smaller portion of the aboveground N pool is allocated to stems (Aerts and De Caluwe 1989). Nevertheless, stems of *Molinia caerulea* have a high N resorption efficiency (75%) as well (Aerts and De Caluwe 1989).

Based on that study, we calculated that including the stem N pool in the non-leaf N pool only caused a minor underestimation (smaller than half the standard error) in the calculation of the contribution of leaf N resorption to late seasonal N requirement. On the other hand, direct reserve formation, or seed production during growth probably increased due to the N treatment. The biomass allocation data (not shown) indicated that the allocation of biomass towards roots and panicles increased in response to the fertilization. The same response was also observed for aboveground N allocation to panicles in *M. caerulea* by Aerts and de Caluwe (1989). So under high N availability, the leaves of *M. caerulea* became less important as a source of N for the late seasonal N requirement, probably in favor of direct reserve formation in roots and seed production before leaf senescence.

However, stored N and not seed production is the fraction directly beneficial for regrowth of the standing vegetation in the following season (Bausenwein *et al.* 2001). The relative contribution of resorbed leaf N to storage formation was calculated using a labelling study with *M. caerulea* (Thornton and Millard 1993). The data presented in that study consist of changes in the ¹⁵N pools in plants that had received labelled N during one year, and unlabeled N the second year. With these data we calculated that at low N availability 33% of the remobilised N from leaves was redirected to storage organs and the rest to seeds, whereas at high N availability, 85% of the remobilised leaf N went into storage. Using these values and the assumption that not only a part, but all seeds detached during leaf senescence, we estimated that the contribution of resorbed leaf N to storage formation was only about 10% under low nitrogen conditions, but up to 25% under high nitrogen conditions (uptake and direct storage formation during growth is responsible for the other part). This means that storage formation became a more important sink for the resorbed leaf N at the end of the growing season. Under natural, unfertilised conditions, Pfadenhauer and Lütke Twenhöven (1985) found that the N pool of reserve forming organs increased with 44% during aboveground senescence of *M. caerulea*, but uptake from the soil N pool could also have contributed substantially to this (Millard 1996). In conclusion, it appears that under fertilised conditions remobilisation of leaf N is less important in late seasonal N economy, and it mostly contributes to N reserve formation.

The observed shift in the source of N to meet the late seasonal N requirement (from recycling toward direct uptake during growth) is in agreement with the hypothesis that mechanisms to conserve nutrients become less efficient when the availability of growth-limiting nutrients increases (Feller *et al.* 1999). Recycling strategies are then replaced by accumulation or reserve formation strategies during active growth. Such changes have to be evaluated from a whole-plant perspective and in the light of alternative allocation patterns (Chapin *et al.* 1990), because N resorption efficiency is not the sole conservation mechanism that influences the importance of N recycling. This might be the reason that N resorption efficiency does not show a clear-cut response to increased nutrient availability: both increased and decreased resorption efficiency have been found and often no response at all (Aerts 1996). It may be clear from our experiment that without a change in N resorption efficiency, a decrease in the importance of the leaf N resorption for the whole plant's N economy is found in response to N fertilization. For evaluating such responses, allocation patterns (including allocation to roots and rhizomes) are important as well. Therefore, the percentage of resorbed leaf N contributing to the late seasonal N requirements is a more meaningful and more accurate parameter than N resorption efficiency alone. Of course, we recognize that it will not always be possible to include all roots when conducting this kind of experiments in the field.

In conclusion, our data show that in the graminoid *M. caerulea* N fertilization decreased the contribution of leaf resorption to the late seasonal N requirements, and that there were neither effects of CO₂ nor a NxCO₂ interaction. The main reason for this N effect was a decreased N allocation towards leaves during growth and not reduced N resorption efficiency. Nitrogen fertilization seems to enhance N reserve formation during growth.

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General discussion

The general aim of this thesis was to study the factors that cause variation in leaf nutrient resorption during senescence, namely methodology, responses to environmental change, and intrinsic (biotic) factors that are related to species-specificity and growth form. The experiments described in this thesis have generated ideas how to reduce the variation that is inherent to methodology. Moreover, these experiments have added to our knowledge of how external factors influence nutrient resorption from senescing leaves. Finally, the results also have implications for nutrient cycling.

METHODOLOGY

Mass loss and leaf area shrinkage during leaf senescence can be considerable and can differ substantially among species. This was one of the main arguments put forward in Chapter 2 to emphasize the need to correct for these changes when measuring nutrient resorption efficiency on mass or area basis. To be able to make such corrections special adaptations in the experimental set-up are needed, i.e. to preselect leaves, monitor the changes in the leaf area photographically, and calculate resorption efficiency on leaf area basis with a correction for leaf area shrinkage (see experiment of Trémolières *et al.* (1999)). Another type of experimental set-up that is not inherently generating biased results is to measure the pools of the investigated nutrient on a whole plant basis. However, this might only yield accurate results in pot experiments, as root systems are hard to retrieve completely from soils in a natural situation.

Mass resorption is responsive to external factors

In Chapter 2, a small compilation of data was presented that shows that mass resorption during leaf senescence differs substantially among species. Moreover, different treatments also seem to affect mass resorption during leaf senescence (Chapter 4): mass resorption during leaf senescence (area based) was significantly higher under increased N availability compared to the control (data not shown). However, calculating the results on a leaf area or unit basis instead of mass basis did usually not influence the general conclusions about nutrient resorption that could be drawn from these data. In this experiment, the responses of resorption proficiency to fertilisation were large enough to overrule the counteracting responses of mass resorption during senescence. The data from the experiment with the graminoid *Molinia caerulea* also showed a doubling of leaf mass loss during senescence in response to the CO₂ treatment: 35% ± 8 (SD) under elevated CO₂ and 16% ± 4 (SD) under ambient condition. This response might have been due to the increase in total non-structural carbohydrates (TNC) at elevated CO₂ concentration (Poorter *et al.* 1997).

In conclusion, mass resorption differs not only among species but can also be affected by variation in external conditions. This may also be true for leaf shrinkage, thus making another point to apply the advocated experimental set-up where possible. Such a set-up may especially decrease experimental variation in ecological research, when different species and different environmental conditions are compared.

A new way to express resorption efficiency

The parameter resorption efficiency could gain more physiological and ecological significance by incorporating the Ultimate Potential Resorption (UPR) in its calculation. The UPR of a nutrient is defined as the lowest level to which this nutrient can be reduced in senescing leaves of any species (Killingbeck 1996), and probably defines the biochemical lower limits. The UPR of N has

been estimated to be approximately 3 mg N g⁻¹ DW litter for various species (Killingbeck 1996, Aerts and Chapin 2000, Côté *et al.* 2002), and this fraction of N contains (among other compounds) the chlorophyll catabolites (Matile *et al.* 1999). For P, the UPR is estimated at 70-100 µg P g⁻¹ DW litter (Killingbeck 1996, Aerts and Chapin 2000, Côté *et al.* 2002). The definition and identification of UPR levels implies that litter with these nutrient levels had a resorption efficiency of actually 100% during senescence. The formula for UPR-corrected resorption efficiency (RE*) would then be

$$RE^* = 100\% * \left(1 - \frac{[N]_{\text{absced}} - [N]_{\text{UPR}}}{[N]_{\text{green}} - [N]_{\text{UPR}}}\right)$$

How UPR-correction can affect values of resorption efficiency is illustrated in Figure 6.1, which shows the nutrient content in green and dead leaves of a standard plant species growing in nutrient-rich versus nutrient-poor conditions. From a large literature survey, Aerts (1996) concluded that nutrient availability and nutrient resorption efficiency are hardly related. Therefore, a resorption efficiency of 60% is shown for both situations in Figure 6.1. Calculating the UPR-corrected resorption efficiency (RE*) yields resorption efficiency values that do show a significant difference between the two situations. In theory, such a correction could lead to different conclusions about the relationships between nutrient resorption and other parameters (e.g. nutrient availability, growth forms). However, this may require more research first as to assess whether all species and growth forms share the same UPR, and whether external factors affect UPR.

A new way to calculate resorption proficiency

Another improvement would be to account for the loss of mass or leaf area shrinkage in the parameter resorption proficiency. The reason is that the (mass-based) concentration of the remaining nutrients in litter increases when other bulk components are removed from the senescing leaves (see also Chapter 2). Mass loss can be substantially and differs among species and treatments (see above), which leads not only to underestimation in mass-based nutrient resorption proficiency, but also to additional variation. This may also be the case for leaf shrinkage and area-based resorption proficiency.

Using area based resorption proficiency may also generate biased results, especially when comparing NRP among different species. Differences in leaf thickness among different species will influence such data (Killingbeck 2004). Both methods (mass based as well as area based calculation) thus present difficulties. Differences in litter specific leaf area (SLA) will influence NRP. Variation in SLA was observed between the N treatments and among species in the underlying data from Chapter 4. SLA was also observed to differ among plant groups (Cornelissen *et al.* 1999) and plants with differences in their leaf anatomy (Pyankov *et al.* 1999). Variation in SLA may thus be the result from species specificity, but also from experimental manipulation.

Most illustrative for the whole problem is the potential incorrect classification with respect to the 'completeness of resorption' scheme of Killingbeck (1996). From the levels indicating 'complete N resorption', I could calculate that these leaves have an SLA of 14 mm²/mg. When the same amount of N (indicating 'complete N resorption') would be in a leaf with a smaller SLA as a result of higher mass density (for instance due to species specificity, or variation in mass loss and area shrinkage), the apparently higher N concentration may only indicate 'intermediate' or 'incomplete' resorption.

To reduce the influence of the abovementioned points the nutrient resorption proficiency may be calculated as the amount of a nutrient in the litter but projected to the original leaf mass of

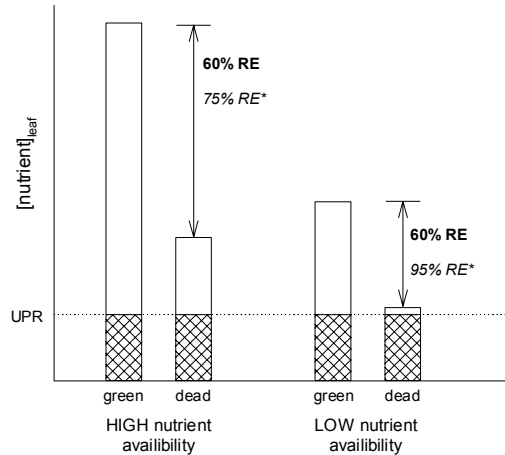


Fig. 1. Hypothetical case of nutrient resorption efficiency (RE) under different nutrient availabilities and the effect on the value of resorption efficiency when corrected (RE*) for the ultimate potential resorption (UPR). See text for further explanation.

the mature leaves. Like for resorption efficiency, the methodology of preselection and leaf area monitoring may also be used to correct resorption proficiency measurements. This might yield less biased nutrient resorption proficiency values when mass loss and or shrinkage are not 'concentrating' the nutrients left behind in the litter.

In conclusion, the parameters and methods to measure resorption can gain accuracy from a correct experimental set-up and from fundamental research on the levels of ultimate potential resorption (UPR). Only then can questions concerning responses to environmental change and species-specificity be answered correctly and with more certainty.

RESPONSES TO EXTERNAL FACTORS

In chapters 4 and 5 the 'old' methods were used to evaluate responses to increases in N availability and CO₂ concentration, as these experiments were designed with the knowledge we had at that time. The N and P resorption efficiencies from senescing leaves were not very responsive to increased N availability or to elevated CO₂ concentration, and N and P resorption proficiencies responded differently to increased N availability.

In the graminoid species *Molinia caerulea* (Chapter 5), elevated CO₂ did not lead to a response in N resorption efficiency. From the '*Molinia*' data the N resorption proficiency can be calculated, which also showed a lack of response to CO₂. On the basis of a meta-analysis (Norby *et al.* 2001), a small but significant decrease in litter N concentration was expected in response to elevated CO₂. As the decrease in green leaf N concentration in response to elevated CO₂ (Cotrufo *et al.* 1998) is much larger than that in litter, also a decrease in N resorption efficiency was expected. However, a significant response of N resorption proficiency to elevated CO₂ is often not detected in single experiments (Norby *et al.* 2001). The large variation to response ratio might therefore also have been a reason for not finding responses in N resorption to elevated CO₂ in the '*Molinia*' experiment. In addition, no CO₂ effect was observed on the contribution of resorbed N from senescing leaves to the late seasonal N economy of *M. caerulea*.

The responses of nutrient resorption efficiency and proficiency to increased N availability are discussed in the paragraphs below, and in addition, a hypothesis to explain this response of N and P resorption proficiency is presented.

Resorption efficiency

The relation between the N status of green leaves and N resorption efficiency has been the subject of much debate that appeared to have been settled with the literature review of Aerts (1996). This review showed that such a relation was weak or non-existing. The data presented in Chapters 4 and 5 are in accordance with this conclusion. However, Côté *et al.* (2002) questioned the possibility of ever finding a (small) correlation through a literature survey as many non-nutritional factors that affect nutrient resorption add variation to such data. They also presented a negative correlation between tree nutritional status and nutrient resorption efficiency. However, their calculations were based on N and P concentrations and not corrected for mass loss. As already mentioned above, mass loss appears to be larger at higher soil N availability (Chapter 4). This could mean that the correlation between nutrient status and nutrient resorption efficiency as found by Côté *et al.* (2002) may be caused by the response of mass loss during senescence. The maximum value of mass loss that could theoretically have led to the reported correlation appeared to be only 30% (15% underestimation in RRE of 70%), as could be calculated from the N data. This mass loss value fits within the range of reported mass loss percentages (see Chapter 2), and at least makes their reported correlation questionable.

It is more likely that there is a relation between N availability and the relative contribution of remobilised N from leaves to the late seasonal N requirements. The results of the experiment with *Molinia caerulea* (Chapter 5) clearly showed a decrease of this parameter in response to N fertilization. This parameter integrates N resorption efficiency and N allocation patterns on a whole plant level. The reported decrease was the result of a response in N allocation, and not in N resorption efficiency. To take allocation patterns into account when evaluating conservation

mechanisms has already been advocated by Chapin (1990), but may not be always possible in field experiments, as complete root systems are hard to retrieve from the soil.

Resorption proficiency

Increased N availability led to decreased N resorption proficiencies in most species that were sampled in the Stordalen experiment (Chapter 4). This is in agreement with the commonly observed pattern (Shaver and Melillo 1984, Kemp *et al.* 1994, Vitousek 1998). As resorption is an energy and resource consuming process for plants (Thomas and Stoddart 1980), it is tempting to suggest that the observed pattern is the result of a decreased resource investment to remobilise N at increased N availability.

In contrast with the response of N resorption proficiency, a variable response to increased N availability was observed for P resorption proficiency among different species (Chapter 4). This lack of a clear pattern was explained by changes in P requirement and 'completeness of resorption' (*sensu* Killingbeck 1996). Relative to N, the demand for P probably increased after N fertilisation. This is based on the observations that N fertilisation led to growth dilution of P in N-limited peat-bog species (Aerts *et al.* 1992b), and that the N:P-ratio often increases after N fertilisation (Chapter 4, Aerts *et al.* 1992b). After N fertilisation, the increased demand for P may have resulted in a tendency to increase P resorption proficiency. However, after N fertilisation species with already complete resorption under control conditions may not have been able to exploit the potentially available P owing to physiological constraints.

The difference in the responses of N and P resorption proficiency to N fertilisation (Chapter 4) fit the idea that the completeness of resorption of a certain nutrient is related to its requirement. There may be a trade off between the need for a certain nutrient and the costs in terms of energy and resource investment. The energy and resource investment will be directed towards the resorption of the limiting nutrient (or alternative beneficial processes), which will then be resorbed to a level indicating complete resorption. (Note that the word 'limiting' will also be used to indicate limitation of the general resource requirement at the timing of leaf senescence, and not only to indicate growth-limitation.)

I hypothesise in the above described line of thinking that the complete resorption of a certain nutrient is highly energy consuming, and that such an amount of energy will largely be invested in the clearly beneficial resorption of the most limiting nutrient. This leads to the following expectations for the response to N fertilisation:

- N resorption proficiency decreases after N fertilisation, unless the plants growing under control conditions had complete N resorption as a result from severe N limitation (arrow 1 in Fig. 6.2).
- P resorption proficiency increases after N fertilisation, unless the plants growing under control conditions were (already) having complete P resorption due to P limitation (arrow 2 in Fig. 6.2).

The arrows in the circle indicate the response of two plants that are neither N nor P limited, but for instance K limited under the control conditions. In this case, N fertilisation will lead to a further relative shortage of K and an increased energy input to resorb as much as possible of the K pool, to the expense of the resorption of other nutrients, like P.

One of the assumptions in the above hypothesis is that N and P resorption are not directly related to each other. The resorption proficiency of N and P are correlated (Killingbeck 1996), but an underlying mechanism for this correlation is unlikely since the biochemical pathways of N and P resorption are different (Hawkins and Polglase 2000). Another point of consideration is that

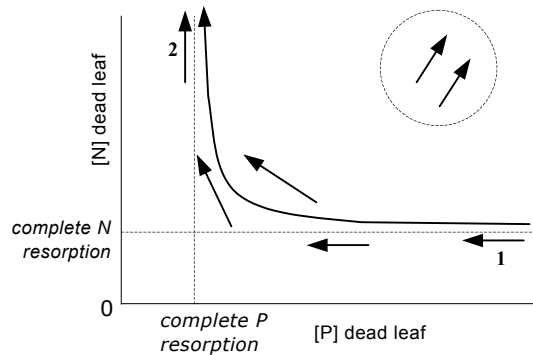


Fig. 2. The response of N and P litter levels (resorption proficiency) to N fertilisation in different transitional situations between N and P limitation. The beginning of the arrows indicates the combination of litter N and P levels in the control situation, and the arrowheads indicate this combination after N fertilisation. See text for further explanation.

there are only indicative levels for completeness of N and P resorption in woody species at the moment. The hypothesis can therefore not yet be tested with non-woody species or additional nutrients. An explanation for the absence of a response to N fertilisation of the N resorption proficiency in *M. caerulea* (graminoid) can therefore not be given with the above hypothesis. More research is needed to establish indicative levels for the classification of completeness of resorption for non-woody species and for other (resorbable) nutrients.

RESPONSES OF NUTRIENT RESORPTION TO COMPONENTS OF GLOBAL CHANGE: IMPLICATIONS FOR NUTRIENT CYCLING

The experiments described in this thesis were conducted with plant species from peatlands. Responsiveness of nutrient resorption to global change could potentially influence nutrient cycling in these ecosystems, in case nutrient input through leaf litter is altered. This can be caused by effects on the chemical composition of the litter and/or by effects on the amount of produced litter.

A response of N resorption proficiency to elevated CO₂ was not observed in *M. caerulea* (see above). Only small changes in litter production are expected in response to elevated CO₂: plant biomass increase in nutrient poor ecosystems might be small (Poorter 1998), but there is no response from the allocation of biomass towards leaves (Poorter *et al.* 1996). An indirect effect of elevated CO₂ is, however, expected to occur through the rise in temperature (IPCC 2001), which may lead to an increased mineralisation rate, and a higher N availability (Rustad *et al.* 2001). This is then in addition to the already higher N availability as a result from increasing N deposition (Vitousek 1994).

Increased N availability leads to changes in N and P resorption proficiency (see above) that contribute to alterations in nutrient cycling. The often reported increase in leaf litter N concentration after N fertilisation (Chapter 4, Shaver and Melillo 1984, Kemp *et al.* 1994, Vitousek 1998) will lead to a higher N input through leaf litter. In addition, the amount of litter also increases in response to N fertilisation (Press *et al.* 1998). This means that the N input through leaf litter shows a positive feedback response to increased N availability, which may result in the speeding-up of the N cycle. The secondary effect of increased N availability on P concentration in litter is also important: it decreased in some species upon N fertilisation (Chapter 4). Combining the patterns in N and P resorption proficiency suggests that, in many species, the N:P ratio of leaf litter will strongly increase in response to increased N availability, thereby possibly leading to a strong P control over the decomposition process. Such a change from N-controlled to P-controlled litter decay has already been observed in southern Sweden and north-west Europe as a result of increased atmospheric N deposition during the past few decades (Aerts and De Caluwe 1997; Bobbink *et al.* 1998). It should be noticed, however, that the level of N fertilisation in the experiment described in Chapter 4 (10 g N m⁻² yr⁻¹) almost certainly overestimates future effects. Nevertheless, it is obvious from these data that the potential changes are substantial, and that the positive feedback mechanism leads to an even further increase in soil N availability in response to increased soil N availability.

Another mechanism that may alter nutrient cycling is the change in species composition and species dominance in response to nutrient fertilisation. When such shifts occur, nutrient input may change as a result of species-specific differences in litter production, but also as a result of a difference in N and P resorption proficiency among PFTs (Chapter 3). In general, Arctic ecosystems respond to fertilisation with a strong increase in biomass of (fast-growing) grasses (Dormann and Woodin 2002). The nutrient concentration in the litter that grasses produce is somewhat lower (Chapter 3), but the concomitant rise in the amount of litter (Press *et al.* 1998) may overrule and nevertheless lead to a positive feedback mechanism. In this case, the intrinsic differences among the NRP of growth forms plays a smaller role in nutrient input through leaf litter than the amount of leaf litter. In conclusion, species shifts may add to the positive feedback mechanism in response to increasing N availability, depending on changes in abundance of functional groups, and affect the rate of nutrient cycling in northern peatlands.

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From leaf to litter: nutrient resorption in a changing environment

Leaf nutrient resorption, the withdrawal of nutrients from senescing leaves, is an important process for both plants and ecosystems. Through this process, plants prevent nutrient losses that would otherwise occur the moment leaves are shed. The resorbed nutrients are reused in tissues such as growing buds, storage organs, or seeds. This process is especially important for the retention of nitrogen (N) and phosphorus (P), as half of the N and P content of leaves is resorbed on average. In natural ecosystems, leaf nutrient resorption controls the soil nutrient input through leaf litter, by decreasing the potentially available nutrient pool in mature leaves. It may thus be clear that if plants alter their leaf nutrient resorption in response to changes in their environment, a modification will take place in the important soil nutrient input from leaf litter. Human-induced changes in the environment have been taking place since the industrial revolution. The burning of fossil fuels to meet the human energy demands has led to a sharp rise in atmospheric CO₂ concentration, and the production and use of fertilisers has led to an increase in soil N availability, not only in agricultural ecosystems. The rising CO₂ concentration is also expected to contribute to global warming, with above-average increases at high latitude sites. In the Northern hemisphere, high latitude sites are largely covered with nutrient poor peatlands. These ecosystems may in addition experience an increase in soil N availability through an increased mineralisation in response to the expected temperature rise. The changes in atmospheric CO₂ concentration and N availability have direct effects on plants, but the nature and magnitude of effects on whole ecosystems are still largely unknown, especially in nutrient poor ecosystems like oligotrophic and mesotrophic peatlands.

It may be clear that the increases in N availability and CO₂ concentration may influence soil nutrient input through leaf litter by affecting leaf nutrient resorption. Therefore, the main goal of this thesis was to investigate the responsiveness in leaf nutrient resorption to the external factors N availability and CO₂ concentration, and examine intrinsic patterns in leaf nutrient resorption that are related to species-specificity and growth form. Two parameters were available to quantify nutrient resorption: nutrient resorption efficiency and nutrient resorption proficiency (NRP). The first parameter expresses, for a certain nutrient, the percentage that is resorbed from the mature green leaf pool. NRP, on the other hand, represents the level to which a certain nutrient has been reduced in litter. Apart from the two main issues (responsiveness to external factors and intrinsic patterns), the methodology of resorption measurement was also examined. In the first part of the thesis I focused on methodology, but this issue was revisited throughout the thesis.

When using the parameter resorption efficiency, one has to be aware that leaf properties that are used as a measurement basis (like leaf area or leaf mass) might change during senescence. This influences the measured value of resorption efficiency. A mathematical model presented in Chapter 2 quantifies the magnitude of the resulting error. This model describes the effect of changes in measurement basis on the difference between the real resorption efficiency (RRE) value and the measured resorption efficiency (MRE). It shows that even moderate senescence-related changes in a measurement basis can lead to considerable underestimations of RRE. Changes in the measurement basis can be substantial, as literature data reports leaf mass loss percentages as high as 40% and leaf shrinkage up to 20% during senescence. Such a level of change seriously compromises the MRE when not corrected for. I calculated that the average RRE for N and P of terrestrial plants must be about 6 to 10 %-points higher than the 50% and 52% reported by Aerts (1996), but deviations can be much greater for individual species. This implies that nutrient resorption from senescing leaves is even more important for nutrient

Summary

retention in terrestrial plants than thought so far. To reduce the methodological error and to minimise the difference between MRE and RRE, the pre-selection of leaves and monitoring of the measurement basis throughout the duration of the experiment are strongly recommended.

Differences in nutrient resorption efficiency among different growth forms are known to be small, but for NRP such data have been incomplete. Therefore, I investigated whether a consistent and region independent growth-form pattern could be found for NRP. The data consisted of N and P resorption proficiencies from 73 different peatland species belonging to five different growth forms growing in three (large-scale) geographically and climatically distinct regions (Chapter 3). N and P resorption proficiency could indeed be consistently organised at the level of growth forms, independently of geographical region: *Sphagnum* mosses had the highest average N resorption proficiency (i.e. lowest litter N concentration), followed by graminoids, evergreens, deciduous species, and forbs. For P, the only difference was that *Sphagnum* mosses were the second most resorption proficient. The differences in absolute values of NRP among regions are probably determined by regional climatic and land use management factors. Given the consistency, growth forms can be used to predict NRP patterns along large-scale environmental and geographical gradients.

To investigate the responses of nutrient resorption to N fertilisation, a field experiment was conducted in sub-arctic Sweden (Chapter 4). A secondary aim was to use different measurement bases to calculate resorption efficiency and proficiency, and evaluate the resulting patterns. I hypothesised that N fertilisation would not affect N nor P resorption efficiency, but that it would lead to lower N resorption proficiency and higher P resorption proficiency. Six dominant plant species were sampled (*Andromeda polifolia*, *Betula nana*, *Empetrum hermaphroditum*, *Eriophorum vaginatum*, *Rubus chamaemorus*, and *Vaccinium uliginosum*). In contrast to the expectations, a general trend of decreased N resorption efficiency occurred in response to N fertilisation, but the expected decrease in N resorption proficiency was seen in all species but one (*B. nana*). P resorption efficiency showed a variable response, and only two out of six species showed the expected increase in P resorption proficiency at increased N availability. The different calculation methods generally produced similar responses of resorption efficiency and proficiency to N fertilisation, except for P resorption efficiency. The results indicate that increased N availability at high-latitudes leads to more N being returned to the soil through leaf litter. However, decomposition of such litter would probably become P-limited.

Chapter 5 deals with the role of leaf nutrient resorption in whole plant N economy and its responsiveness to doubled CO₂ concentration and increased N availability during growth. To investigate the contribution of resorbed leaf N to late seasonal N sinks (storage formation, seed production), a greenhouse experiment was conducted with the perennial deciduous graminoid *Molinia caerulea*. Increased N availability reduced N allocation to leaves, but did not affect N resorption efficiency. As a consequence, the contribution of leaf N resorption to late seasonal N economy decreased. The elevated CO₂ treatment neither affected N resorption efficiency nor the contribution of resorption to the whole plant N economy, and there was no CO₂ x fertilisation interaction. Such interaction effects were expected on the basis of literature about other processes (such as photosynthesis, growth, and N metabolism), but it does not seem to apply to the process of N resorption, at least not in *M. caerulea*. The results also indicate that a shift from recycling strategy towards direct reserve formation during growth must have occurred due to the increased N availability, as the role of leaf N resorption in late seasonal N economy decreased. The conclusion from this experiment is that the internal N cycling in *Molinia caerulea* will not be affected in a CO₂ richer world, but only in an N richer world.

In the general discussion (Chapter 6), the main topics (methodology, responsiveness to external factors and intrinsic patterns) are discussed and new ideas are presented. In the methodological section it is argued that, in addition to the results of Chapter 2, the meaningfulness of the parameter resorption efficiency may improve by incorporating the Ultimate Potential Resorption (UPR) into its calculation. UPR is the lowest level to which a nutrient can be reduced in senescing leaves, and this actually implies that litter with a nutrient level as low as UPR should be considered to have had a resorption efficiency of 100%. However, more research is needed first to explore the responsiveness of UPR to external factors and taxonomic specificity. In the section dealing with the responsiveness of NRP to external factors, a model is presented that predicts the complex patterns in N and P resorption proficiency as observed in Chapter 4. It

is based on the ideas (1) that reaching complete resorption of a remobilisable nutrient is relatively more energy and resource demanding than only reaching incomplete resorption, and (2) that the observed patterns can be explained by a trade-off between the additional costs to reach complete resorption and the value of the extra remobilised nutrient for the plant. To finish, the results described in this thesis are placed in the context of soil nutrient input through leaf litter in nutrient poor peatlands that are exposed to changes in their environment. The responsiveness of nutrient resorption to increased soil N availability will clearly lead to more N becoming available through leaf litter. N resorption is (almost) not responsive to doubled atmospheric CO₂ concentration, and elevated atmospheric CO₂ concentration will therefore not influence the nutrient input (noticeably) in this way. Moreover, the consistent differences in NRP among different growth forms may also contribute to changes in nutrient input through leaf litter, when the growth form composition alters in response to changes in the environment.

Van blad tot (af)val: nutriënten resorptie in een veranderend milieu

Tijdens het afsterven van plantenbladeren worden sommige nutriënten door de plant uit deze bladeren teruggetrokken en naar andere delen getransporteerd, zoals groeiende delen, zaden of opslagorganen. Dit proces wordt ook wel aangeduid als 'resorptie' van nutriënten uit afstervende bladeren, en is belangrijk voor zowel de individuele plant alsook het hele ecosysteem. In planten zorgt dit proces er namelijk voor dat het verlies van nutriënten wordt beperkt. Met name is dit van belang voor het behoud van stikstof (N) en fosfor (P), aangezien ruwweg de helft van de hoeveelheid N en P door de plant uit zijn bladeren wordt teruggetrokken. Op ecosysteemniveau beheerst dit proces de hoeveelheid nutriënten die via dode bladeren in de bodem beschikbaar kan komen. Door nutriënten resorptie uit afstervende bladeren is die hoeveelheid echter altijd kleiner dan wat er in potentie beschikbaar zou zijn vanuit groene bladeren. Het moge dus duidelijk zijn dat kwantitatieve veranderingen aan het resorptie proces uit afstervende bladeren tot verschuivingen in de nutriëntentoevoer aan de bodem kan leiden. Zulke veranderingen zouden kunnen plaatsvinden naar aanleiding van de recente milieuveranderingen.

Menselijke handelen heeft ertoe geleid dat het milieu sinds de industriële revolutie aan ongekend snelle veranderingen onderhevig is. Het verbranden van fossiele brandstoffen om aan de energiebehoefte van de mens te voldoen heeft ervoor gezorgd dat de atmosferische koolstofdioxide (CO₂) concentratie sterk is gestegen. Een verdubbeling van het niveau van vóór de industriële revolutie wordt zelfs nog deze eeuw verwacht. Het gebruik van industrieel geproduceerde N bemesters heeft bovendien een verhoogde N beschikbaarheid in de bodem veroorzaakt, en niet alleen in agrarische systemen. Door de verhoging van de CO₂ concentratie in de atmosfeer wordt verder een temperatuurstijging op aarde verwacht, met bovengemiddelde stijgingen in de gebieden gelegen op de hogere lengtegraden. Deze (sub)arctische gebieden zijn op het noordelijk halfrond vooral bedekt met nutriëntenarme veengebieden. Door de hogere temperatuurstijging die daar wordt verwacht zal ook waarschijnlijk de mineralisatie sneller verlopen en ook daar de N beschikbaarheid in de bodem toenemen. Al deze milieuveranderingen hebben directe gevolgen voor plantengroei, maar de aard van de effecten op ecosystemen, en met name die in oligotrofe en mesotrofe veengebieden, zijn nog grotendeels onbekend.

Indien de verhoogde N beschikbaarheid in de bodem en CO₂ concentratie in de atmosfeer een effect zouden hebben op de resorptie van nutriënten uit afstervende bladeren, dan zou dit de nutriënten toevoer aan de bodem kunnen veranderen. De doelen die dit proefschrift dan ook beoogt is te onderzoeken in hoeverre het resorptie proces reageert op veranderingen in N beschikbaarheid en CO₂ concentratie. Bovendien wordt in dit proefschrift ook de intrinsieke patronen van resorptie geanalyseerd die zich op het niveau van soort en groeivorm afspelen. In het onderzoek waren twee parameters voorhanden om resorptie van nutriënten uit afstervende bladeren te kwantificeren: nutriënten resorptie efficiency and nutriënten resorptie proficiency (NRP). De eerste parameter geeft in procenten aan hoeveel van een bepaald nutriënt is geresorbeerd ten opzichte van het gehalte in groen blad, en NRP geeft daarentegen aan hoeveel van een bepaald nutriënt in dood blad is achtergebleven. Het gebruik van deze parameters en de methodologie is, afgezien van de hiervoor beschreven doelen, ook een belangrijk onderwerp in dit proefschrift. Nadat het eerste deel van het proefschrift er volledig aan geweid is, komt het in de andere hoofdstukken nog herhaaldelijk aan bod.

Bij het gebruik van de parameter resorptie efficiency moet men erop bedacht zijn dat bladafmetingen die gebruikt worden als meetbasis (zoals oppervlakte of massa) kunnen veranderen tijdens het afsterven van het blad. Dit beïnvloedt namelijk de waarde van de resorptie efficiency. De grootte van de aldus veroorzaakte afwijking is weergegeven met een

Samenvatting

model dat in Hoofdstuk 2 wordt gepresenteerd. Dit model beschrijft de grootte van het verschil tussen de werkelijke waarde en de gemeten waarde van resorptie efficiency als gevolg van veranderingen in de meetbasis. Hieruit blijkt dat zelfs kleine verandering in de meetbasis tot een aanzienlijke onderschatting van de resorptie efficiency kan leiden. Uit literatuurgegevens is duidelijk dat massaverlies tijdens bladsterfte zelf een waarde kan bereiken tot 40%, en bladkrimp een waarde tot 20%. In combinatie met de gemiddelde gemeten resorptie efficiency voor N (50%) en P (52%), kon met het model worden berekend dat de werkelijke N en P resorptie efficiency waarschijnlijk 6 tot 10%-punten hoger ligt; in individuele gevallen kunnen verschillen zelfs veel hoger uitvallen. Deze uitkomst betekent bovendien dat het proces van N en P resorptie uit afstervende bladeren nog belangrijker is voor het behoud van deze nutriënten in landplanten dan tot nu toe gedacht. Met een aanpassing in de experimentele opzet zou de verandering in de meetbasis gekwantificeerd kunnen worden, en dus rekenkundig gecorrigeerd. De aanpassing houdt in dat de bladeren ver voor het afsterfproces worden geselecteerd, gemerkt en fotografisch vastgelegd tot na het afsterven om zo de veranderingen in het bladoppervlakte te kunnen bepalen.

Uit de literatuur blijkt dat er amper verschillen zijn in de N en P resorptie efficiency gemiddelden tussen verschillende groeivormen, maar voor de parameter NRP was dit nog niet uitgebreid uitgezocht. De vraag die dus in hoofdstuk 3 wordt behandeld is of er een consistente en bijgevolg regio-onafhankelijke indeling in groeivormen te maken is op basis van de gemiddelde waarden van N en P resorptie proficiëntie. De dataset die hiervoor is gebruikt bestond uit N en P resorptie proficiënties van 73 soorten behorende tot vijf verschillende groeivormen en afkomstig uit drie klimatologisch verschillende geografische regio's. De ordening in zowel N als P resorptie proficiëntie gemiddelden bleek consistent en regio-onafhankelijk: *Sphagnum* mossen hadden de hoogste N resorptie proficiëntie (d.w.z. de laagste N concentratie in dood blad), gevolgd door grassen, groenblijvers, bladverliezers en kruiden. Het enige verschil in deze volgorde voor P resorptie proficiëntie was dat de *Sphagnum* mossen de op twee na hoogste resorptie proficiëntie hadden. Het uiteenlopen van de absolute patronen tussen de verschillende regio's is waarschijnlijk veroorzaakt door de verschillen in klimaat en ecologische beheersmaatregelen. Wegens het consistente karakter kan het aggregatieniveau van groeivormen echter gebruikt worden om NRP patronen te voorspellen over grote geografische gradiënten.

Om effecten van verhoogde N beschikbaarheid op de resorptie van N en P te kunnen onderzoeken is een veldexperiment uitgevoerd in subarctisch Zweden (Hoofdstuk 4). Daar is al gedurende vier jaar een bescheiden stukje veengebied blootgesteld aan N bemesting ten bate van experimenten. Het experiment beoogde bovendien een methodologische component te onderzoeken, en wel in hoeverre de verschillende meetbases om N en P resorptie efficiency en resorptie proficiëntie op te berekenen wel een eenduidig beeld geven van de effecten van N bemesting. De uitgangshypothesen voor de effecten van N bemesting luiden dat de N en P resorptie efficiency niet zou worden beïnvloed, maar dat N bemesting wel tot een lagere N resorptie proficiëntie en een lagere P resorptie proficiëntie zou leiden. Zes dominerende plantensoorten werden voor het experiment bemonsterd, en wel *Andromeda polifolia*, *Betula nana*, *Empetrum hermaphroditum*, *Eriophorum vaginatum*, *Rubus chamaemorus*, en *Vaccinium uliginosum*. In tegenspraak met de verwachtingen bleek er een algemene trend te zijn voor verminderende N resorptie efficiency na N bemesting, maar de N resorptie proficiëntie was wel in alle soorten (behalve *B. nana*) afgenomen. De P resorptie efficiency vertoonde een wisselvallige respons op N bemesting, en slechts twee van de zes soorten had een verhoogde P resorptie proficiëntie. In het algemeen leidde het gebruik van de verschillende meetbases tot gelijkgestemde conclusies over de effecten van N bemesting, behalve bij het berekenen van P resorptie efficiency. De resultaten geven aan dat een verhoogde N beschikbaarheid in subarctische veengebieden zal leiden tot een grotere N toevoer aan de bodem via afgestorven blad. De decompositie van deze bladeren zal waarschijnlijk P gelimiteerd raken.

Hoofdstuk 5 behandelt de effecten die een verdubbelde CO₂ concentratie en verhoogde N beschikbaarheid tijdens de groei heeft op de rol van N resorptie in de totale N-huishouding aan het einde van het groeiseizoen (aanleg van reserves en productie van zaad). Om dit te onderzoeken is een kasexperiment uitgevoerd met het bladverliezende en overblijvende *Molinia caerulea* (Pijpestrootje) waarin de veranderingen in de N-pools tijdens het bovengronds afsterven van de planten is gemeten. Het bleek dat er relatief minder N in de bladeren werd geïnvesteerd

bij een verhoogde N beschikbaarheid, maar dat de N resorptie efficiency niet was veranderd. Dit leidde ertoe dat de bijdrage van geresorbeerd N uit bladeren minder belangrijk werd in de totale N huishouding. De behandeling met verdubbelde CO₂ concentratie had geen effect op N resorptie efficiency, noch op de bijdrage van geresorbeerd N uit blad aan de totale N-huishouding. Interactie-effecten tussen N bemesting en CO₂ concentratie op deze parameters bleven ook achterwege, hoewel zulke effecten wel werden verwacht op basis van literatuurgegevens over andere processen, zoals fotosynthese, groei en N metabolisme. De respons op de verhoogde N beschikbaarheid (kleinere rol voor bladeren als bron van N) duidt er bovendien op dat er een verschuiving heeft opgetreden van een recycling georiënteerde strategie richting een opslag formatie georiënteerde strategie in de N-huishouding. De conclusie uit dit experiment is dat de interne N recycling in *M. caerulea* niet zou veranderen in een CO₂ rijkere wereld, maar wel in een N rijkere wereld.

In de algemene discussie (hoofdstuk 6) worden de resultaten uit dit proefschrift bediscussieerd rond drie thema's (methodologie, respons na veranderingen in N en CO₂ beschikbaarheid en gevolgen hiervan voor de nutriëntencycli in noordelijke veengebieden) en nieuwe ideeën gepresenteerd. Zo wordt in het methodologische deel geargumenteed dat de parameter resorptie efficiency aan betekenis zou kunnen winnen indien de *Ultimate Potential Resorption* (UPR) erin zou worden verrekend. De UPR is namelijk het absolute bodemniveau tot waar een nutriënt kan worden geresorbeerd. Ondanks dat de UPR waarde voor N en P hoger is dan het nulniveau, vertegenwoordigt dit per definitie dus eigenlijk de waarde die wordt behaald bij 100% resorptie efficiency. De erkenning hiervan zou kunnen bijdragen aan een verdere zinvolle evaluatie van ecologische patronen, net als het verrekenen van de veranderingen in de meetbasis (hoofdstuk2). Eerst is er echter meer onderzoek nodig naar de variatie in UPR waarden die het gevolg zou kunnen zijn van externe en taxonomische factoren. In het deel dat over de effecten van externe factoren op NRP gaat, wordt een model gepresenteerd dat reactie op N bemesting van N en P resorptie proficiëncy voorspelt zoals beschreven in hoofdstuk 4. Dit model combineert de ideeën dat (1) complete resorptie bereiken extra energie kost ten opzichte van onvolledige resorptie, en (2) dat de patronen in N en P resorptie proficiëncy kunnen worden verklaard met een model waarin een afweging wordt gemaakt tussen de kosten voor een volledige resorptie van een bepaald nutriënt, en de waarde die de plant hecht aan het geresorbeerde nutriënt op het moment van bladsterfte. Dit model voorspelt dus dat als er een tekort is in de plant van een bepaald resorbeerbaar nutriënt op het moment van bladsterfte, indien mogelijk dit nutriënt compleet geresorbeerd zal worden. Tot slot worden de resultaten uit dit proefschrift in de context geplaatst van nutriëntentoevoer aan de bodem in nutriëntenarme veengebieden die onderhevig zijn aan veranderingen in het milieu. De respons van N resorptie uit afstervende bladeren op verhoogde bodem N beschikbaarheid zal er duidelijk toe leiden dat er nog meer N in de bodem komt via afgestorven bladeren. N resorptie reageert echter amper op een verdubbelde CO₂ concentratie, en hierdoor wordt niet verwacht dat verhoogde atmosferische CO₂ concentratie een duidelijk effect heeft op de N toevoer aan de bodem via afgestorven bladeren. De consistente verschillen tussen de NRP van verschillende groeivormen zullen wel bijdragen aan veranderingen in de nutriënten toevoer via afgestorven bladeren wanneer veranderende milieumomstandigheden leiden tot een andere plantensamenstelling.

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